CONCENTRATION OF Cu, Cd, Zn, Fe and Mn IN THE NILE RIVER WATER AND ITS CONTRIBUTION TO THE MEDITERRANEAN

by

A. Osman EL-RAYIS and M.A.H. SAAD
Oceanography Dept., Faculty of Science,
Alexandria University, Alexandria (Egypt)

Abstract

Concentration of dissolved Cu, Cd, Zn, Fe and Mn were determined in thirty-one surface and subsurface water samples collected from fourteen stations spread along the course of the river Nile, from Cairo to the Rosetta mouth. Few variations in the metal concentrations were observed in the water column along the course of the river. The relative abundance of the metals is in the order of Zn > Fe > Cu > Mn > = Cd.

The contribution of these metals in the river to the Eastern Mediterranean is estimated and discussed.

*
* *

Introduction

The river Nile divides near Cairo into two main branches, the Rosetta and Damietta branches, west and east of its delta respectively.

Following the erection of the Aswan High Dam in 1964, the outflow of the Nile water to the Mediterranean became restricted to the Rosetta branch, the Damietta branch remaining blocked by a permanent earthen dam near its mouth, the Farascour dam. The outflow from the Rosetta branch, is controlled by a barrage, 35 km south of its mouth, the Edfina barrage, allowing the release of about 3.5 billion m3/year of river water to the Mediterranean Sea in winter,(SHARAF EL-DIN 1977). The mixed water mass in the sea extends vertically to depths of 50 m below the surface and horizontally to distances of about 25 km (GERGES, 1980). According to Gerges, this mixed water mass moves eastwards from Rosetta. This mixed water, however, has been traced by BALLESTRA et al. (1980) in the Levantine basin at lat. 32° 57'N and long. 31° 56'E, during measurements of the transuranic elements, plutonium and americium in the Eastern Mediterranean Sea in 1977.

Within the framework of the Co-ordinated Mediterranean Pollution Monitoring and Research Programme (U.N.E.P.), several aspects of pollution in the Egyptian Mediterranean are being currently studied. One of the major problems is the estimation of the input of pollutants from various land-based sources, including rivers, to the Mediterranean. Although rivers are known to contribute substantially to the total input of pollutants, studies on the amounts of pollutants carried by the Mediterranean rivers to date still appear to be inadequate for quantitative work (OREGIONI et al., 1978 and SPIVACK et al., 1983).

Table 1. - Results of dissolved trace metals ($\mu g\ l^{-1}$) and chlorosity ($g\ Cl\ l^{-1}$) values in the Nile and estuarine waters of Rossetta branch, June 1978.

Copper in the Nile water

Station No.	I	II	III	IV	V	VI	VII	VIII	IX	X	Mean
Maximum depth (m)	7.0	5.0	4.0	2.0	7.0	7.5	7.0	4.0	14.0	8.0	
Surface	-	1.6	1.1	1.3	1.3	1.2	-	1.4	1.7	0.9	1.31
Bottom	1.6	1.2	1.2	1.0	1.1	-	1.7	0.9	1.3	1.6	1.29
Average	1.6	1.4	1.15	1.15	1.2	1.2	1.7	1.15	1.5	1.23	1.30

Copper in the estuarine water

Station No.	XI	XII	XIII	XIV	Mean
Maximum depth (m)	5.0	3.0	7.0	8.0	
Surface	1.5	1.4	3.2	1.4	1.88
Middle	-	-	1.4	2.6	2.00
Bottom	1.5	1.3	1.3	1.8	1.48
Average	1.5	1.35	1.97	1.93	1.79

Cadmium in the Nile water

Station No.	I	II	III	IV	V	VI	VII	VIII	IX	X	Mean
Surface	0.35	0.49	0.13	0.30	0.38	0.31	0.66	0.39	0.43	0.44	0.388
Bottom	0.58	0.27	0.65	0.35	0.45	0.36	0.34	0.46	0.16	0.56	0.418
Average	0.47	0.38	0.39	0.33	0.42	0.34	0.50	0.42	0.30	0.47	0.403

Cadmium in the estuarine water

Station No.	XI	XII	XIII	XIV	Mean
Surface	1.21	0.39	0.31	0.36	0.57
Middle	-	-	0.52	0.52	0.52
Bottom	0.44	0.39	0.70	0.35	0.47
Average	0.83	0.39	0.51	0.41	0.52

Table 1. - (Continue)

Zinc in the Nile water

Station No.	I	II	III	IV	V	VI	VII	VIII	IX	X	Mean
Surface	4.0	2.8	3.8	58.1	14.2	3.4	6.8	5.4	6.2	10.0	11.47
Bottom	6.7	4.5	-	6.8	2.9	3.7	-	3.7	4.9	5.9	4.89
Average	5.35	3.65	3.8	32.45	8.6	3.6	6.8	4.6	5.6	6.5	8.18

Zinc in the estuarine water

Station No.	XI	XII	XIII	XIV	Mean
Surface	55.1	9.8	7.4	3.3	18.9
Middle	-	-	4.3	12.0	8.2
Bottom	18.3	6.5	1.0	6.0	8.0
Average	36.7	8.2	4.2	7.1	11.7

Iron in the Nile water

Station No.	I	II	III	IV	V	VI	VII	VIII	IX	X	Mean
Surface	2.1	4.4	1.4	2.3	2.3	1.1	1.8	2.4	3.3	3.3	2.44
Bottom	2.8	2.1	2.5	1.5	2.2	2.6	4.4	2.8	1.8	2.5	2.52
Average	2.45	3.3	2.0	1.9	2.3	1.9	3.1	2.6	2.6	2.6	2.48

Iron in the estuarine water

Station No.	XI	XII	XIII	XIV	Mean
Surface	2.2	2.8	1.9	1.6	2.13
Middle	-	-	1.4	1.9	1.65
Bottom	2.3	2.8	2.2	6.8	3.53
Average	2.3	2.8	1.83	3.43	2.44

Manganese in the Nile water

Station No.	I	II	III	IV	V	VI	VII	VIII	IX	X	Mean
Surface	0.1	0.7	0.9	0.4	0.6	0.5	0.4	0.4	0.5	0.4	0.49
Bottom	0.5	0.4	0.5	0.4	0.3	0.4	0.4	-	0.5	0.5	0.43
Average	0.3	0.55	0.7	0.4	0.45	0.45	0.4	0.4	0.5	0.4	0.46

Table 1. (Continue)

Manganese in the estuarine water					
Station No.	XI	XII	XIII	XIV	Mean
Surface	0.6	0.5	0.5	0.4	0.5
Middle	-	-	0.4	0.4	0.4
Bottom	0.9	0.4	0.5	0.4	0.55
Average	0.75	0.45	0.47	0.4	0.48

Chlorosity in the Nile water											
Station No.	I	II	III	IV	V	VI	VII	VIII	IX	X	Mean
Surface	-	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03
Bottom	-	0.02	0.03	0.03	0.04	0.04	0.03	0.03	0.03	0.04	0.032
Average	-	0.02	0.03	0.03	0.03	0.035	0.03	0.03	0.03	0.04	0.031

Chlorosity in the estuarine water					
Station No.	XI	XII	XIII	XIV	Mean
Surface	4.91	5.45	6.08	6.59	5.758
Middle	-	-	11.91	13.18	12.550
Bottom	6.08	17.24	20.79	21.80	16.480
Average	5.495	11.35	12.93	13.86	11.596

Table 2. - Correlation coefficients between chlorisity and each of the trace metals in Rosetta branch waters.

Element		Cu	Cd	Zn	Fe	Mn
Chlorosty	River water n= 17	-0.21	0.18	0.16	0.25	-0.30
	Rosetta branch n=29	0.32	0.19	0.00	-0.19	-0.08

n= Number of observations.

The value of significance is > 0.5 or < -0.5.

With the aim of filling this gap, trace metals in the water of the river Nile were measured and the contribution of Cu, Cd, Zn, Fe and Mn by the river Nile to the Mediterranean was estimated.

Material and methods

Rosetta branch is about 250 km in length, an average width of 270 m and an average depth of 7 m.

Thirty-one surface and subsurface water samples were collected, in June 1978, from fourteen stations ; ten at upper stream locations and four downstream or in the estuarine zone from Edfina barrage, (figure. I). The water samples were collected with a plastic sampler of eight litres capacity, and kept in polyethylene Jerry cans, prewashed with acid and rinsed with metal-free water (purities of 10^{-2} µg/l for the metals studied). The samples were filtered through 0.45 um pore size, clean membrane filters (Millipore). Cu, Cd, Zn, Fe and Mn in the filtered waters were preconcentrated using chelating resin, Chelex-100, (RILEY and TAYLOR, 1968), but in ammonia form. This by allowing the filtered water to flow through the Chelex resin in a glass column. the flow rate did not exceed 300 ml/hour. The column was then washed with 150 ml of matel-free water. The metals on the resin were eluted with 70 ml 2M redistilled HNO_3 "Merk". The eluated solution was collected in a 100 ml capacity silica crucible and evaporated to dryness on a hot plate at a low temperature, 95°C. 1 ml of 6M HNO_3 was added and when the residue has dissolved, the solution was transfered quantitatively to a 25 ml measuring flask using metal-free water. Cu, Cd, Zn, Fe and Mn were determined in the solution using flame atomic absorption spectrophotometry (Schimatzu-AA-360-11). The coefficient of variation was 8.5, 3.8 and 1.92 for Cu, Cd and Zn respectively. Chlorosity of the water samples was determined by titration with silver nitrate solution.

Results and discussions

The results of dissolved Cu, Cd, Zn, Fe and Mn measurements and chlorosity values of the Nile water as well as those in the estuarine water of Rosetta branch are listed in table 1.

In the Nile water (average chlorosity 0.031 g/l), the range of concentrations was between 0.9-1.7, 0.13-0.66, 2.8-58.1, 1.1-4.4 and 0.1-0.9 ug/l for Cu, Cd, Zn, Fe and Mn respectively. In the estuarine water (average chlorosity 11.60 g/l) , the concentrations ranged between 1.3-3.2, 0.31-1.21, 1.0-55.1, 1.4-6.8 and 0.4-0.9 µg/l for Cu, Cd, Zn, Fe and Mn respectively. Cu, Cd, Fe, and Mn show slight variations in concentrations relative to that for Zn in both the fresh and estuarine waters. Most of the values in these waters are close to their overall means, which are given in table 1. The two exceptionally high concentration values of Zn 58.1 and 55.1 ug/l, observed in the surface waters of stations III and XI, could be due to contamination during collection of the two samples. Excluding these two values, the Zn concentration ranges from 2.8 to 14.2 and from 1.0 to 18.3 ug/l in the river and estuarine waters respectively. The values for Zn, however, are generally higher compared to those for the other four metals in the branch waters. The abundance of these metals in the waters is in the order Zn > Fe > Cu > Mn >= Cd.

During 1979, M. Fahmy (personal communication) sampled the Nile water at a station east of the station II of the present study in March, June, September and December for metal analysis. He observed the same order of abundance as mentioned above and the percentage standard deviation for Fe, Mn, Zn and Cd during the four seasons were 22, 30, 45 and 9 respectively. Zn also showed the highest fluctuation in the concentrations relative to the other metals studied. He attributed the fluctuation mainly to the biological activity in the Nile water.

Table 2 shows the corellation coefficient between the chlorosity and each of the trace metals studied in the Rosetta branch. In general, no significant relationship was found between the metal concentration and chlorosity, even in the estuarine part of the branch. This suggests that their

level in the sea-water off the branch mouth is more or less similar to that found in the Nile water. And remobilization or adsorption of metals to or from the water to the surfaces of suspended solids during their transfer from fresh to saline water, seem not to be clear here. This phenomenon has been reported for other estuaries in other areas such as the Rhine estuary, by DUINKER (1980). The obscurity of this phenomenon in the Nile estuary could be due to the low level of the Nile suspended silts. As an effect of the Aswan High Dam, the Nile water became relatively clear during most of the year, its suspended solids content falling to a few mg per litre (FAHMY, 1981). The values of suspended matter before 1964 were as much as 3000 mg/l, (SHARAF EL-DIN, 1976). Most of the suspended silt is being deposited behind the dam. Further investigations into the level of those metals in the Mediterranean coastal water off Rosetta mouth is needed. The nearest area investigated for these metals is Abu Kir bay, west of Rosetta mouth (SAAD et al. unpublished data). figure I. The levels of these metals in this bay (table 3) are considerably higher than those found in Rosetta branch waters. This bay, however, receives wastes from food canning factories, paper mills, fertilizer industries and agricultural run-off from lake Edku. These could be responsible for the high values of the metal concentrations observed in this bay.

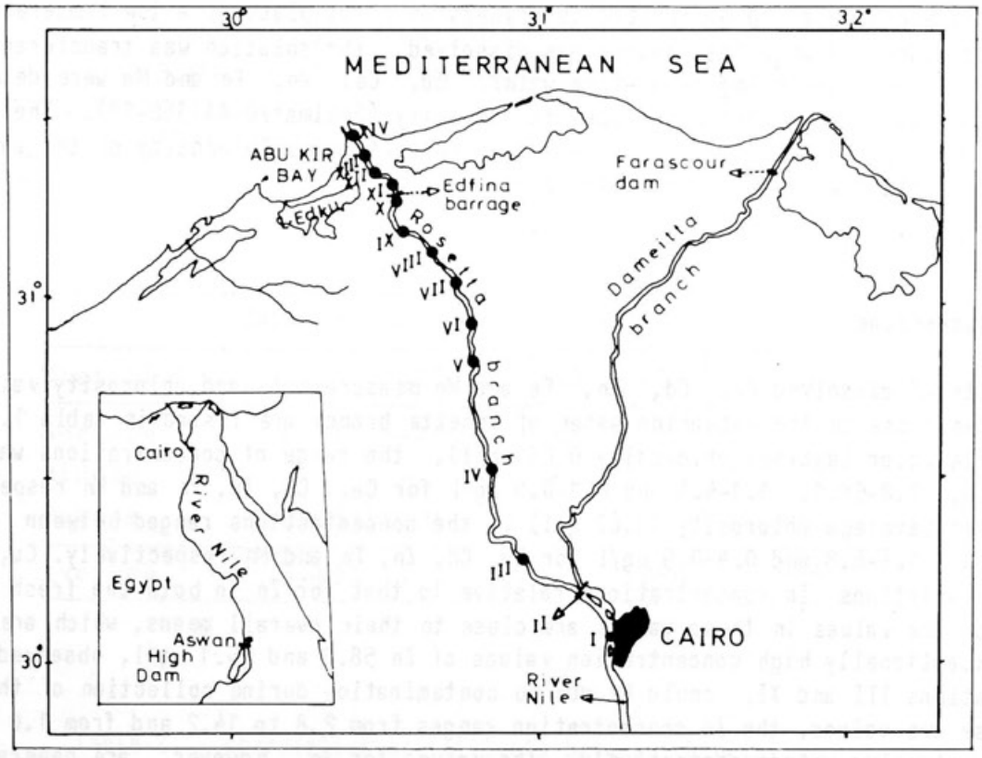


Fig.1 - Map of Rosetta branch showing the positionsof stations.

The average metal concentrations in world rivers (table 3), reported by CHESTER and ASTON (1977) and MARTIN and WHITFIELD (1983) are generally higher than in the Nile water, but the order of abundance of the metal Zn > Cu > Cd in the Nile and other world river waters is similar. The most recent values for river water reported by MARTIN and WHITFIELD (1983) are higher for Zn, Fe and Mn than in the Nile water. It is difficult, however, to compare the level of elements from one river to another, simply because each river has it own chemical characteristics and concentrations which depend mainly on the nature of its catchment area. Fe, for example, in the world rivers reaches much

higher concentrations (670 or 40 ug/l) compared to that in the Nile water (2.5 ug/l). This may be due to the fact that Nile water is of relatively low organic content and confirms the effectiveness of the dams in precipitating the colloidal Fe. The increase of organic matter content dissolved in water favours the possible formation of Fe-organo dissolved complexes which can pass with some fine colloidal-Fe through 0.45 um-pore size membrane filters, (RILEY and SKIRROW, 1975).

Table 3. - Average concentration (µg/l) of some trace metals in the Nile water, in other world rivers and in Abu Kir Bay.

	Trace metal					Reference
	Cu	Zn	Cd	Fe	Mn	
River Nile	1.3	8.2	0.4	2.5	0.5	Present Work
World rivers	5	10	1.0-3.4§	670	5	Chester & Aston (1976)
	1.5	30	0.02	40	8.2	Martin & Whitfield (1983)
Abu Kir Bay	4.2	25.5	0.32	6.7	3.7	Unpublished data

§ Data from Royle (1973) : Cd-river Rheidal, Wales, U.K.

Annual contribution of dissolved metals by Nile water to the Mediterranean Sea

The volume of water discharged to the Mediterranean by the river Nile annually has fluctuated with time depending on the effectivness of the dams built on the river course since 1902. Before 1902, i.e. before building the first Aswan Dam, it was 100 billion m3 annually, decreasing to an average 35 billion m3 from 1902 to 1964, i.e. before erecting the new Aswan High Dam, and to 3.5 billion m3, on average, annually after 1964.

Table 4. - The total amount of fresh water and of some heavy metals discharged from the river Nile to the south east Mediterranean Sea, 1978.

Period	The annual discharged amount to the Mediterranean					
	Fresh water (billion m^3)	metal (in tons)				
		Cu	Cd	Zn	Fe	Mn
After erection of Aswan High Dam	3.5§	4.6	1.4	28.7	2.3	1.8

§ After Sharef El-Din (1977).

The present amounts of the various metals, in metric tons, based on the present work, discharged with the Nile water to the south-east Mediterranean Sea is calculated and shown in table 4. As mentioned before, the Nile effect is mostly confined first to an area of the sea 25 km from the Rosetta mouth before reaching other eastern parts of Eastern Mediterranean Sea.

Summary

Thirty-one water samples collected from Rosetta branch, the main discharging branch for the Nile water to the Mediterranean Sea, were analysed for dissolved Cu, Cd, Zn, Fe and Mn. The branch consists mainly of fresh water except in its last 35 km before joining the sea, where there is a mixture of waters from the Nile and the sea. The concentrations of each of the metals in the fresh and in the estuarine waters are more or less alike, suggesting that both the fresh and the saline waters bear similar levels of the five metals. The levels are comparable with those reported by CHESTER and ASTON (1977) and MARTIN and WHITFIELD (1983) for other world rivers. On the basis of the present study, the river Nile is annually contributing 4.6, 1.4, 28.7, 8.8 and 1.8 tones of dissolved Cu, Cd, Zn, Fe and Mn respectively, to the Mediterranean Sea. The present amount of water discharged annually is about one tenth and one thirtieth of amounts prior to 1964 and 1902 respectively.

NATURAL AND MAN-MADE SURFACE FILMS AT THE AIR/SEA INTERFACE CRITERIA BASED ON MEASUREMENTS OF PHYSICAL PHENOMENA

by

Velimir PRAVDIC and Djurdjica DRAGCEVIC
Laboratory of Electrochemistry and Surface Phenomena
Center for Marine Research Zagreb
The Rudjer Boskovic Institute, Zagreb, (Yugoslavia)

Abstract

The dynamic phenomena of surface tension and surface potential of natural and man-made surface films at the sea-water/air interface offer some interesting insight into the energetics of such films. Using a combined laboratory unit for the simultaneous measurement of surface tension and surface potential under the same rate of perturbation, the mechanical and electrical responses were obtained. Analysing these responses an estimate was obtained on the energy barrier such surface films represent to the penetration by gases, and to the exchange of aerosols and other particulate matter through it. By application of thermodynamics to such films in the steady state, data were obtained on the free and bound energy. It seems that mechanical perturbation (winds and waves) generate electrification of the surface and consequently influence, at least to a minor degree, the character of aerosol generated.

*
* *

Introduction

Research on the exchange of matter and energy between the atmosphere and the sea has dominated several fields of marine chemistry, environmental and meteorological science, and has become one of the several projects in the MED POL programmes. The most comprehensive review of such studies can be found in several reviews (PROSPERO, 1975, LISS, 1975, LISS and SLINN, 1983).

The formation of aerosols and the transport of dissolved/particulate matter from the sea to the atmosphere is mediated by the film forming organics present either as natural detritus or as anthropogenic releases (LEMLICH, 1972 ; CLIFT, 1978). The surface film is a barrier for the transport between the sea-water and the atmosphere (BLANCHARD, 1963 ; BEZDEK and CARLUCCI, 1974 ; LISS and MARTINELLI, 1978). It has been found that this barrier is comprised not only of chemical structural forces, but also of electrical field gradients (Davies and Rideal, 1963).

The present paper highlights some of the results which were obtained in a study of natural seawater films collected in the Rijeka Bay (the northern Adriatic). These films were studied in an apparatus constructed in this laboratory (DRAGCEVIC and PRAVDIC, 1981) featuring simultaneous dynamic

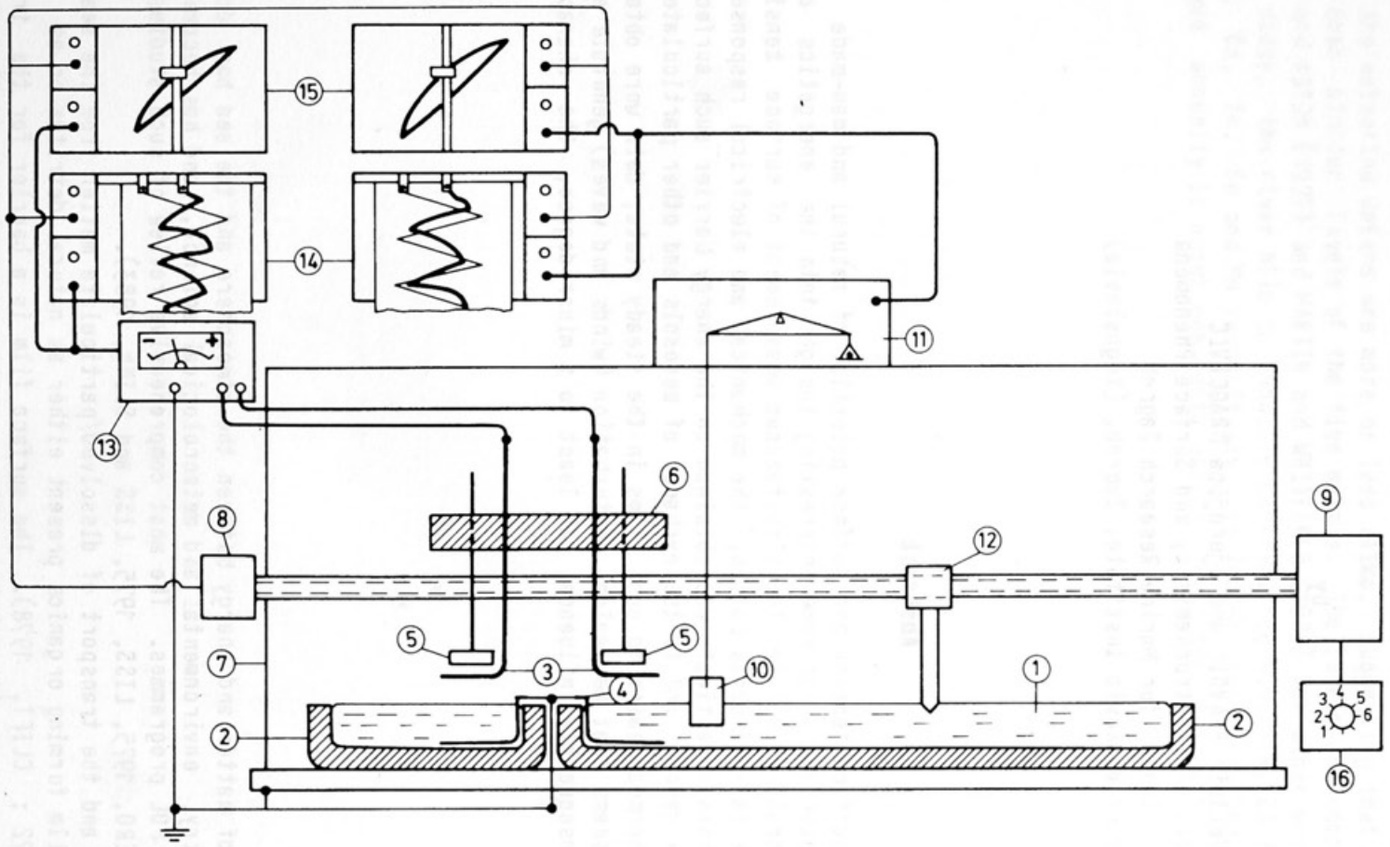


Fig. I. The schematic of the dynamic surface tension-surface patential apparatus : (1) sample trough : (2) reference trough : (3) gold electrodes potential sensors · (4) electrical ground connection ; (5) radioactive ionization probes ; (6) electrode holder, vertically adjustable ; (7) Faraday's cage : (8) position tranducer : (9) sweep motor and drive : (10) Wilhelmy plate-surface tension sensor ; (11) surface tension electro balance ; (12) P.T.F.E. surface sweeper : (13) differential electrometer : (14) and (15) recording devices : (16) sweep rate controller.

perturbation measurements of surface tension and of surface potential. Its aim was to indicate that the surface film is capable of storing and transforming mechanical and electrical energy, and that the barrier to the transport of gases and particulate matter is both structural (entropic) and electrical (dipolar).

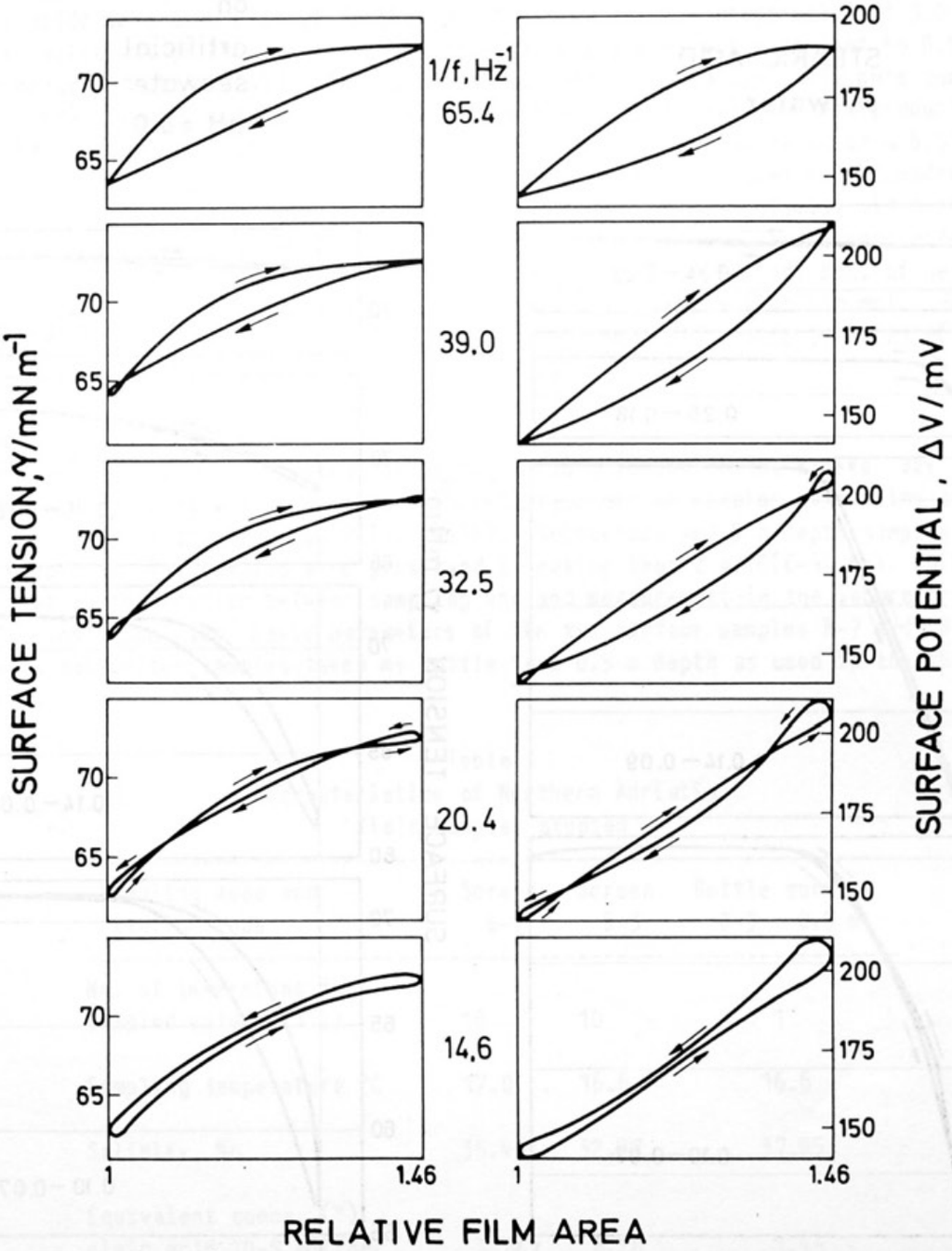


Fig. II. Surface tension (left) and surface potential (right) of the field sample (bottle) plotted against relative area in the measuring trough at five perturbation rates.

Furthermore, such studies as represented here and reported in part before (DRAGCEVIC et al., 1979 ; DRAGCEVIC and PRAVDIC, 1980, 1981) allow the interpretation of phenomena in surface films based on quantitative comparison with model systems. They are not designed or meant to be a substitute for chemical analysis, but rather a complement in understanding the energetics of such films.

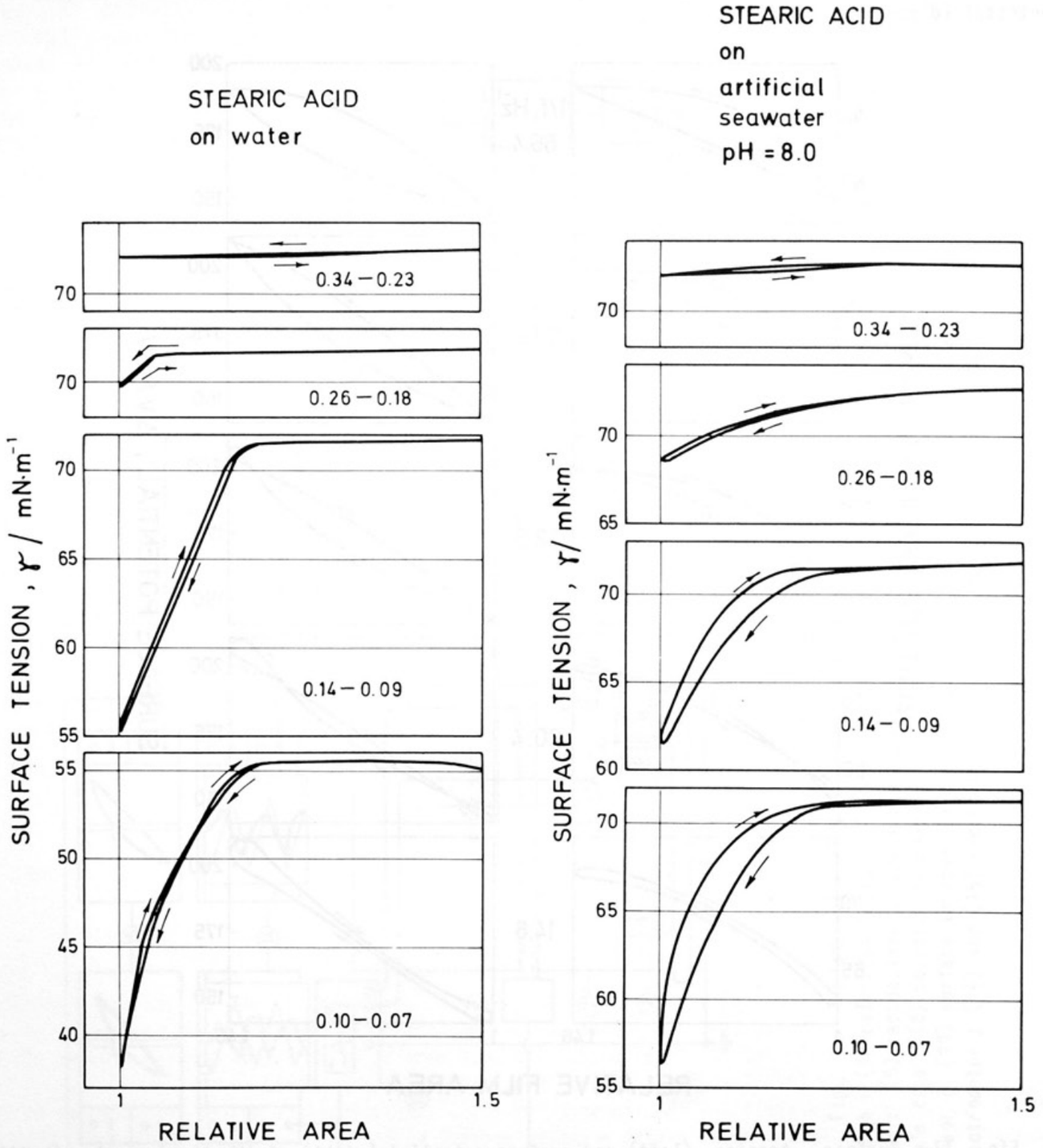


Fig. III. Dynamic surface tension for stearic acid against the relative film area in the trough on (a) water, and (b) artificial sea-water at 38 ppt salinity. Figures inside show concentrations of stearic acid in nm2 per molecule in compressed and expanded film. Perturbation rate : 57.6 Hz-1.

Experimental

Chemicals and film forming agents

Stearic (n-octadecanoic) and oleic (cis-octadecen (9, 10) oic) acids (Kemika, Zagreb) and polyethylene glycol (mol. weight M = 4000, Merck-Schuchardt, Fed. Rep. of Germany) were high purity grade chemicals. The acids were used without further purification for the preparation of 3.0 x 10(E-4) mol. dm(E-3) stock solutions in ethanol, whereas polyethylene glycol was dissolved to 0.5 g dm(E-3) in water. Sodium dodecylsulphate (SDS) (BDH laboratories, London) was a specially pure commercial chemical (99.0 per cent). It was recrystallized twice from absolute ethanol. The end product was chromatographically pure, that is, better than 99.99 per cent SDS. It was prepared as a 6.51 x 10(E-4) mol dm(E-3) stock solution of SDS in water. Artificial sea-water was prepared using quadruply distilled water, the last two stages from a fused silica still, tested for conductivity (less than 2 x 10(E-6) S cm-1) and for the absence of organics by surface tension-surface area hysteresis effects (to within 0.02 mN. m-1). All the salts were of analytical purity grade (MERCK, Fed. Rep. of Germany) and were used without further purification. Artificial sea-water had a composition (in mol. dm(E-3)) : 0.56 NaCl, 0.05 MgSO(4), 0.01 CaCl(2), 2.2 x 10(E-3)NaHCO(3) and 0.22 x 10(E-3) Na(2)CO(3). The final solution had a pH of 8.0 and corresponding to a salinity of 38 ‰.

Field samples

Sea-water samples were collected at hydrographic stations in the Rijeka Bay (the northern Adriatic). Surface sampling was done using a Garrett-type screen sampler consisting of a frame of 85 x 70 cm of 16 mesh stainless steel (GARRETT, 1965). Subsurface and 5 m depth samples were taken by Niskin water samplers. The samples were preserved by making them 2 x 10(E-4) mol. dm(E-3) in HgCl(2) to prevent microbial degradation between sampling and and measurement in the laboratory.

Table 1 identifies the basic parameters of the two surface samples B-7 and B-3 sampled by screen, and of a subsurface samples taken my bottle from 0.5 m depth as used in the IGOSS and MED POL I pilot project.

Table 1
Characteristics of Northern Adriatic
field samples studied

Sampling mode and station code	Screen B-7	Screen B-3	Bottle surface B-3 : 0.5 m
No. of immersions for sampled volume (1 l)	10	10	1
Sampling temperature °C	17.0	16.6	16.6
Salinity, ‰	35.46	32.77	37.05
Equivalent concn. (*) oleic acid 10-8 mol/dm2	3.78	8.26	1.44
Surface tension (**) mNm-1	58.15	55.45	71.88

(*) Analysis on concn. equivalents (PRAVDIC and DRAGCEVIC, 1981) by standard addition of oleic acid to artificial sea-water of the same salinity until the same surface tension as sample.
(**) Surface tension of the sample measured at maximum compression of film, transferred as indicated in text, and measured dynamically at lowest perturbation rate (57.6 Hz-1).

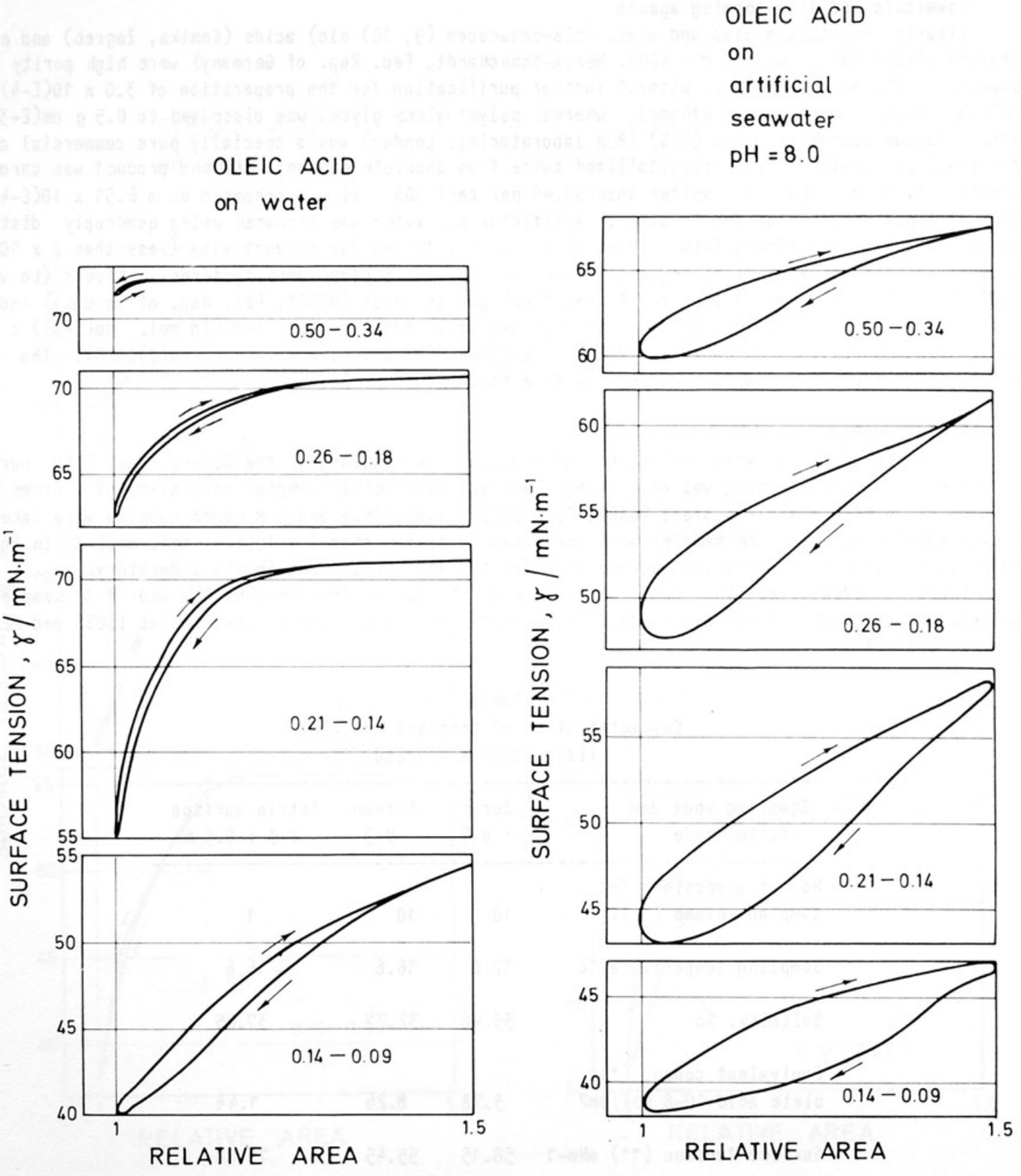


Fig. IV. Same as for figure III, except for oleic acid as film forming agent.

Transfer of the field sample into the measurement trough.

The sample was collected and transferred to a one litre bottle. It was vigorously shaken and poured into the measuring trough. The trough had a volume of 760 cm3 and an exposed surface area of 380 cm2, yielding a surface-to-volume ratio of 0,50 cm-1. Thus it was a standardized procedure which can always be applied to relate to concentrations prevailing in the field.

Techniques and methodology

The combined apparatus for surface tension and surface potential measurement, constructed in this laboratory is schematically shown in figure I. It comprises a Wilhelmy Pt-sensor attached to an electrical microbalance (Cahn Division, Ventron Corp., USA) whose output is fed to a recording device (shown as chart recorders in figure I). The registration of the surface potential consists of two identical gold electrodes situated close to the surface of the sample (large trough) and of the reference solution (small trough). The reference solution used was synthetic sea-water of the same salinity as the sea-water sample used. The electrodes are connected to a differential high impedance electrometer, and the resulting voltage difference is fed to the recording device (shown again as chart recorders in figure I). The sample in the measuring trough can be perturbed by a PTFE surface sweeper in a predetermined way, simulating dynamic conditions at sea.

The result of such measurements, shown in figure II, is a recording of the surface tension and surface potential against the relative surface (compression ratio). The phenomenology of such hysterese curves allows for identification of the nature and of the origin of surface films by comparison with various standards. These standards have been produced by spreading films of stearic and oleic acids, crude oil, hexadecane, sodium dodecylsulphate and polyethyleneglycol on natural and artificial sea-water. The area enclosed by the hysteresis curve has dimensions of energy and is, eventually, the amount of either total surface energy (surface tension) or its part, electrical energy (dipolar reorientation) stored and released by such films during dynamic perturbation (DRAGCEVIC, 1983; DRAGCEVIC AND PRAVDIC, to be published).

RESULTS AND DISCUSSION

The phenomenology of surface films is described in a sequence which would best reveal its response to dynamic perturbation.

Types of surface films

In order to illustrate the phenomenology of dynamic surface tension it is necessary to emphasize the importance of the concentration of the film, the nature of the substrate, and the perturbation rate. Two model substances were studied first in an attempt to describe the dynamic surface tension behaviour of the sea-water surface. In figures IIIa and IIIb the model substance is stearic acid and the results are shown of studies at various surface concentrations.

In figure IIIa the dynamic surface tension of four concentrations of stearic acid on water (of salinity S = 0) is shown. The numbers indicate the calculated area per molecule in nm2 at maximum expansion and compression of the film. For the lowest concentrations (top curve) there is almost no hysteresis in the compression-expansion cycle. At the next higher concentration one finds the incipient hysteresis and a kink in the curve showing the breakdown of the compressed surface film. At even higher concentrations the hysteresis is still small, but the film shows typical inelastic breakdown behaviour, marked by the sharp break in the surface tension - surface area curve (ADAMSON, 1976; DAVIES and RIDEAL, 1963).

The result of the studies of stearic acids films on artificial sea-water are shown in figure IIIb. At the same concentrations, as in figure IIIa, one immediately observes an increasing elasticity of these films, appearing as a rounding-off of the compression-expansion curves. It is a phenomenon associated with the formation of calcium mono- and distearate in the surface film, a process

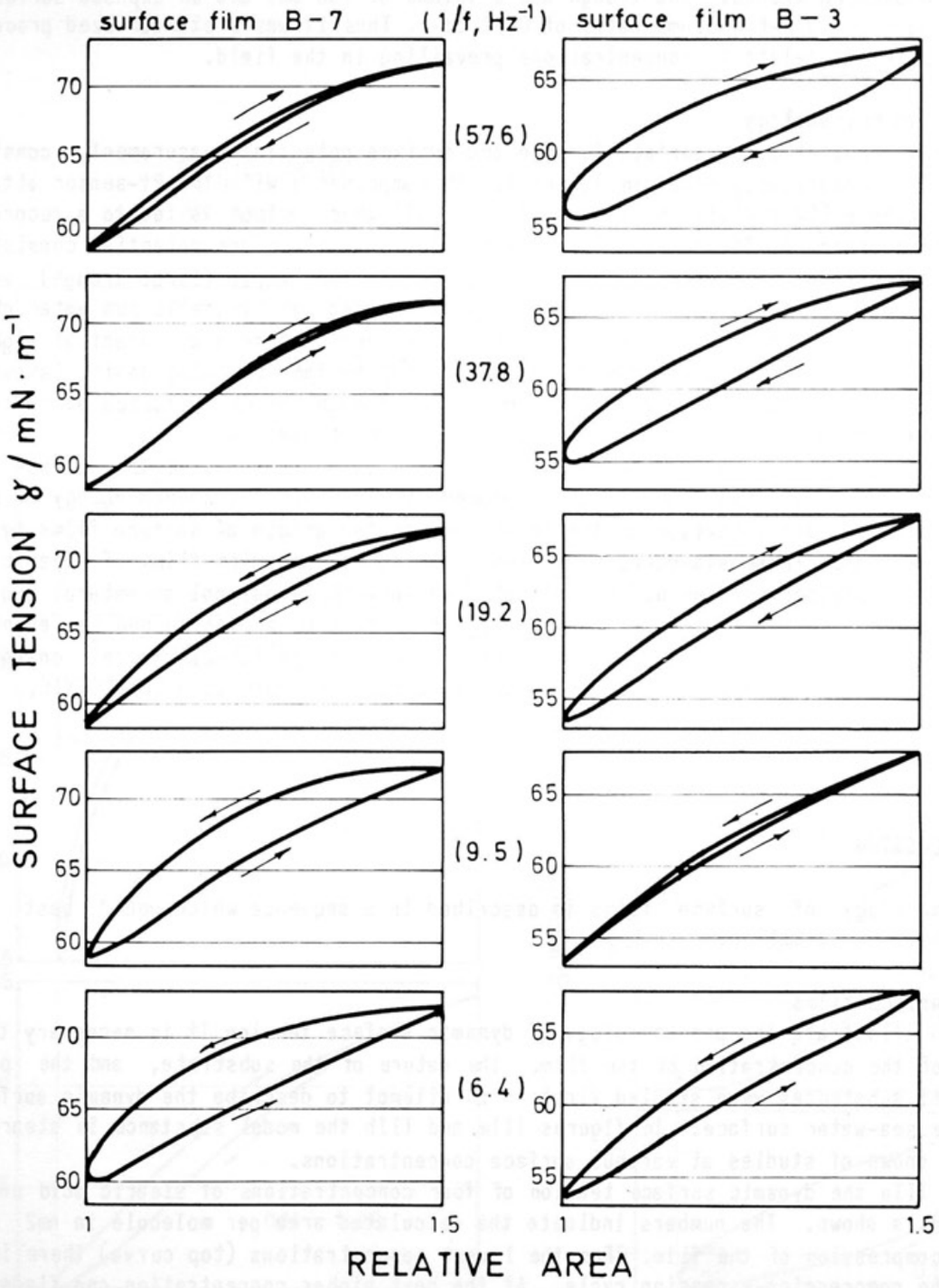


Fig. V. Surface tension vs. relative area for two original sea surface films at various perturbation rates.

known to happen in sea-water (NEUMAN, 1975). These films represent a model for most of the films of natural origin formed by lipid-like detrital material.

Figures IVa and IVb represent the experiments done with oleic acid, at concentrations in the same range as with stearic acid. The phenomenology of hysteresis immediately indicates more "soft" forms, and considerably increased hysteresis areas. Such behaviour is characteristic of anthropogenic pollutants, stemming mostly from crude oil derivatives and detergents. One of the features is characteristic : the reversal of the anticlockwise direction of the hysteresis loop to the clockwise happens at lower concentrations for oleic acid than for the rigid films of stearic acid. Thus the indication of the approximate concentrations at which in a surface film such a hysteresis loop changes sign is also a criterion of the origin of the sea surface film.

In figure V the results are given for the two field samples. Both films were subjected to increasing perturbation rates (top to bottom in figure V). The point at which perturbation rates induce the hysteresis loop to change direction is also one of such criteria. Film B-7 is more rigid at low perturbation rates and changes sign at the very next rate. Film B-3 is less rigid at low perturbation rates, more so at high, and shows the reversal at some high perturbation rate. On the basis of the information available such a criterion is also useful in characterizing sea surface films with respect to their origin. It seems that B-7 is the lipid-like film, and the B-3 one softened by sources of anthropogenic origin.

On the hysteresis loop area

The hysteresis loop area has a dimension of energy. Several hundreds of such hysteresis areas were integrated and studied during this work. The surface tension hysteresis areas range between $+3 mJ/m^2$ at low perturbation rates to $-1 mJ/m^2$ at the high ones, referring to those accessible in the apparatus as used here (cf. figure V). The surface potential hysteresis loops similarly range from +8 to -10 mV m^2 per m^2. In these latter values the missing factor is capacity and the dielectric constant in the film, to enable calculations of the electrical energy stored. A calculation assuming 10 nm for the film thickness would indicate only negligible effects such as temperature rise due to the conformational energy, probably negligible in comparison with heating due to random friction. On the contrary, both the absolute changes in the surface potential (some 50 mV per cycle, cf. figure II) and the calculation of the capacity available at moderate to high surface coverages support the idea of BLANCHARD (BLANCHARD, 1963), that at dynamic fields of 5000 Vs/cm the electrification of the interface must be considerable. However, exact calculations are dependent on too many adjustable parameters to allow quantitative conclusions on the total charge transfer possible.

Conclusion

The studies on surface films made by the techniques and methodology of dynamic surface tension and surface potential, as described in the paper, offer a wealth of information on their behaviour, origin and characteristics. Used as background information and related to experiments directly measuring gas exchange rates, it would enable one to make significant predictions on the influence of sea surface films on a particular marine ecosystem. It seems that data of this kind would be highly useful in shallow, nearshore areas, and indeed in areas where a significant load of urban and insdutrial pollution, including heated effluents may seriously strain the receiving capacity of the ecosystem.

Acknowledgements

This work has been supported by the Authority for Scientific Research of the SR of Croatia (Yugoslavia). The original equipment purchases were made through Grant 68X0104 (P.L. 480) from the US Environmental Protection Agency, Washington, D.C. Mr. Z. PEHARAC is thanked for the collection of sea surface samples, and Mr. M. MILUNOVIC for constructing and servicing the electric and electronic equipment.

Références

ADAMSON, A.W. (1976). Physical chemistry of surfaces. Wiley-Interscience, New-York, 3rd ed. pp. 50 and 129 ff.

BEZDEK, H.F. and A.F. CARLUCCI (1974). - Concentration and removal of liquid microlayers from sea-water by bursting bubbles, Limnol. Oceanogr. 19 : 126-132.

BLANCHARD, D.C., (1963). - The electrification of the atmosphere by particles from bubbles in the sea, Progress in Oceanogr. 1 : 72-202, Pergamon.

CLIFT, R., J.R. GRACE and M.E. WEBER, (1978). - Bubbles, drops and particles. Academic Press, New York.

DAVIS, J.T. and E.K. RIDEAL, (1963). - Interfacial phenomena, 2nd ed., Academic Press, New York, pp. 11 and 225 ff.

DRAGCEVIC, Dj., (1983). - Dynamic properties of surface films at the sea-water/atmosphere interface. Ph.D. thesis, University of Zagreb.

DRAGCEVIC, Dj. and V. PRAVDIC, (1980). - Steady-state relaxation times for surfactant films at the water/air interface, Croat. Chem. Acta 53 : 1-7.

DRAGCEVIC, Dj. and V.PRAVDIC, (1981). - Properties of the sea-water-air interfaces. 2. Rates of surface film formation under steady state conditions, Limnol. Oceanogr. 26 : 492-499.

DRAGCEVIC, Dj., M. VUKOVIC, D. CUKMAN, and V. PRAVDIC, (1979). - Properties of sea-water-air interface. Dynamic surface tension studies. Limnol. Oceanogr. 24 : 1022-1030.

GARRETT, W.D., (1965). - Collection of slick-forming materials from the sea surface, Limnol. Oceanogr. 10 : 602-605.

LEMLICH, R., (1972). - Adsorptive bubble separation techniques, Academic Press, New York.

LISS, P.S., (1975). - Chemistry of the sea surface microlayer. In : J.P. RILEY and G. SKIROW (eds.), Chemical Oceanography, 2nd ed., v.2, Academic Press, New York, p. 193-243.

LISS, P.S. and F.N. MARTINELLI, (1978). - The effect of oil films on the tranfer of oxygen and water vapour across an air-water interface, Thalassia Jugosl. 14 : 215-220.

LISS, P.S. and W.G.N. SLINN, (1983). - Air-sea exchange of gases and particles. D. Reidel Publishing Company, Dordrecht.

NEUMAN, R.D., (1975). - Calcium binding in stearic acid monomolecular films, J. Colloid Interface Sci. 53 : 161-171.

PROSPERO, J.M. (ed. Chairman), (1975). - Background papers for a Workshop on the tropospheric transport of pollutants to the Ocean the National Research Council, Ocean Sciences Boards, Miami Florida, pp. 351.

PRAVDIC, V. and Dj. DRAGCEVIC, (1981). - Natural and man-made surface films at the sea-water/air interface. Studies of samples collected in the Rijeka Bay (Northern Adriatic), Ves Journées Etud. Pollutions, C.I.E.S.M., 73-79.

ATMOSPHERIC CONTRIBUTION TO MARINE POLLUTION IN THE BAY OF NAPLES (*)

by

Antonino PALUMBO and Michele IANNIBELLI
Dipartimento di Geofisica e Vulcanologia
Universita' di Napoli
Largo S. Marcellino N° 10 Napoli - (Italia)

Abstract

The contribution of atmospheric metal pollution to marine pollution, in the Gulf of Naples has been analysed. The present paper refers only to three heavy metals : Fe, Cu and Zn. A comparison between the atmospheric input and the outfalls input coming from the hinterland has been made.

The results show a contribution of the atmosphere to the sea in the following percentages :

Fe : 31.1 % Cu : 10.5 % Zn : 11.5 %

Further investigations must be conducted to confirm this estimate.

*
* *

Introduction

Concentrations of atmospheric pollutants at ground level are obviously markedly lower than the data observed at the mouth of any sewer or drain.

On the other hand, the sea-body surfaces included in the above-mentioned draining areas are markedly reduced compared to coastal water extensions, with a higher percentage of atmospheric pollutants, and to offshore water expanses, nevertheless characterized by long-range transport. At this stage of our research in some areas we might experiment with a comparison between two pollutant inputs : coastal water and the atmosphere.

In the Bay of Naples marine pollution was determined as amongst the highest in Italy (MONCHARMONT, 1973). The whole area is surrounded by many urban and industrial centres where atmospheric pollution reaches considerably high values (PALUMBO et al., 1978).

It is difficult however, to quantify the chemical components due to scarce concentrations. Considering these facts the Gulf of Naples may represent an interesting sampling site for a comparative study of the two main pollutant inputs : the hydric (coming from the hinterland) and the atmospheric.

---

* This paper refers to the special agreement N.18.444 A/CNS/83

The Gulf of Naples is a bay with a wide entrance facing west to the Tyrrhenian Sea (YAMAZI, 1964) with a mean depth of 170 m : the area is 870 km2 and the volume is 150 km3 (CARRADA et al., 1980). Such a discrepancy between these last two parameters reduces the influence of local wind stress, increasing the power of internal flushing.

The Bay of Naples is entirely a volcanic system where the Vesuvius and the Flegrei Fields are the active vents and the mainland, together with the islands of Ischia and Procida form the system rim. Because of this structure, and because of the presence of the island of Capri, alongshore transport between the Gulf and close coastal embayments is restricted, as well as the exchange of waters between the Bay and the Mediterranean Sea. (BELLAMY et al, 1974).

Density (DE MAIO et al., 1983) and tidal currents (PALUMBO et al., 1981) are very low. These may be the causes of the increased level of toxic metals in the Bay.

monitoring station	Pb	Cd	Zn	Cu	Fe
	(milligrams/sq.meter 30days)				
1) Ischia	0.79	0.05	1.58	0.26	7.63
2) Bacoli	3.68	0.26	5.00	0.26	40.14
3) Lucrino	6.19	0.39	17.63	0.66	23.29
4) Napoli	5.79	0.39	4.74	1.32	38.43
5) Torre del Greco	1.71	0.13	3.16	0.53	4.74
6) Castellammare	2.90	0.26	4.61	1.18	19.74
7) Vico Equense	2.69	0.16	8.26	2.19	5.03
8) Sorrento	0.78	0.01	1.18	0.39	3.95
9) Capri	1.11	0.03	0.79	0.11	0.79
Mean value	2.85	0.13	5.22	0.77	15.97

Table 1.

The local climate, lacking extensive precipitation and slightly windy, affects the hydrology of the Bay, thus provoking in the summer slightly higher salinities and the formation of layering. Between early spring and late autumn, which mark the beginning and the end of the layering, respectively, a clearly defined thermocline may be noticed.

In winter, with the coming of violent seasonal winds, vertical mixing makes almost all the waters of the bay isothermal (HAPGOOD, 1960).

Material and methods

Hydric input - Atmospheric input

Because of the difficulty of obtaining reliable emission data, it was impossible to set up a diffusive model which would have provided a sea-level distribution of pollutants.

The physical processes by which atmospheric pollutants reach the surface of sea are extremely complex. The present research is based on a statistical approach which led to very promising results. Further observational data are needed to obtain more significant determinations.

The following pollutants : Pb, Cd, Zn, Cu and Fe, found in great abundancy in the Bay of Naples (DE ROSA et al., 1982), were examined.

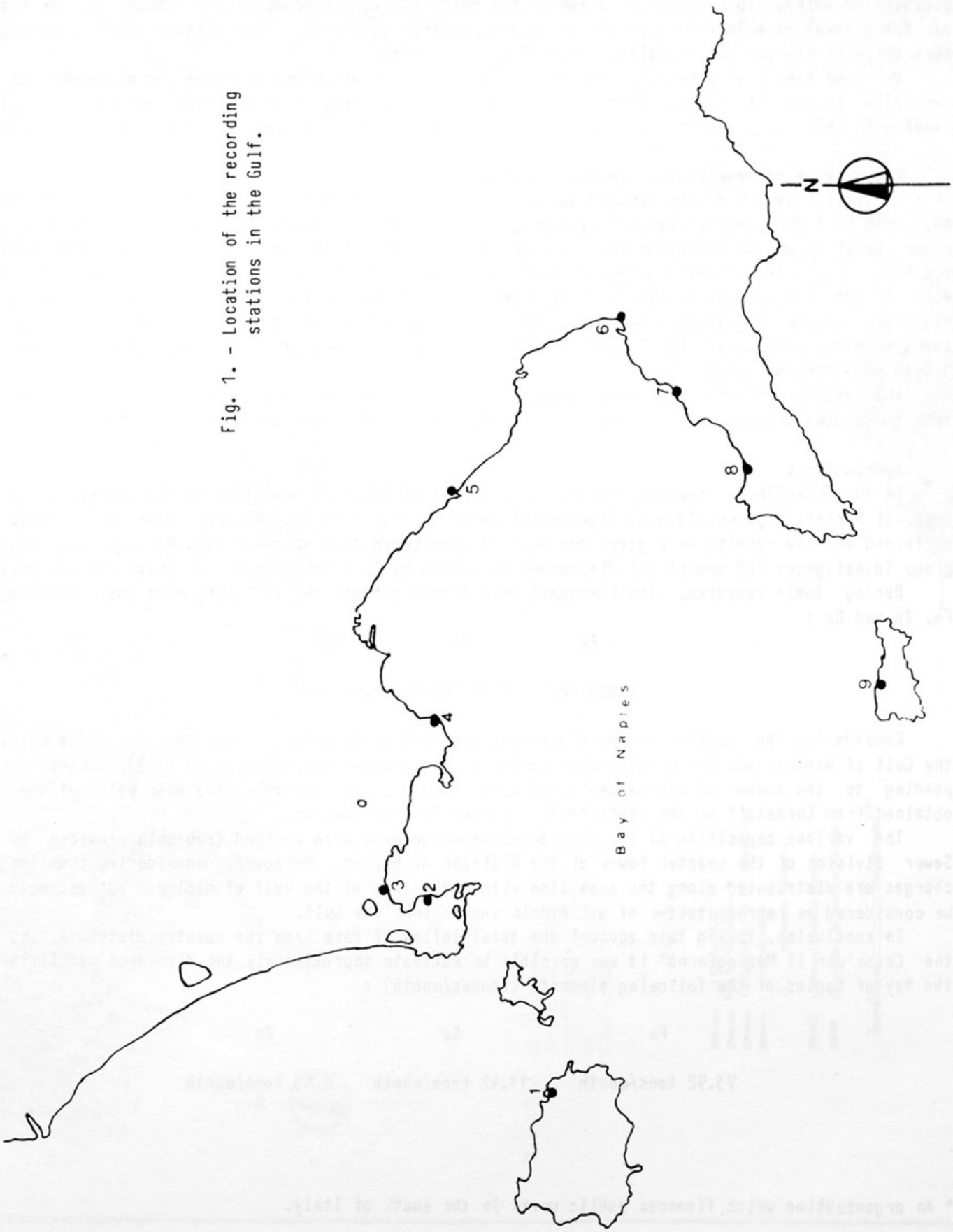


Fig. 1. - Location of the recording stations in the Gulf.

The areas of investigation, all located by the seaside, are reported in figure I. During the two-month experiment (April and August, 1983) glass plates permanently filled with rain and/or bidistilled water, to simulate a permanent sea surface, were exposed to the atmosphere. The results of the present experimental approach are not comparable to others. Some authors have investigated separately on dry and wet deposits. (GEORGII et al., 1984).

We have been trying to determine the overall fall-out of pollutants from the atmosphere to the sea. The present technique of the plates suits our aims better than any other method. The plates exposed to the atmosphere collect rainfall, dust, and anything that is deposited on the sea surface.

Preparation of samples for elemental analysis

A suitable amount of the exposed volume of water taken from the various stations was adjusted to pH 1 with 66 % HNO_3 (analytical guide, Merck). After removal of insoluble material by filtration, the clear solution was concentrated under vacuum at 30 °C 1/10 of the original volume and then analyzed for Pb^{++}, Cd^{++}, Zn^{++}, Cn^{++} and Fe^{++} content by standard absorbtion technique. In the case of samples with a very low content of metals (less than 0.02 ppm) the concentration factor was increased to 1/100 to secure significant determinations of metals well within the limit of sensitivity of the lamps used for each metal. As a control the same volume of deionized bidistilled water adjusted to pH 1 with 66 % HNO_3 was used.

The results indicating the total amount (mg) of each analysed element fallen from the atmosphere into the plates, reduced to a one-square metre surface for 30 days, are reported in table 1.

Hydric input

In 1973 EUROSTAFF, commissioned by the District of Naples, investigated 123 discharges in the Gulf of Naples (figures IIa,b,c) from Castellamare di Stabia to Capo Miseno. Chemical analyses were performed and the results were given for most of them (more than 50 per cent). Moreover, our research group investigated 127 additional discharges neglected by Eurostaff because of their smaller volume.

During their research, the Eurostaff investigators found the following mean concentrations of Fe, Zn and Cu :

Fe	Zn	Cu
2.900 mg/l	0.341 mg/l	0.448 mg/l

Considering the location of the discharges analysed by Eurostaff, covering almost the whole of the Gulf of Naples, and the considerable number of the above-mentioned samples (123), roughly corresponding to the number of discharges examined here (127), we have taken the mean value of the data obtained from Eurostaff as the concentration values for our samples.

The various capacities of the discharges examined here were derived from data provided by the Sewer Division of the coastal towns of the District of Naples. Moreover, considering that the discharges are distributed along the coastline within the area of the Gulf of Naples, our estimate can be considered as representative of all hydric inputs into the Gulf.

In conclusion, taking into account the total inflow of data from the coastal districts, and from the Cassa per il Mezzogiorno* it was possible to estimate approximately the discharge quantities in the Bay of Naples of the following elements (tonnes/month) :

Fe	Cu	Zn
73.92 tons/month	11.42 tons/month	8.69 tons/month

* An organization which finances public works in the south of Italy.

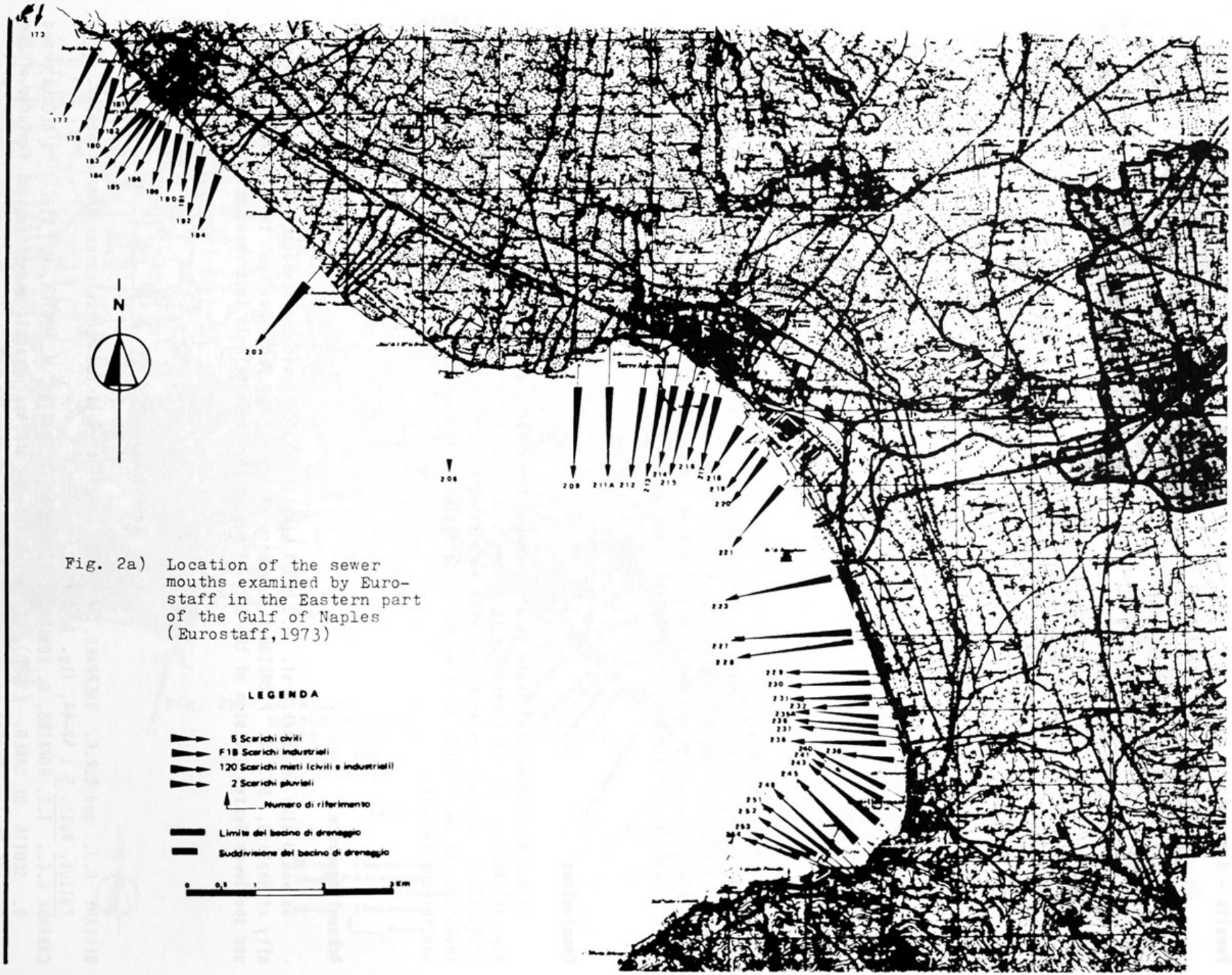


Fig. 2a) Location of the sewer mouths examined by Eurostaff in the Eastern part of the Gulf of Naples (Eurostaff,1973)

Results - discussion

The data reported in table 1 show high values in the over-populated and industrialized areas.

At the extreme seaward edge of the Bay, in Capri, Sorrento and Ischia, more scarcely populated and with little industry, the values are much lower. So we have subdivided the surface of the Bay into two parts : the most polluted narrow coastal sea-strip (about 62 km long, 1 km wide) and the remaining area.

For the atmospheric contribution to the nearby coastal sea-strip the mean values of stations 2,3,4,5,6,7, were assumed. For the offshore marine area the mean values of Sorrento, Capri and Ischia were assumed. The total atmospheric input for the whole Bay of Naples was then found to be:

Fe	Cu	Zn
23 tons	1.2 tons	1 ton

The ratio between the meteorological input and the hydric is :

Fe : 31.1 % Cu : 10.5 % Zn : 11.5 %

It is interesting to note that the above values are considerably lower than the actual ones for the hydric input, as before 1973 discharges were not controlled by law. At present they are reduced because of the law control. Moreover, the discharges observed are mostly public pluvial ducts in which private buildings and small factories have had built their discharges. Consequently there is quite a considerable atmospheric component, even in the discharges from the hinterland.

Conclusions

It has been shown that there is a noticeable atmospheric contribution to marine pollution in the Bay of Naples. It is reasonable to assume that such a contribution can be found elsewhere. The statistical approach experimented with here provided satisfactory and promising results. Further investigation and efforts are required to determine more accurately the pollutants conveyed into the sea through the air.

Acknowledgements

We would like to thank Prof. G. PROTA (Institute of Organic and Biological Chemistry - University of Naples), Dr. O. PIOMBINO (Laboratory of Hygiene and Prophylaxis of the District of Naples) and the Sewer System Division of the City of Naples for their help in this research.

References

BELLAMY D.J. and C.R.C. SHEPPARD, (1974). - Pollution of the Mediterranean around Naples. Mar. Pollut. Bull. 5 : 42-44, fig. 3, tab. 2.

CARRADA G.C., T.S. HOPKINS, G. BONADUCE, A. IANORA, D. MARINO, M. MODIGH, M. RIBERA D'ALCALA' and B. SCOTTO DI CARLO, (1980). - Variability in the hydrographic and biological features of the Gulf of Naples. Marine Ecology 1 : 105-120.

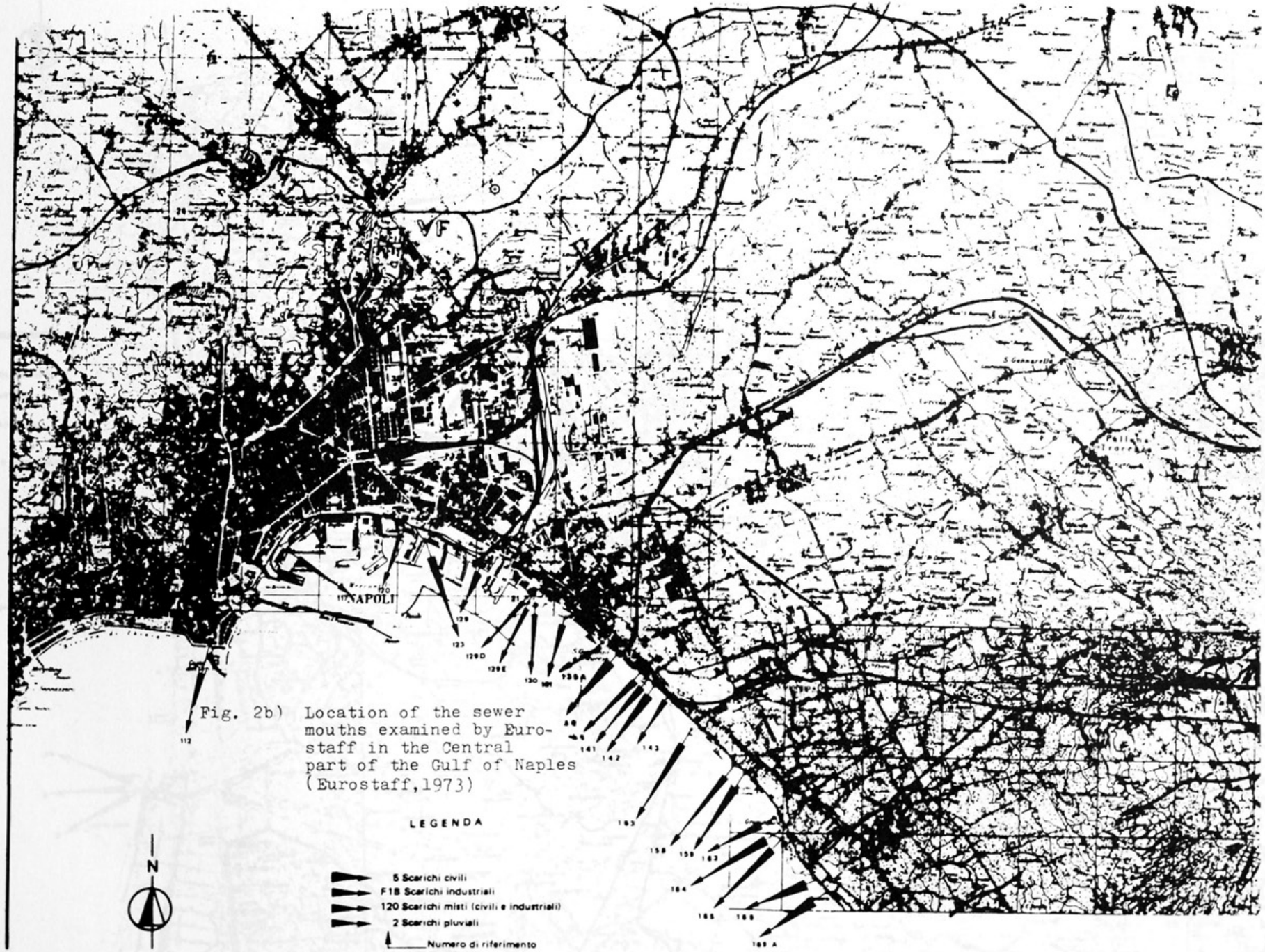


Fig. 2b) Location of the sewer mouths examined by Eurostaff in the Central part of the Gulf of Naples (Eurostaff,1973)

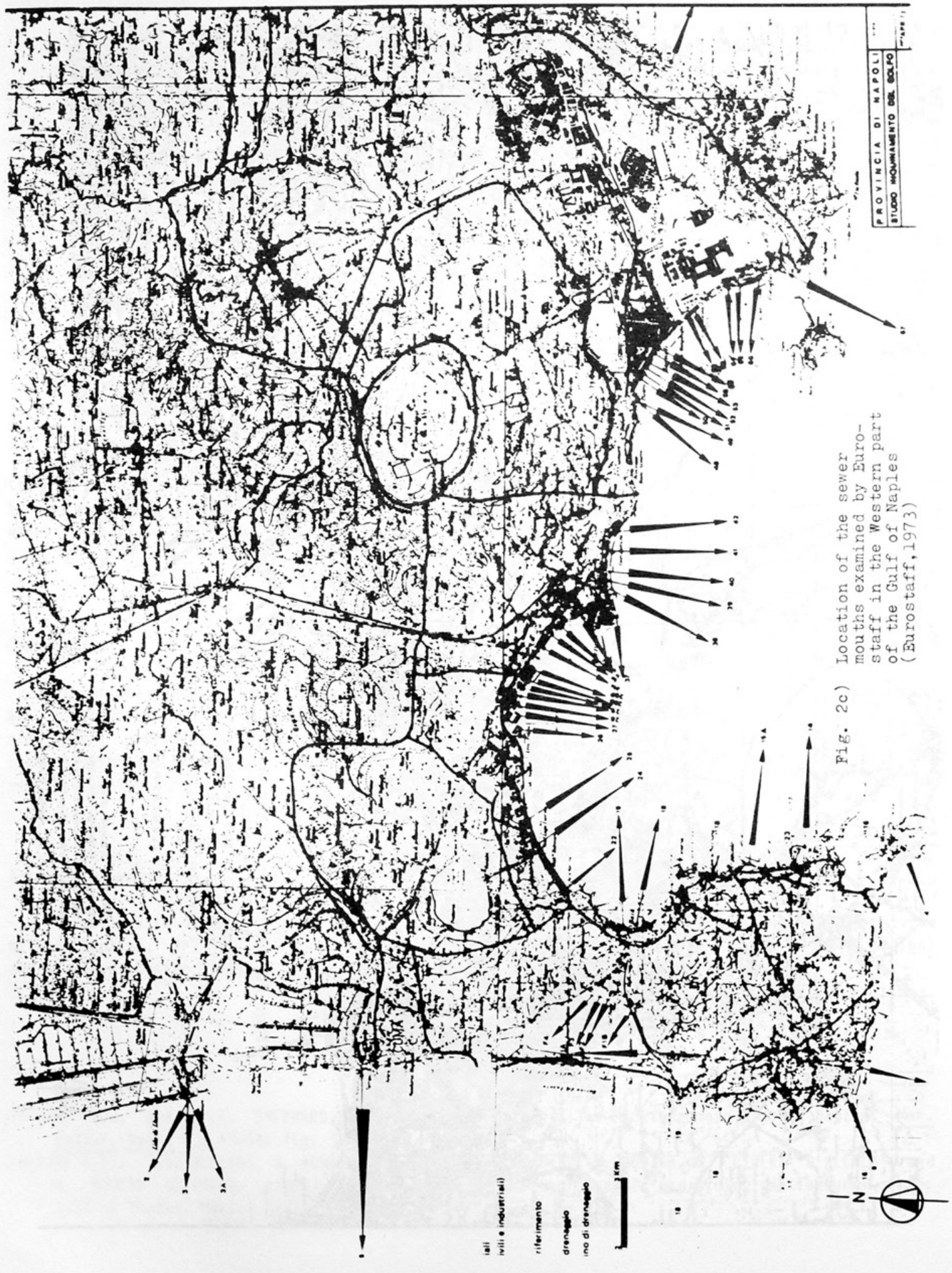


Fig. 2c) Location of the sewer mouths examined by Eurostaff in the Western part of the Gulf of Naples (Eurostaff,1973)

DE ROSA S., V. DAMIANI and E. AMBROSANO, (1982). - Indagine sullo stato di contaminazione dei sedimenti superficiali del tratto del Golfo di Napoli tra Capo Posillipo e Torre del Greco. C.N.R. Risorse Biologiche e Inquinamento Marino. Atti del Convegno : 625-642.

DE MAIO A., M. MORETTI, E. SANSONE, M. VULTAGGIO and G. SPEZIE, (1982). - Dinamica delle acque del Golfo di Napoli ; presentazione dei dati ottenuti dal 1977 al 1980. Annali Istituto Universitario Navale Napoli 49, App. 2 : 1-305.

EUROSTAFF, (1973). - Studio sull'inquinamento del Golfo di Napoli. Amministrazione Provinciale di Napoli.

GEORGII H.W, C. PERSEKE and E. ROHBOCK, (1984). - Deposition of acidic components and heavy metals in the Federal Republic of Germany for the period 1979 - 1981. Atmospheric Environment 18 : 581-583.

MONCHARMONT U. (1977). - Bibliografia sulla biologia delle acque marine del Golfo di Napoli. Relazione per l'I.R.S.A. (C.N.R.) 292 pp. (not published)

PALUMBO A. and A. MAZZARELLA, (1982). - Mean sea level variations and their pratical applications. Journal of Geophysical Research, 87 : 4249-4256.

PHYSICO-CHEMICAL INVESTIGATION IN THE COASTAL AREA BETWEEN THE ARNO AND SERCHIO RIVERS (TUSCANY, ITALY)

by

C. BLUNDO, L. LORETI, R. PAGNOTTA
M. PETTINE and A. PUDDU
Water Research Institute
National Research Council Rome - Italy

Abstract

Pinewoods in S. Rossore, situated along the Tyrrhenian coast between the Arno and Serchio rivers mouths, have shown signs of deterioration in recent years. A research project was set up by a Commission of experts to investigate the causes and the extent of the phenomenon.

In the framework of this project the Water Research Institute was charged with the investigation of coastal water quality because pollutants occurring in the coastal waters could affect coastal vegetation by air-borne dispersion.

In 1983 an evaluation was therefore made of quality of coastal water and of the final stretches of the rivers flowing into this area.

On the basis of the physico-chemical characterization of these waters, the Arno river appears to be the main source of pollution. The intensity of coastal water quality alteration depends on the distance from the mouth. At a distance of 2-3 miles from the Arno, the concentration of pollutants reaches typical Mediterranean coastal water levels.

*
* *

Introduction

Public opinion in Italy was recently alarmed by the degradation of coastal vegetation in the S. Rossore estate. A Commission of experts was consequently set up to co-ordinate a series of studies carried out on various sectors likely to be responsible for this degradation, i.e. soil, water and air.

Special attention was paid to the quality of coastal water owing to its possible indirect influence on coastal vegetation through the air-borne transport of potentially dangerous substances in the form of aerosols.

Indeed, among the various possible causes of the deterioration of coastal vegetation special emphasis was given to pollutants occurring in coastal water, mainly surfactants, being transported to the pinewoods by sea-spray (LAPUCCI 1968; GRIEVE and PITMAN,1976; GELLINI et al., 1983).

Before considering the processes of aerosol formation and the transport of related pollutants, the definition of the physico-chemical characteristics of air and water systems was considered to be a first priority step.

The present article reports the results obtained in the 1983 investigation for:

I. pollutant loads discharged into the sea from rivers;
II. modes of in-sea distribution of fresh river water;
III. characterization of coastal water quality.

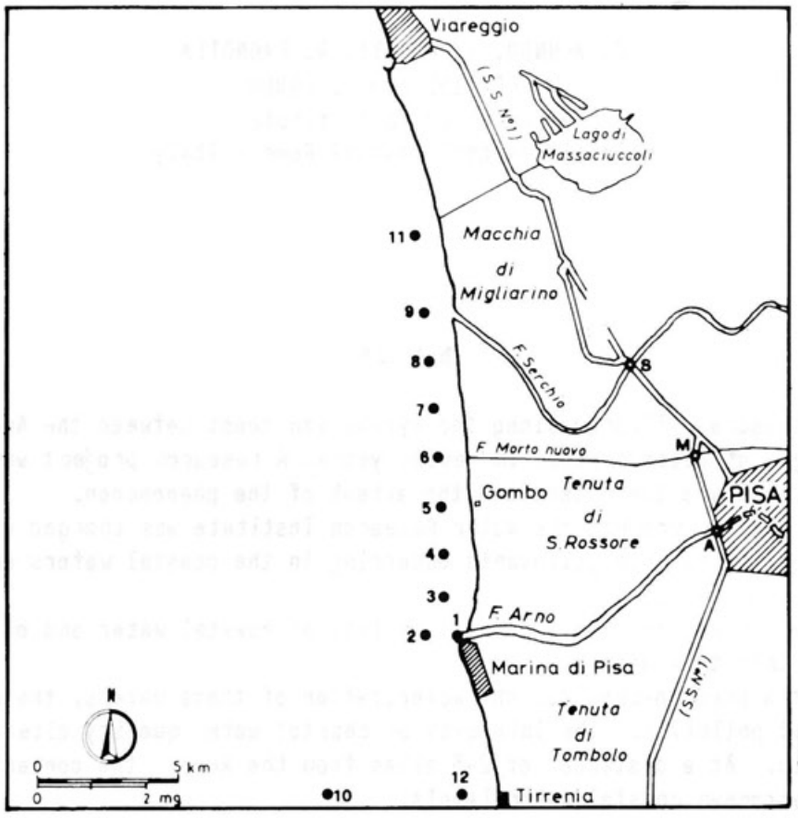


Figure I. - The sampling stations

Methodology

Six surveys were carried out in the period October 1982 - December 1983.

Fifteen sampling stations were set up in the survey area, as shown in figure 1.

At stations 1 to 12, surface samples were taken using wide-necked plastic bottles so that the surface film could also be included in the sample, except at station 10 (reference point) where the samples were collected with a Niskin bottle at a depth of ten metres.

Stations A, M and S were situated respectively near the mouths of the Arno river (10 km from the mouth), the Morto Nuovo river (8 km from the mouth) and the Serchio river (10 km from the mouth).

Immediately after the sample had been taken, the temperature was measured using a mercury column thermometer and the pH determined using a portable pH meter. Samples were stored in the dark at a constant temperature of 4° C until filtered.

Filtration was carried out within one day of sampling using 0.45 μm Millipore filters. Two aliquots of each sample, kept respectively in polyethylene bottles for the analysis of surfactants and in glass bottles for the analysis of hydrocarbons, were acidified to pH 2 immediately after sampling.

Experimental analysis included conductometric measurements of conductivity in river water samples and of salinity in sea-water samples, chemical oxygen demand (COD), biological oxygen demand (BOD), and determination of total organic carbon (TOC), anionic surfactants (MBAS), non ionic surfactants (BIAS) and oils (SOOM), as well as nutrients.

The analytical methods used for COD, BOD, surfactants and oils are those reported by the I.R.S.A. handbook (1972), while the manual of STRICKLAND and PARSONS (1972) was used for all nutrient determinations, except for silicates and total phosphorus (TP), for which GRASHOFF's procedures (1976) were used. Total inorganic nitrogen (Ntoti) has been calculated by summing nitrate, nitrite and ammonia values. TOC was determined after the samples had been digested in closed vessels through the infrared determination of the CO developed.

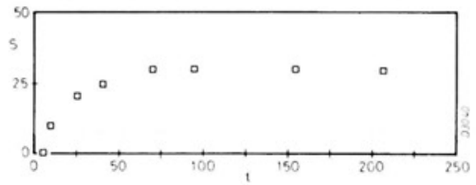


Figure II. - Salinity concentration (g/l) in the plume of the mouth of Arno river at different times (minutes) on 5 may 1983.

Results

The quality characteristics of the water at the mouth of the above three rivers can be obtained from the data listed in table 1. Using the classification criteria proposed by the Royal Commission on Sewage Disposal (LESTER, 1969) for BOD and NH_4^+ and by I.R.S.A. (1977) for phosphorus and nitrogen, the situation was found to be extremely serious in the case of the river Morto Nuovo, satisfactory for the Serchio and polluted for the Arno.

In the case of the latter river, there is an abrupt deterioration in water quality just downstream from the city of Florence, which draws on the river to satisfy more than 80 per cent of its water requirements. The situation of oxygen supersaturation near the source of the Arno deteriorates into one of deficiency near its outfall (BALLONI et al., 1979).

The river Arno has a low mean flow rate, subject to considerable drops and consequent decreases in its dilution capacity in dry weather, a slow downflow, and consequently diminished re-aeration, due to the flatness of most of its bed, all of which adds up to an oxygen saturation value at the mouth of about 30 per cent in summer.

The organic matter and nutrient concentrations found at the mouth vary considerably according to the flow conditions. With low flow rates (6-7 m^3/sec) very high concentrations may be found, i.e. up to about 17 mg/l for TOC and about 70 mg/l for COD. In medium and medium-high flow rate conditions, the above values drop to 6.1 $\pm$ 1.2 mg/l and 26.0 $\pm$ 5.8 mg/l, respectively.

As for surfactants, which several sources indicate as responsible for the degradation of the coastal vegetation, the river Morto shows the highest concentration of MBAS. However, considering that the mean flow rate of the Morto ($\sim$ 1.2 m^3/s) is much lower than of the Arno ($\sim$80 m^3/s), it is the latter river that makes the greatest contribution ($\sim$ 900 t/y) to surfactant input in the coastal area.

The effect exerted by these rivers on the quality of the coastal waters off the S. Rossore estate thus appear to be significant only in the case of the Arno. Indeed, the Morto Nuovo, owing to the small quantity of water it conveys, and the Serchio, owing to its satisfactory water quality, have no effect on the quality of these waters.

For the purpose of studying more thoroughly the in-sea diffusion dynamics of substances transported by the Arno river, a series of samples were taken from a body of river water as it gradually mixed with the coastal sea-waters. This body of water was tagged by releasing a series of drogues at the river mouth. Samples were then taken at fixed time intervals following plume centre drogue movement. Results show that the flow direction of the fresh water plume is not influenced by the

Table 1. - Mean values (Vm) and standard deviation (δ) of the investigated parameters in the rivers Arno, Morto and Serchio in the period October 1982-December 1983.

PARAMETER	ARNO		MORTO		SERCHIO	
	Vm	σ	Vm	σ	Vm	σ
Flow (m^3/S)	76.1	70.2	-	-	-	-
MBAS (mg/l)	0.38	0.36	1.70	0.91	0.07	0.03
BIAS (mg/l)	0.19	0.17	0.26	0.19	0.09	0.05
SOOM (mg/l)	0.52	0.41	1.31	1.13	0.18	0.20
PO_4^{3-} - P (mg/l)	0.50	0.52	1.43	0.22	0.02	0.02
TP (mg/l)	0.86	0.75	1.69	0.26	0.06	0.04
NO_2^- - N (mg/l)	0,07	0.06	0.13	0.21	0.01	0.01
NO_3^- - N (mg/l)	0.99	0.51	0.40	0.18	0.37	0.13
NH_4^+ - N (mg/l)	1.13	0.47	3.25	1.43	0.06	0.04
SiO_2 - Si (mg/l)	2.25	1.25	3.19	1.54	1.58	1.02
TOC (mg/l)	7.83	4.78	6.21	1.18	2.91	1.17
BOD (mg/l)	9.03	2.71	8.83	4.15	5.15	3.23

coastal current up to 1-2 miles offshore. This is, in fact, the average distance generally covered by the drogues released at the river mouth. As the distance away from the outfall increases, the river water gradually merges with the Ligurian sea-water, following the local surface currents running S-N along the coast.

Figure II shows the salinity behaviour on the central line of the plume obtained on 5 May 1983.

The deficit between the salinity on the central line of the plume (Sp) and the ambient value of sea-water (Sw) decreased with the travel time in minutes (t), according to the following exponential equation:

$$\frac{Sw - Sp}{Sw} \cdot 100 = 91.7 \cdot e^{-0.014t}$$

The concentrations of the chemical parameters show an inverse correlation with respect to salinity.

With regard to coastal water quality the mean values and their relative standard deviations referring to the most significant parameters measured at the stations listed in figure I are shown in table 2.

On the basis of the data obtained the following observations may be made:

a) As far as the measured physical characteristics of the water are concerned, the transparency appears to be fairly homogeneous (range values between 1 and 4 m) and the salinity somewhat variable. On the basis of the latter parameter, stations 1 and 2 stand out from the others since they are situated at the mouth of the Arno and thus have lower salinity values than the other, more strictly coastal (stations 3 ÷ 9 and 11). A further distinction can be made within this group concerning stations 3 and 9 whose salinity, in some cases less than 30 g/l, appears to have been more greatly affected by the river water.

b) The trophic state of the water can be analysed by checking the concentrations of the nutrients against the classes proposed by VOLLENWEIDER (1965) for lacustrine water, which are held to be valid also for coastal sea-water.

Such a comparison reveals the oligotrophic nature of stations 4 ÷ 12 (mg/l of $N_{tot.}$ < 0.1 and PO_4^{3-} P < 0.01). On the other hand, the conditions shown by stations 1 and 3 range from eutrophic to oligo-mesotrophic. The same comparison carried out on the basis of individual rather than mean values showed that only stations 5,6 and 9 can occasionally attain an oligo-mesotrophic state.

c) As far as organic substances are concerned, except for stations 1 and 2, TOC values fall between 1.5 ÷ 3 mg/l, which can be considered normal for sea-water (WANGERSKY , 1975).

Analysis of SOOM and BIAS data reveals fairly low and homogeneous values. The oil values in particular are much lower than the maximum acceptable limit (2 mg/l) for bathing and shellfish farming (SWPCB, 1957). The MBAS values show that the coastal water can be classified as "sufficiently pure" (values less than 0.1 mg/l) in accordance with proposed quality standards (MAJORI, 1968). However, both station 1 (end stretch of Arno river) and station 2 (500 m from the mouth) occasionally exceed this limit.

The values recorded for the coastal zone at the mouth of the Arno were then compared with the results of other surveys on different parts of the Italian coast (MAJORI 1968, 1981, for Upper Adriatic; DE RENZI et al., 1978, for the Ligurian sea; I.R.S.A., 1983, unpublished data, and Amministrazione Provinciale - Roma, 1981, for the coast of Lazio).

This comparison shows that the values recorded off the S. Rossore estate do not differ greatly from those of the other coastal areas in which recorded MBAS values mostly lie in the 0.01 to 0.1 mg/l range.

Table 2. - Mean values (Vm) and standard deviation (δ) of the investigated parameters in the coastal area in the period October 1982-December 1983.

STATION	SAL (g/l)		MBAS (mg/l)		BIAS (mg/l)		SOOM (mg/l)		TP (mg/l)		$N_{tot.\ i.}$ (mg/l)		SiO_2 - Si (mg/l)		TOC (mg/l)	
	Vm	δ	Vm	δ	Vm	δ	Vm	δ	Vm	δ	Vm	δ	Vm	δ	Vm	δ
1	8.1	6.5	.12	.08	.03	.03	.37	.43	.16	.03	1.36	.38	1.13	.86	4.42	.92
2	21.7	9.6	.09	.05	.05	.05	.26	.39	.17	.08	.88	.52	.66	.32	3.84	1.27
3	35.3	4.7	.07	.03	.06	.07	.23	.28	.02	.01	.14	.18	.09	.05	2.17	.57
4	36.9	1.1	.04	.02	.12	.12	.17	.24	.02	.00	.04	.02	.06	.02	1.96	.66
5	36.2	2.1	.04	.03	.06	.03	.18	.26	.02	.02	.07	.09	.06	.03	2.02	.35
6	36.0	1.3	.04	.02	.06	.04	.14	.18	.08	.01	.08	.06	.13	.12	2.17	.26
7	36.2	.8	.05	.01	.10	.11	.10	.10	.03	.01	.04	.04	.07	.06	1.43	.27
8	36.4	.7	.04	.04	.10	.07	.13	.19	.01	.00	.03	.02	.07	.04	1.63	.50
9	32.0	6.1	.07	.03	.08	.07	.09	.09	.09	.10	.08	.08	.29	.41	1.69	.56
10	37.6	.1	.04	.04	.03	.03	.10	.13	.02	.01	.02	.01	.05	.01	1.66	.51
11	35.8	.8	.02	.01	.22	.15	.14	-	-	-	.03	.01	.06	.01	2.06	.47
12	37.9	-	.03	-	.12	-	.36	-	.02	-	.05	-	.12	-	1.86	-

Conclusions

Analysis of the results obtained indicates that qualitative changes do occur in the coastal waters compared to water not subjected to river discharge.

The highly dynamic situation characterizing the area makes it possible to disperse load so that conditions up to 2 - 3 miles offshore rapidly return to values that are normal for the Tyrrhenian coast.

As far as the hypothesis according to which surfactants could affect coastal vegetation, being transported to the pinewoods by sea-spray, the results on surfactant concentration in coastal water do not enable any cause-effect to be proposed to explain the observed damage to vegetation. MBAS values in the coastal area investigation do not differ greatly from those of other coastal areas in which vegetation is not damaged at all or shows only less signs of déterioration.

Anyway, it is worth noting that in particular meteorological conditions, i.e. when strong W - SW winds are blowing, GELLINI et al. (1983) found a significant increase of surfactant concentration in aerosols.

Since collection of samples in the sea surface microlayer under the above conditions is not possible due to the roughness of the sea. field verification of the dynamic of aerosols enrichment is also precluded.

In the framework of the project studies are now under way, by using artificial systems (wind channel) in order to define a model of surfactant enrichment in aerosols in different wind strength conditions and to verify the effect of the related sea-spray on pinewoods.

Acknowledgements

The authors wish to thank Mr F. BACCIU and Mr. D. MASTROIANNI for their valuable assistance in the analysis of samples. They also wish to thank Prof. R. PASSINO, Director, and Dr. T. LA NOCE, Head of Water Quality Department, Water Research Institute, for their encouragement and guidance.

References

Amministrazione Provinciale - Roma (1981). - Indagine sullo stato di inquinamento delle acque superficiali. Edizioni delle Autonomie S.r.l., Roma.

BALLONI, W., M. VINCENZINI and G. FLORENZANO (1979). - Biocenosi algali ed eutrofizzazione nel fiume Arno. In Atti del convegno sulla eutrofizzazione in Italia Roma, 3-4 ottobre 1978. C.N.R. AC/2/45-70.

DE RENZI G.P., R. PALMERINI MORELLI, P. ORLANDO, S. VOLTA and C. DARDANELLI, (1978). - Research into the content of oil droplets, detergents and bacteria in the sea-water and sea beds of the North Tyrrhenian Sea. IV Journées Etud. Pollutions. C.I.E.S.M., Antalya 24-27 nov. 1978, 123-128.

GELLINI R., F. PANTANI, P. GROSSONI, F. BUSSOTTI, E. BARBOLANI and C. PRINALLO, (1983). - Survey of the deterioration of the coastal vegetation in the park of San Rossore in central Italy. Sonderdruck aus European Journal of Forest Pathology, Band 13, Heft 5-6, 296-304.

GRASHOFF K., (1976). - Methods of sea-water analysis. Verlag Chemie Weinheim, New York.

GRIEVE A.M. and M.G. PITMAN, (1976). - The condition of Norfolk bland Pinas on the Adelaide beachfront. Search, 7, 275-276.

I.R.S.A., (1972). - Metodi analitici per le acque. Quad. Ist. Ric. Acque, 11.

I.R.S.A., (1977). - Indagini sulla qualita delle acque del fiume Po. Quad. Ist. Ric. Acque, 32.

LAPUCCI P.L., (1968). - The pollution of sea-waters by detergents as a contributory cause of delete-

rious effects on Thyrrhenian pinewoods. Proc. Conference on oil Pollution of the Sea. Rome 7-9 Oct., 1968.

LESTER W.F., (1969). - Standard based on the quality of receiving water. Wat. Poll. Control, 68, 324-332.

MAJORI L., (1968). - Research experiments on the pollution of the waters of the Upper Adriatic. Proc. Int. Conference on Oil Pollution of the Sea. Rome, 7-9 Oct. 1968.

MAJORI L., G. NEDOCLAN, F. DARIS, G.B. MODONUTTI and C. CAMPELLO, (1981). - Ves. Journées Etud. Pollution - C.I.E.S.M., Cagliari, 9-13 Oct. 1980, 585-594.

STRICKLAND J.D.H. and J.H. PARSONS, (1972). - A practical handbook of sea-water analysis. Fisheries Research Board of Canada, Ottawa.

S.W.P.C.B. (State Water Quality Control Board), (1957). - Water quality criteria. The Resource Agency of California, Sacramento, CAL.

VOLLENWEIDER R. (1965). - Materiali e idee per una idrochimica delle acque insubriche, Mem. Ist. It. Idrobiol., 19, 213-286.

WANGERSKY P.J., (1975). - Measurement of organic carbon in sea-water. Advance in Chemistry Series, 147, 148-162.

THE EFFECT OF SEWAGE DISCHARGE ON SOME CHEMICAL CHARACTERISTICS OF SEA-WATER

by

Saad D. WAHBY and M.M.A. SHRIADACH
Institute of Oceanography and Fisheries
Alexandria (Egypt)

Abstract

The eastern harbour of Alexandria is affected by the sewage pumping station at Kayet Bey discharching about 64 x 106 m3 of sewage annually, in addition to a number of secondary outfalls inside the harbour itself.

The distribution of salinity and oxygen showed that stations affected by sewage discharge have lower salinity and in most cases lower oxygen content. The annual average oxygen content of the stations near the main outfall was 3.8L-1, increasing to 5.28 ml L-1 as we get away from the out fall. Oxygen studies revealed that sewage discharge accelerates the rate of oxygen consumption up to 100 per cent within five days incubation in stations near the outfalls. Also the distribution of oxidizable organic matter is related to sewage discharge and to the spreading of low density water inside the semi-enclosed harbour.

The annual average oxidizable organic matter for the eastern harbour area as a whole was found to be equivalent to 13.445 mg 02L-1, while for an area away from sewage discharge it ranged between 0.11 mg 02L-1 in winter and 1.13 mg 02L-1 in summer.

*
* *

The eastern harbour of Alexandria (figures I and II) has an area of about 500 ha and a depth between 3.5 and 9 m. It is a semi-enclosed basin subjected to sewage pollution through a main pipe lying 350-400 m west of the entrance. These are eleven secondary outfalls situated inside the harbour itself. About 150,000 m3/day of untreated sewage discharge into the sea thus affecting the chemical composition of sea-water in the area.

This paper discusses the effect of this sewage discharge on the distribution of salinity, oxygen content, rate of oxygen consumption and oxidizable organic matter content.

The distribution of salinity (figure IV) shows that the surface salinity of the harbour is significantly lower than those at the bottom throughout the year. This is due to the effect of fresh water of lower salinity discharged through the main pump as well as to the subsidiary outfalls situated inside the harbour, so stratification is clear. The annual average salinity for surface waters shows a remarkable difference between the entrance of the harbour (35.85 ‰), and stations lying inside the harbour away from the effect of direct sewage discharge (37.193 ‰).

The discharge of sewage into the sea is an important factor in disturbing the normal oxygen distribution, especially in protected areas and near the outfalls. In the eastern harbour of Alexandria the surface oxygen values varied between 2.02 ml L-1 (37.34 per cent saturation) and 8.21 ml L-1

(187.87 per cent saturation). The average annual value was 4.92 ml L-1 (99.55 per cent saturation) for the surface water. Figure V gives the horizontal distribution of oxygen for surface and bottom waters. In January 1980 surface waters had lower oxygen values than bottom ones. This is due to the spreading of sewage-diluted water, as given in the salinity data.

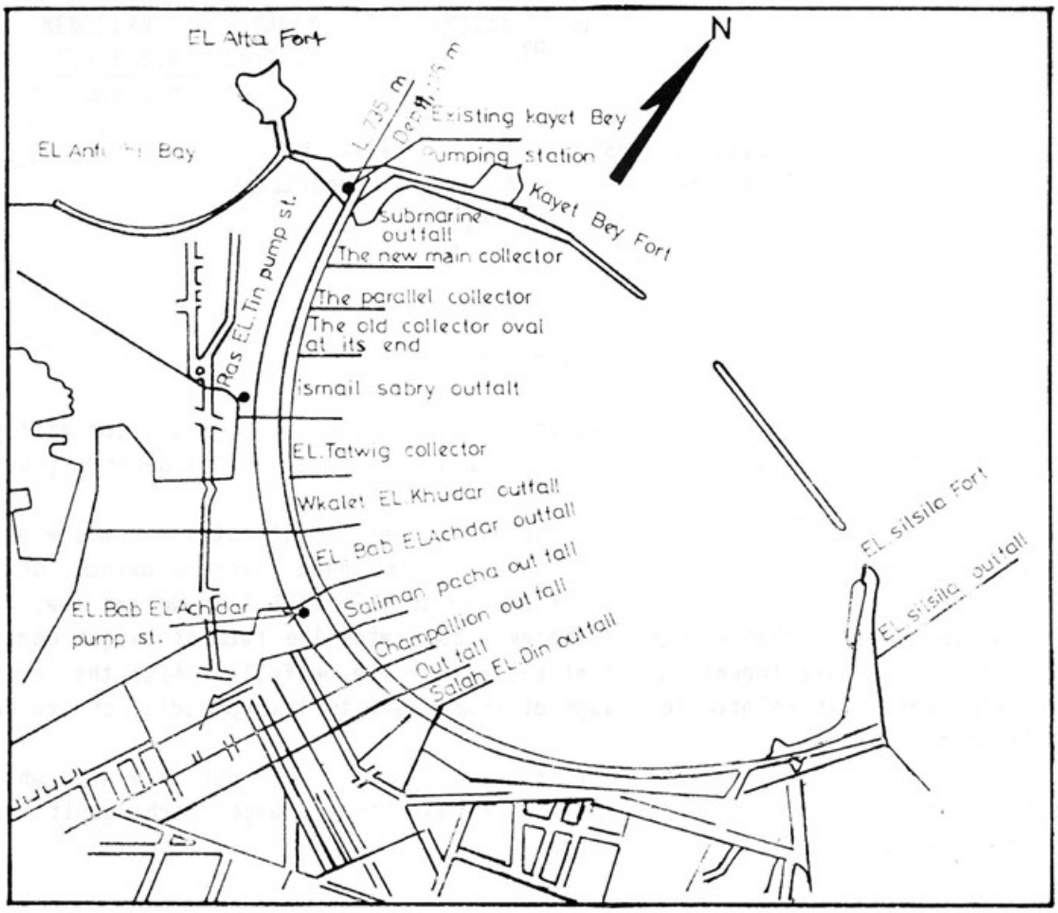


Fig. I. - The eastern harbour area showing the main pumping station at Kayet Bey and subsidiary openings.

In March, and evidently due to photosynthetic activity, the surface water is richer in oxygen, a phenomenon which is reserved in May when bottom water, especially at stations near the outfalls have higher oxygen content. In this month the distribution of salinity is similar in both surface and bottom waters, and stratification is not present. In July and September, low salinity water spreads on the surface enriching it with oxygen. In November low salinity water invades the harbour through its opening, as is clear from figure IV, causing a marked increase in the oxygen content especially at the centre of the harbour where salinity is lowest. In January 1981, and due to the disappearance of stratification, bottom waters of the harbour had higher oxygen values than surface ones. Oxygen and salinity studies reveal that sewage discharge brings about a certain degree of eutrophication causing an increase in the oxygen content correlated with the spreading of low salinity water.

This eutrophication is confirmed by oxygen consumption experiments which showed that in station I near the main outfall percentage oxygen consumption after an incubation of five days at 20° C was 100 per cent throughout the year, except in July. A higher oxygen consumption rate was recorded in stations affected by sewage discharge, either through the main outfall or through secondary outfalls inside the harbour itself and it proved to be related to the spreading of fresh water in the area.

Surface water affected by sewage discharge have higher oxygen consumption values (monthly average between 24.9 and 78.8 per cent) than bottom water (monthly average values between 8.4 nad 43.5 per cent.

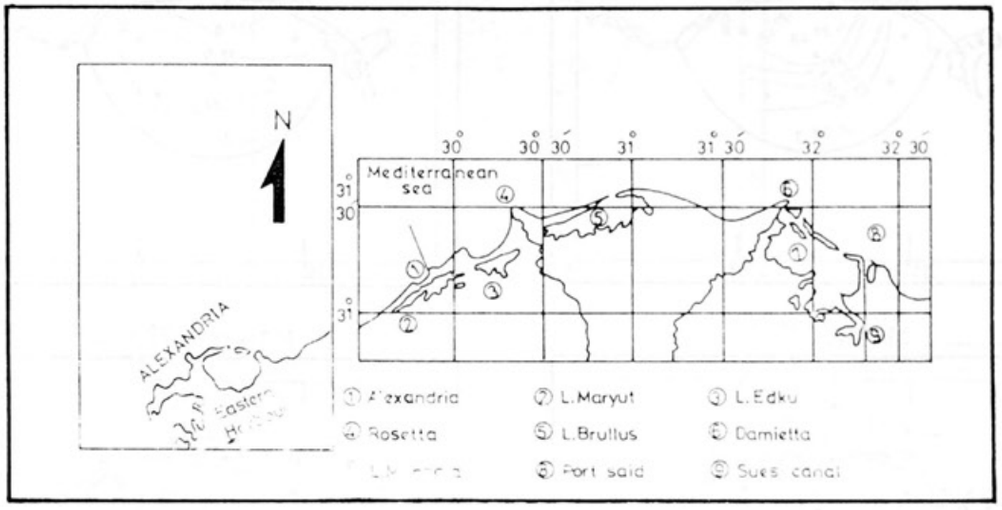


Fig. II. - Area investigated

The average value of oxidizable organic matter of the eastern harbour as a whole was found to be 13.44 mg O2L-1. The annual average of surface water was 14.52 mg O2L-1, while that of the bottom water was 12.37 mg O2L-1. As a rule the oxidizable organic matter of the surface water was higher than that of the bottom water throughout the year figure VI which is due to the oxidation of less dense sewage water at the surface.

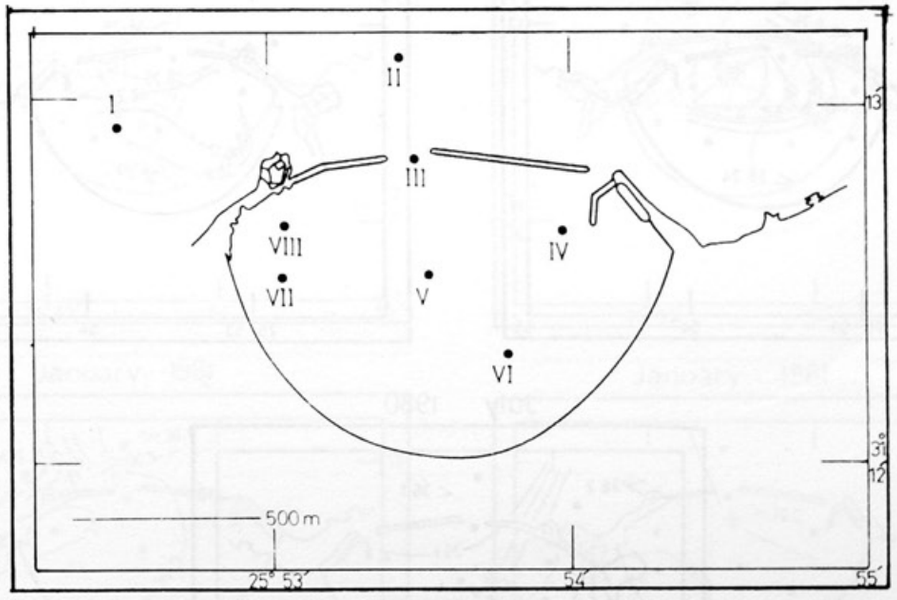


Fig. III. - Sampling stations

It was found that the eastern harbour has higher organic matter content than many of the marine areas around Alexandria studied by several authors, EL-DEEB 1977, EMARA 1969, EL-AWADY 1972, MAHMOUD 1979.

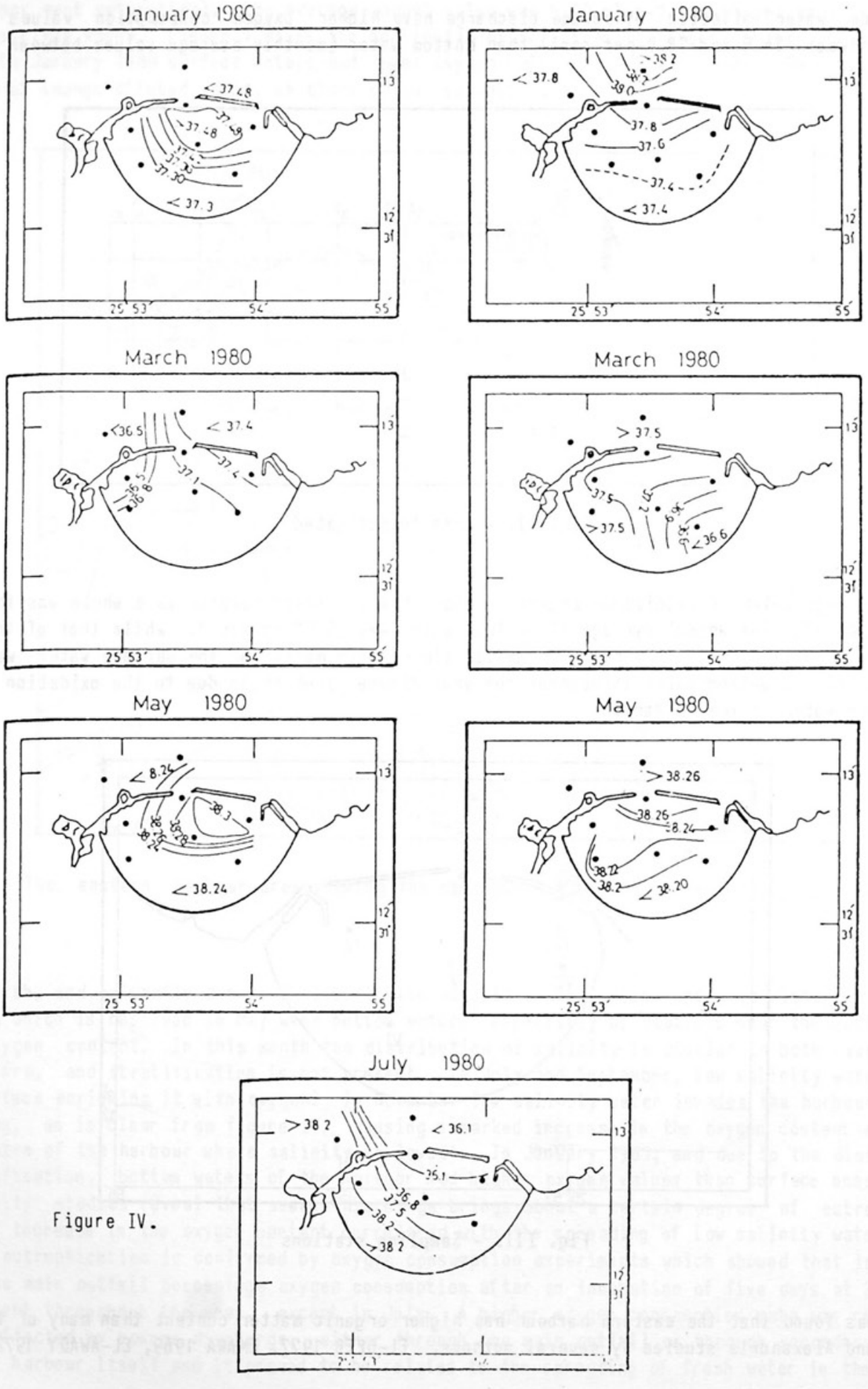

January 1980
January 1980
March 1980
March 1980
May 1980
May 1980
July 1980
25° 53'
54'
55'
13'
12'
31'

Figure IV.

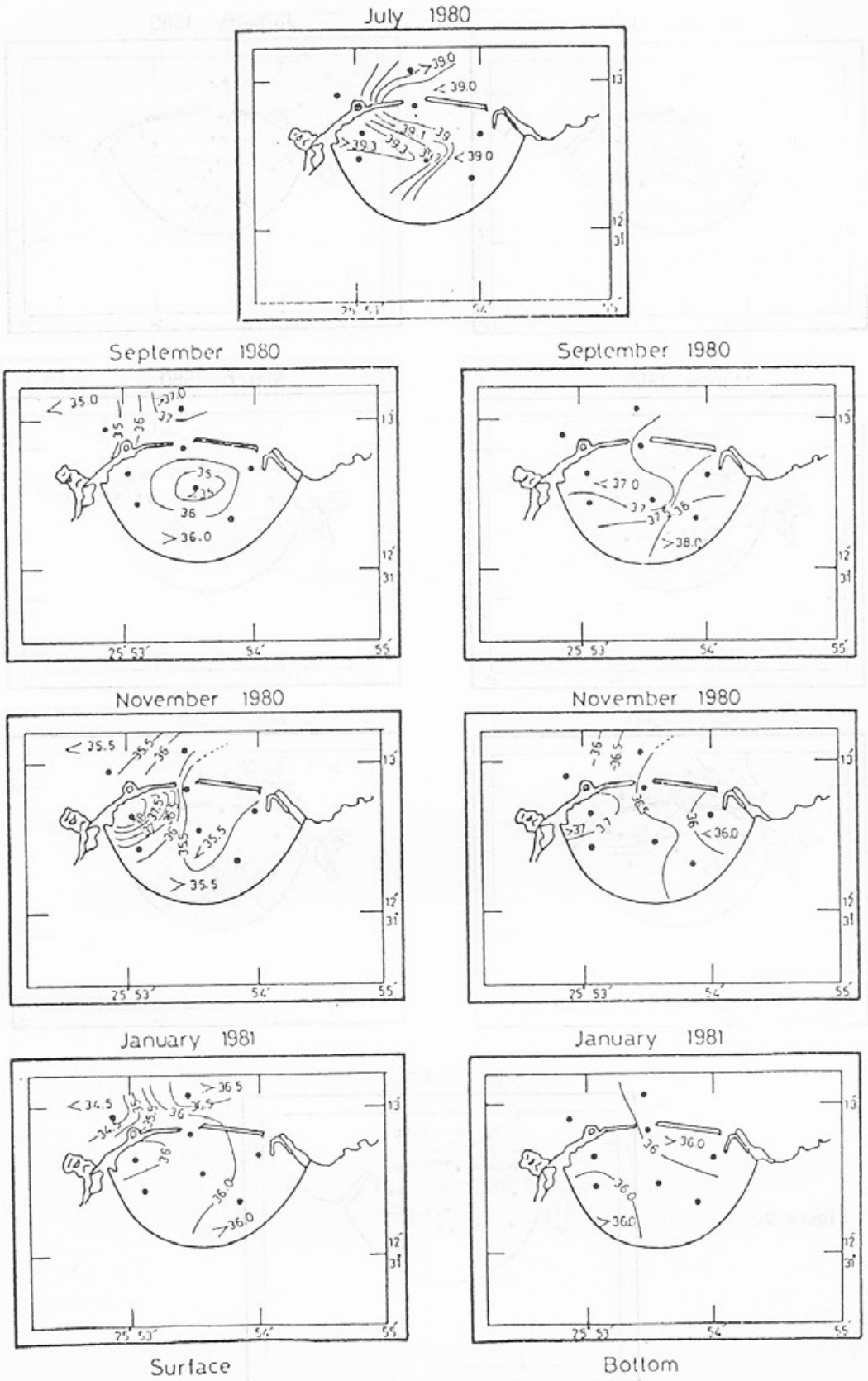


Figure IV. - Distribution of salinity (S ‰) during 1980.

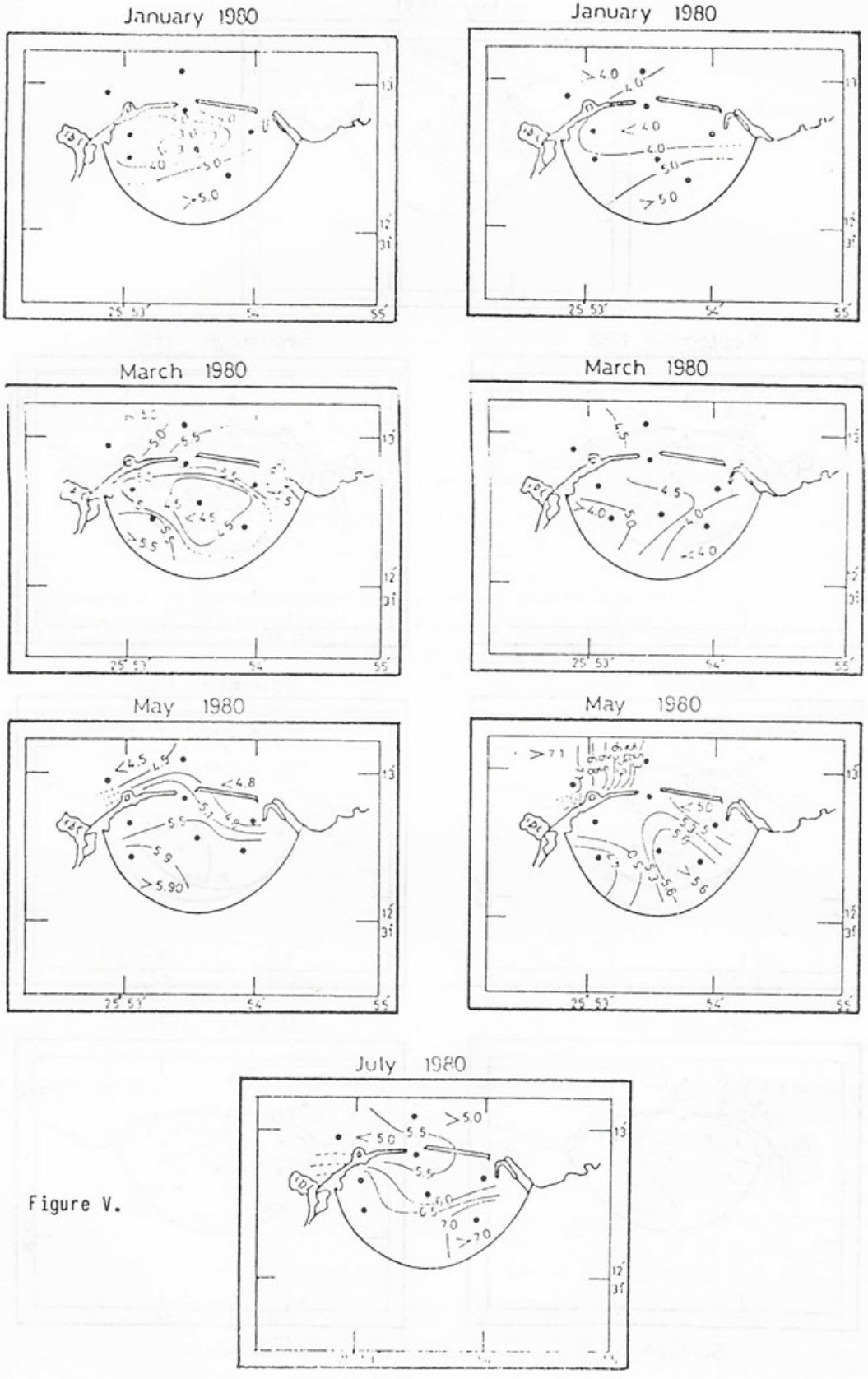

January 1980
January 1980
March 1980
March 1980
May 1980
May 1980
July 1980

Figure V.

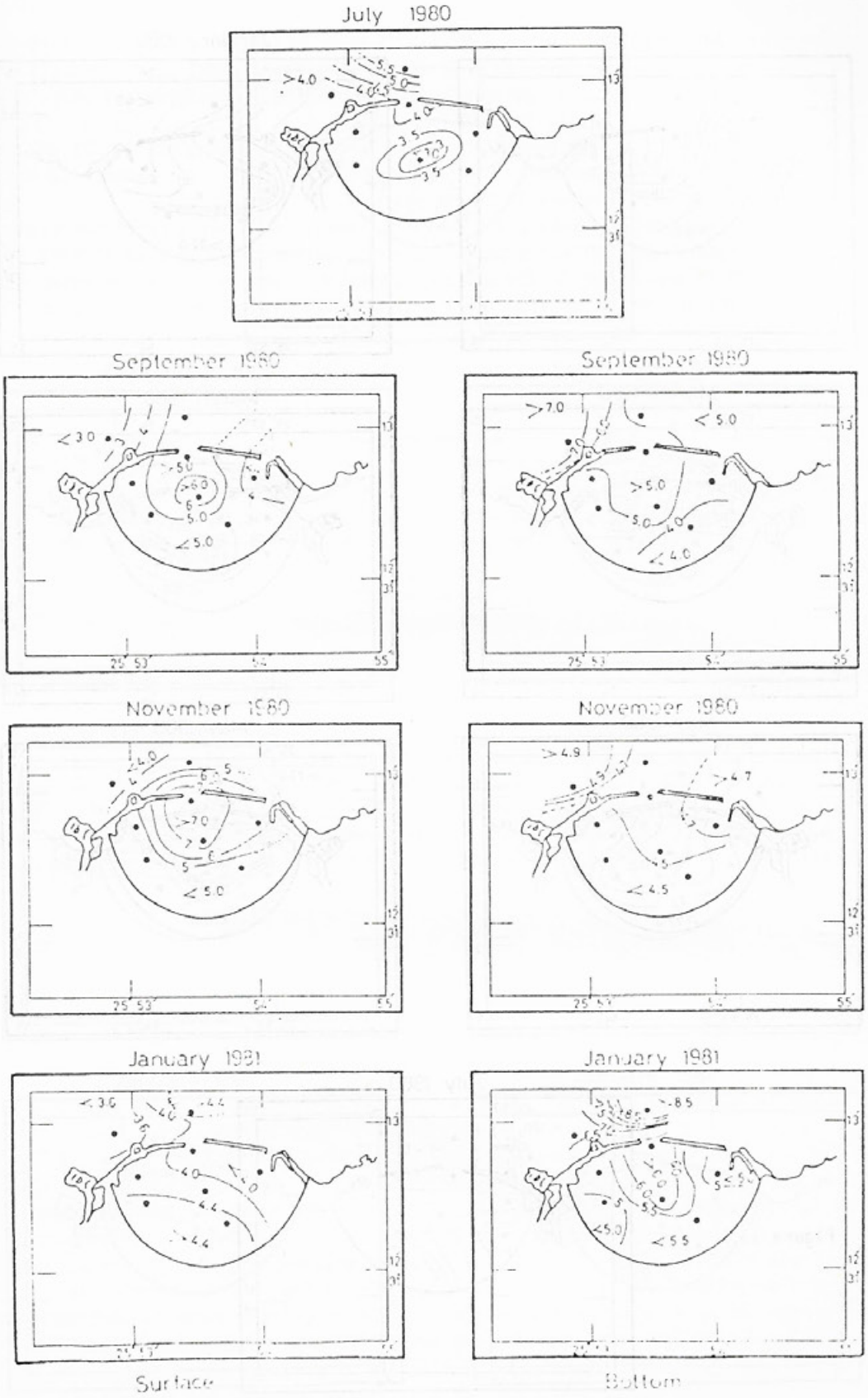


Figure V. - Distribution of dissolved oxygen (mlL-1) during 1980.

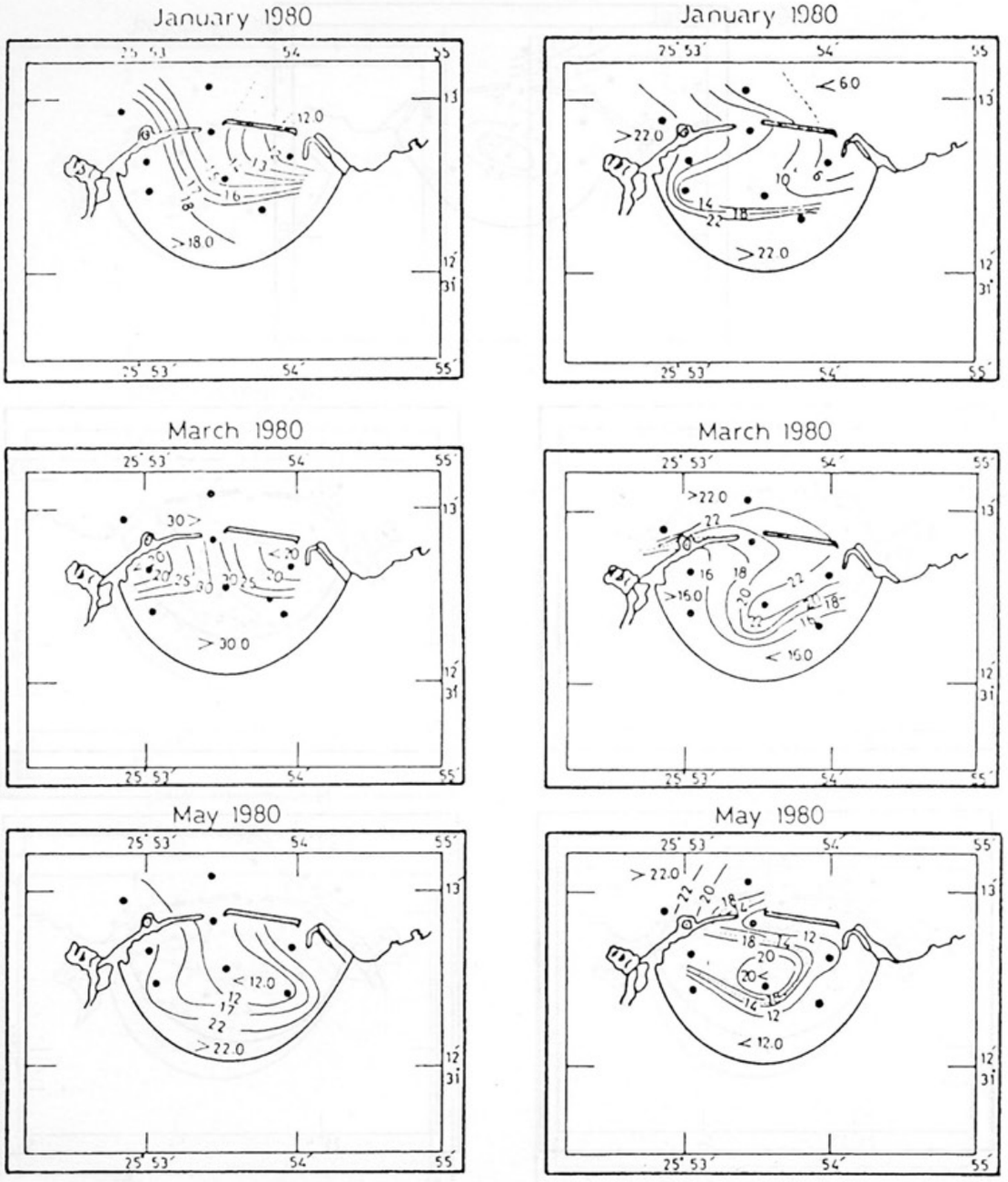

January 1980
January 1980
March 1980
March 1980
May 1980
May 1980
July 1980
25° 53'
54'
55'
13'
12'
31'

Figure VI.

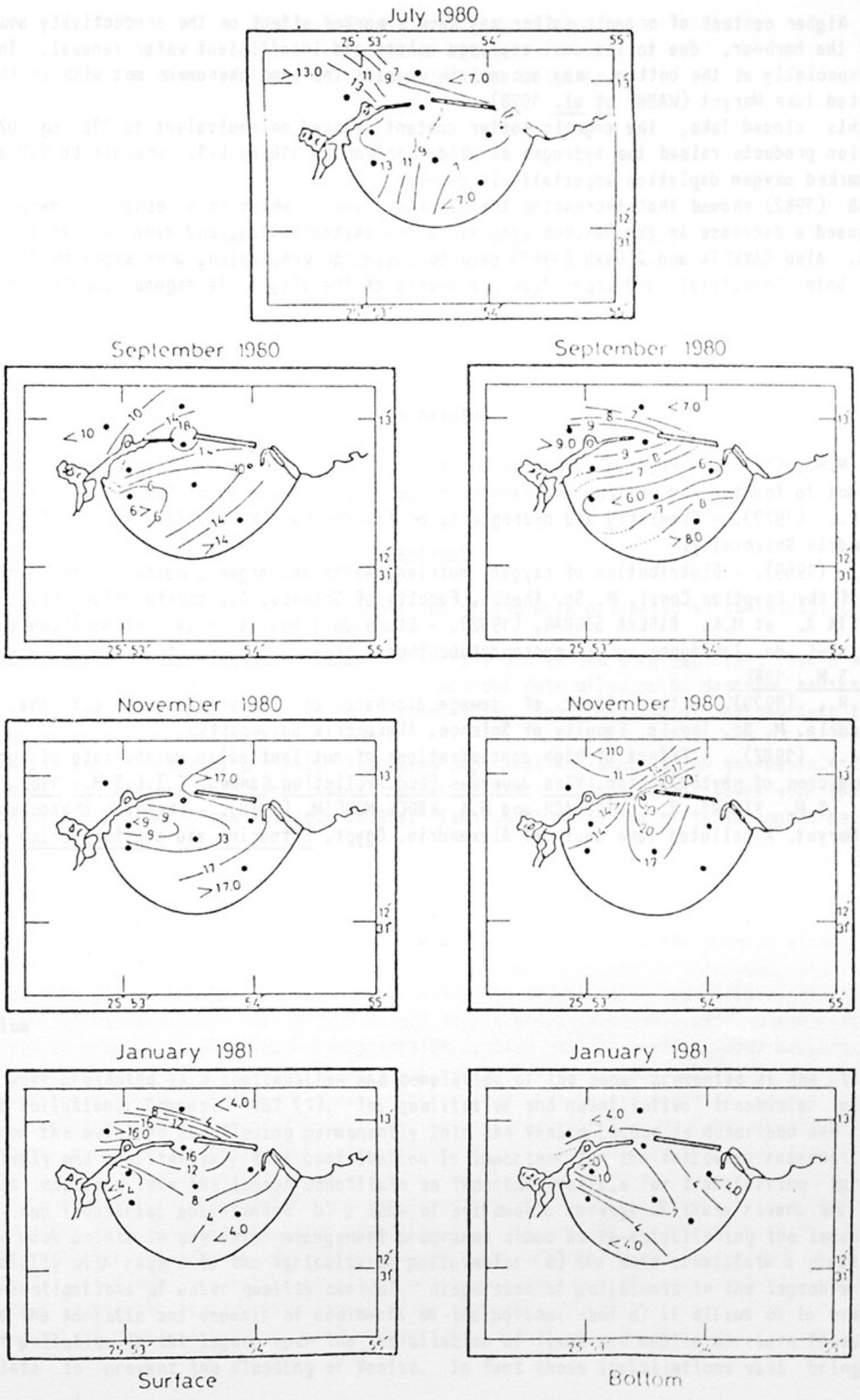


Figure VI. - Distribution of Oxidizable organic matter (mg O_2L-1) during 1980.

This higher content of organic matter may have a marked effect on the productivity and general ecology of the harbour, due to its semi-enclosed nature and insufficient water renewal. The organic matter, especially at the bottom, may accumulate causing the same phenomena met with in the highly eutrophicated lake Maryut (WAHBY et al. 1978).

In this closed lake, the organic matter content reached an equivalent to 172 mg O2L-1, its decomposition products raised the hydrogen sulphide content to 116 mg L-1, ammonia to 7.7 mg L-1 and caused a marked oxygen depletion especially in summer.

SULTAN (1982) showed that increasing the nutrient level, which is a result of sewage decomposition, caused a decrease in the maximum crop of marine phytoplankton, and even stopped the growth of some forms. Also GARRETA and SIGUAN (1982) came to a similar conclusion, when studying the effect of pollution both industrial and urban from the sewers of the city of Tarragona (Spain) on benthic seaweeds.

References

EL-AWADY, M.M., (1972). - Physical and chemical characteristics of Alexandria western harbour waters relevant to fouling and antifouling paints, M. Sc. Thesis, Faculty of Science, Cairo University.

EL-DEEB M.K., (1977). - Chemistry and hydrography of Abu-Kir bay. M. Sc. Thesis, Faculty of Science, Alexandria University.

EMARA H.I., (1969). - Distribution of oxygen, nutrient salts and organic matter in the Mediterranean Sea off the Egyptian Coast. M. Sc. Thesis, Faculty of Science, Alexandria University.

GOMEZ GARRETA A. et M.A. RIBERA SIGUAN, (1982). - Etude de l'impact de la contamination du polygone industriel de Tarragone sur le macrophytobenthos. VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M., 1982.

MAHMOUD T.H., (1979). - The effect of sewage discharge on the water quality off the coast of Alexandria. M. Sc. Thesis. Faculty of Science. Alexandria University.

SULTAN H.A., (1982). - Effect of high concentrations of nutrient salts on the rate of growth and maximum crop of phytoplankton. VIes Journées Etud. Pollution Cannes, C.I.E.S.M., 1982.

WAHBY S.D., S.M. KINAWY, T. EL-TABBACH and M.A. ABDEL-MONEIM, (1978). - Chemical characteristics of Lake Maryut. A polluted lake south of Alexandria, Egypt. Estuarine and coastal marine science 7, 17-28.

LAGOON OF VENICE: FRESH WATER AND POLLUTANT TRANSFER

by

S. BERNARDI*, F. COSTA*, S. VAZZOLER*, Z. VINCENZI*,
R. CECCHI**, G. GHERMANDI** and G. MAGNONI**
* Istituto per lo Studio della Dinamica
delle Grandi Masse-C.N.R., S. Polo 1364
30125 Venezia (Italy)
** Istituto di Fisica e Osservatorio Geofisico
dell'Università
Via Campi 213/A - 41100 Modena (Italy)

Abstract

This paper is a continuation and the completion of the work presented at the XXVIII Congress and Plenary Assembly of I.C.S.E.M., Cannes, 1983.

Data of some periodical field measurements carried out in the more important rivers and canals which empty into the lagoon are reported. These recorded data allow us to describe and estimate the quantity of freshwater pollutants (nitrogen, phosphorus, heavy metals) discharged into the lagoon from the watershed during the year.

For convenience, the data presented have been separated for each lagoon sub-basin. The Venice Lagoon, which is divided into three natural water basins, in fact communicates with the Adriatic through the Lido, Malamocco and Chioggia inlets. Each inlet feeds one of these lagoon basins, after which they are named.

*
* *

Introduction

The work presented is a continuation and completion of the paper presented at the 6th Workshop on Marine Pollution, Cannes, 1982 (1). The qualitative and quantitative freshwater contribution coming from the mainland and flowing permanently into the Venice Lagoon is described and evaluated. Quantitatively and qualitatively this contribution is important for the following reasons: a) rivers and canals emptying into the lagoon constitute an important vehicle for transferring agricultural, municipal and industrial pollutants: b) a lack of systematic surveys of these rivers and canals is one of the weak points in any water management programme aimed at re-establishing the lagoon environment especially with regard to the agricultural pollutants: c) the data constitute a preliminary to further investigations of water quality control, dispersion of pollutants in the lagoon and from the lagoon to the Adriatic and deposit of sediments on the bottom; and d) it allows us to evaluate the effects of pollution in the lagoon upon the installation of fixed and mobile barriers foreseen at the three inlets to prevent the flooding of Venice. In fact these installations will bring about a

reduction in the capacity of exchange between the lagoon and the Adriatic Sea, consequently decreasing the exchange of water especially in the areas bordering the lagoon where the water courses of the hinterland empty.

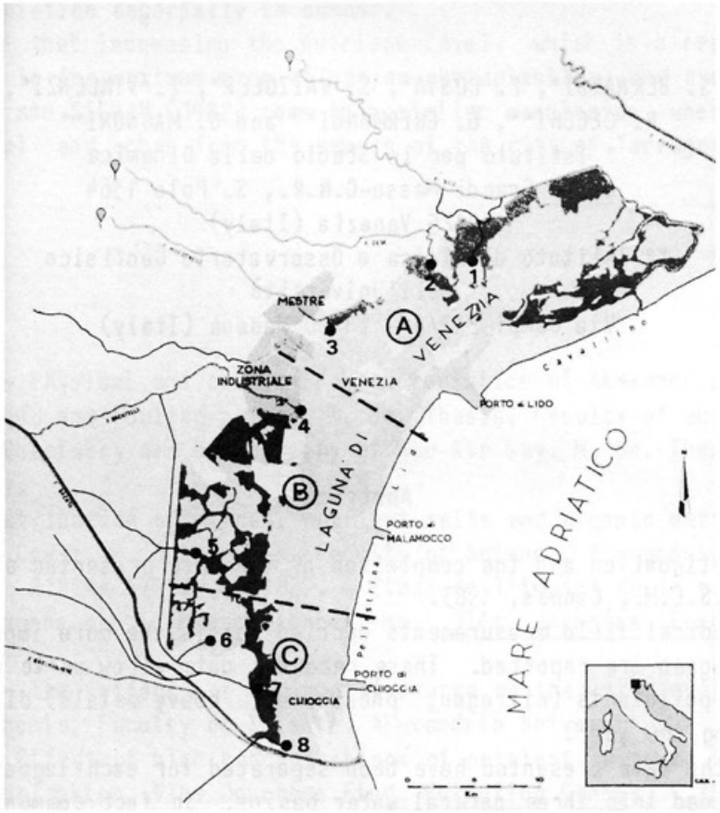


Figure 1

Rivers and canals flowing the into lagoon

Shaped like a half-moon, the lagoon of Venice has a maximum length of 52 km and a width which varies between 8 and 14 Km. The total surface area is 586 km2 and the mean depth is 0.5 m (figure I.) Linked to the sea through three inlets, Lido, Malamocco and Chioggia, the lagoon is divided into three natural water basins. Each of which feeds a lagoon basin from which it is named.

The Lido basin, or north lagoon, has the largest surface expansion (about 270 km2) and includes the city of Venice. The basin of Malamocco, or central lagoon (about 160 km2) includes a large part of the Venetian industrial zone. Finally the Chioggia basin, or south lagoon, has a surface area of about 116 km2.

An investigation extended to the entire area bordering the lagoon permitted the identification of 24 points where water flows out from the drainage basin and description of each water course and how it reaches the lagoon (2). From this investigation only eight natural channels and agricultural canals flowing into the lagoon were selected. The eight outlets are: the Silone Canal, River Dese and Canal Osellino (Marzenego) in the north lagoon; Naviglio del Brenta Canal and Canal of Lova in the central lagoon; and Taglio Nuovissimo Brenta, Montalbano Canal and Trezze Canal in the south lagoon (figure I).

Field work

Field measurements were carried out at each of the eight outlets with the use of a houseboat especially equipped to measure river discharge (3). Measurements were taken under normal weather conditions and were repeated over three periods during the year to have an idea of the seasonal effects. The main difficulty in measuring arises from the tidal oscillations. During each 15-day period, a long time series of measurements was made at half-depth at the mouths of each water course. Moreover vertical profiles were taken every four hours (4). Pollutants were determined by off-line analysis of water samples automatically collected in the upper river layer every four hours.

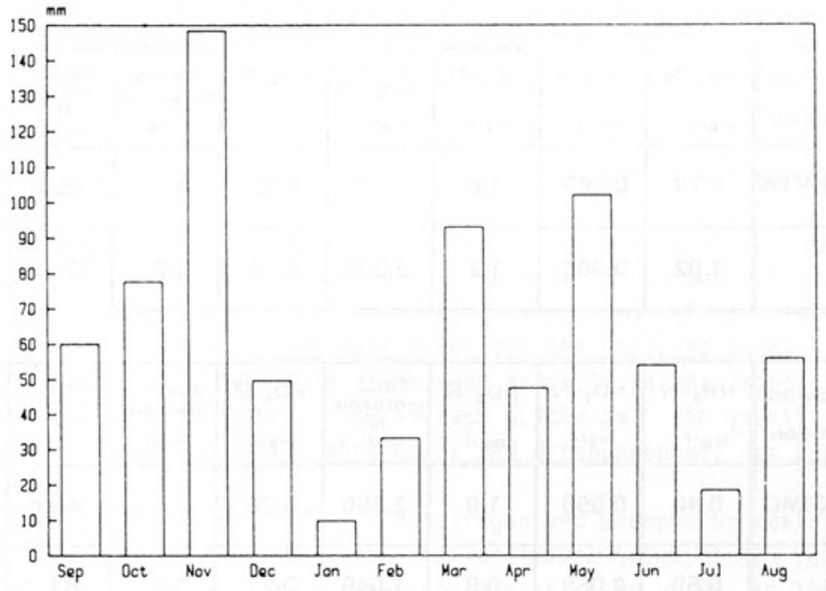


Figure 2

Methods and determination of pollutants

Among the many pollutants it was decided to limit measurements to nutrients and some heavy metals. In the field operations from September 1982 to August 1983, monthly water samples were collected at each of the eight outlets. For each sample, in situ determinations were made for the following parameters : air-sea temperatures, salinity, pH and dissolved oxygen. Samples kept under refrigeration during transport were tested in the laboratory for ammonia, nitrite, nitrate and total orthophosphates. Standard methods were used in carrying out the analyses (5).

Samples were collected in polyethylene bottles and acidified with HNO_3 to determine some heavy metals. These were detected by exposing a precipitate of the water sample to accelerated protons which excite the x-ray emission of the heavy atoms (PIXE) (6,7).

Monthly samples were collected at a mid-point of the river down to a 20 cm depth. The sampling day corresponds to spring and neap tide situations and the hour to the minimum low tide. Salinity profiles of the Dese and Silone carried out in preceding field measurements showed that the salt wedge is always one metre below the water surface and recedes considerably during the minimum low tide. The sampling sites in figure I correspond to a section of the river mouth.

a)

WATER COURSES FLOWING INTO THE NORTH LAGOON	NH_3-N mg/l	NO_2-N mg/l	NO_3-N mg/l	Total inorganic N mg/l	PO_4-P mg/l	Average Discharge m^3/s	Total inorganic N tn/yr	PO_4-P tn/yr	N / P
SILONE	0.37	0.064	1.4	1.834	0.15	9.3	537.9	44.	12
DESE	1.07	0.200	1.2	2.470	0.31	3.1	241.5	30.3	8
OSELLINO (MARZENEGO)	1.12	0.221	1.2	2.541	0.45	1.7	136.2	24.1	6

b)

WATER COURSES FLOWING INTO THE CENTRAL LAGOON	NH_3-N mg/l	NO_2-N mg/l	NO_3-N mg/l	Total inorganic N mg/l	PO_4-P mg/l	Average Discharge m^3/s	Total inorganic N tn/yr	PO_4-P tn/yr	N / P
NAVIGLIO BRENTA	0.73	0.082	1.8	2.612	0.32	4.	329.5	40.3	8
LOVA	1.02	0.086	1.2	2.306	0.55	1.9	138.2	33.	4

c)

WATER COURSES FLOWING INTO THE SOUTH LAGOON	NH_3-N mg/l	NO_2-N mg/l	NO_3-N mg/l	Total inorganic N mg/l	PO_4-P mg/l	Average Discharge m^3/s	Total inorganic N tn/yr	PO_4-P tn/yr	N / P
TAGLIO NOVISSIMO	0.40	0.050	1.9	2.350	0.25	7.4	548.4	58.3	9
MONTALBANO	0.59	0.059	0.9	1.549	0.21	1.7	83.	11.3	7
TREZZE	0.53	0.077	1.1	1.707	0.22	1.6	86.1	11.1	8

Table 1 a, b, c.

LAGOON OF VENICE	AVERAGE DISCHARGE m^3/sec	TOTAL N INORGANIC tn/yr	ORTHOPH. P tn/yr	N / P
NORTH LAGOON (%)	14.1 (46)	915.6 (43.6)	98.4 (39)	9
CENTRAL LAGOON (%)	5.9 (19)	467.7 (22.4)	73.3 (29)	6
SOUTH LAGOON (%)	10.7 (35)	717.5 (34)	80.7 (32)	9
TOTAL	30.7	2100.8	252.4	8

Table 2.

Results and discussion

For convenience the data presented are divided for each lagoon basin. The graphs in figure II give the recorded monthly rainfall values expressed in mm during the test period.

The special legislation for the city of Venice (8, 9) established the maximum values allowed for the effluents discharged into the lagoon. Yet even today no regulation on the allowable limits in the proximity of the outlets emptying into the lagoon and sea exists.

Considering the trend of the single nutrients the situations were as follows :

- Orthophosphates. Keeping in mind that in stream currents the total phosphorus/orthophosphates ratio rarely exceeds 2, it is found that for the lagoon quite a number of values exceed both limits or at least the lower limit allowed by the law (0.45-1.51 ppm as phosphorus). In particular, the canals of Osellino, Navaglio Brenta and Lova had quite high values concentrated in the months of May and September 1983 which exceed the limits.
- Nitrate never exceeds the allowable limits fixed wihtin the range of 4.5-11.2 mg/l.
- Nitrite values fall within the range of 0.033-1.6 mg/l in the north lagoon : 0.015-0.17 mg/l in the central lagoon : and 0.010-0.19 mg/l in the south lagoon.

The law established the values between 0.3-0.6 mg/l for nitrogen. A higher limit was exceeded in the Dese and the Osellino during the month of October 1983.

- Ammonia ranges between 0.18-2.3 mg/l in the north lagoon ; 0.35-1.98 mg/l in the central lagoon : and 0.10-1.23 mg/l in the south lagoon with a pH between 7.4 and 8.3 for the whole lagoon.

The law prescribes a range between 1.53-3.88 mg/l for values not correlated to pH, the lower limit of which is exceeded many times in the Dese River and the Osellino Canal.

The quantitative evaluation of nutrients discharged annually in the lagoon is reported in table 1 a, b, c, in which yearly mean values, the average discharge, the quantity of total inorganic nitrogen (sum of ammonia, nitrite, nitrate) phosphorus and orthophosphate are reported for the north, central and south lagoon respectively.

Finally the reported values in table 2 of the nitrogen and phosphorus contribution in the entire lagoon summarize the situation. The north lagoon has an annual input of 915 tons of total inorganic nitrogen and 98 tons of phosphorus with a nitrogen-phosphorus ratio of 9. In the central lagoon, 467 tons of nitrogen and 73 tons of phosphorus are discharged with a ratio of 6 ; and in the south lagoon, 717 tons of nitrogen against 80 of phosphorus with a ratio of 9. Overall, about 2100 tons of nitrogen and 252 tons of phosphorus are discharged annually with a ratio of 8.

Data on the heavy metals are reported in tables 3 a, b, c and 4.

Conclusions

The lagoon can be considered a quasi-closed system and the matter transferred by canals and rivers can be calculated with a reasonable approximation. This matter remains in the lagoon for some-time before being transferred to the Adriatic Sea and possibly it can be trapped on the bottom and successively released from it.

If the quantity of pollutants discharged in the lagoon from the drainage basin during the year can be estimated, it is very difficult to estimate the exchange between the lagoon and Adriatic Sea. In fact, the volume of water exchanged during each tide cycle is very high with respect to the net flux towards the sea. An eventual measure would require a maximum precision (1 ‰) and a longer temporal series with respect to those measurements carried out on the rivers and canals investigated. Unfortunately the experimental conditions of the three inlet mouths are less favourable than those of the internal canal network.

Measures of deposit and release of matter on the lagoon bottom require numerous samplings in order to be significant. With the purpose of completing the estimation of a matter budget within the lagoon system, a measurement programme should be set up on a long-term basis at the sea inlets. A

a)

WATER COURSES FLOWING INTO NORTH LAGOON	Fe ppb	Ni ppb	Cu ppb	Hg ppb	Pb ppb
SILONE	241.3	2.3	3.8	0.7	2
DESE	616.9	3.7	6.0	1.4	7
OSELLINO	644.0	3.7	7.4	*	11

b)

WATER COURSES FLOWING INTO CENTRAL LAGOON	Fe ppb	Ni ppb	Cu ppb	Hg ppb	Pb ppb
NAVIGLIO BRENTA	614.9	10.1	9.3	*	7
LOVA	576.4	3.6	6.8	0.9	10

c)

WATER COURSES FLOWING INTO SOUTH LAGOON	Fe ppb	Ni ppb	Cu ppb	Hg ppb	Pb ppb
TAGLIO NOVISSIMO	425.4	3.3	5.5	*	2
MONTALBANO	397.0	3.4	5.5	1.8	8
TREZZE	310.2	5.0	4.6	2.5	6

Table 3 a, b, c.

LAGOON OF VENICE	Fe tn/yr	Ni tn/yr	Cu tn/yr	Hg tn/yr	Pb tn/yr
NORTH LAGOON	165.9	1.3	2.1		1.9
(%)	(40)	(32)	(38)		(41)
CENTRAL LAGOON	112.0	1.5	1.6		1.5
(%)	(27)	(37)	(29)		(33)
SOUTH LAGOON	136.2	1.25	1.8		1.2
(%)	(33)	(31)	(33)		(26)
TOTAL	414.1	4.05	5.5	*	4.6

Table 4.

* The mean value and the amount of Hg present in the quantity discharged have not been calculated since the available data refer to extreme situations.

research programme should also be initiated at the same time to study the mechanisms of deposition and release of polluted material on and from the lagoon bottom.

Parameter	Unit of Measure	Maximum Allowed
pH	unit	5.5 - 9.0
Temperature	°C	30
Ammonia	mg N/l	1.5 - 3.88
Nitrite	mg N/l	0.3 - 0.6
Nitrate	mg N/l	4.5 -11.2
Total phosphates	mg P/l	0.45- 1.51
Iron and manganese	µg/l	2000-4000
Nickel	µg/l	2000
Copper	µg/l	50
Mercury	µg/l	5
Lead	µg/l	100

Table 5. - Maxima allowed for water discharge in the Venice Lagoon.

References

(1) S. BERNARDI, F. COSTA, S. VAZZOLER, R. CECCHI, G. GHERMANDI and G. MAGNONI, (1982). - Transfer of freshwater and pollutants into the Lagoon of Venice, Communication VIth Workshop on Marine Pollution of Mediterranean, Cannes, 1982.

(2) M. MENEGHIN, and G. ZAMPERONI, (1982). - Morfologia dei corpi idrici sversanti nella laguna di Venezia, ISDGM-CNR, Venice, TR 117.

(3) G. ARCARI, F. COSTA and F. SIMONATO, (1982). - "Rivo Alto"-Unità di controllo dell'ambiente lagunare, ISDGM-CNR, Venice, TR 116.

(4) E. ANGELI, G. ARCARI, S. BERNARDI, R. CECCHI, F. COSTA, G. GHERMANDI, M. MENEGHIN, R. RUGGERI, F. SALVIATI, R. SANTANGELO, F. SIMONATO, S. VAZZOLER, G. ZAMPERONI and R. ZENNARO, (1981). - Metodologia per la misura diretta delle immissioni di acqua dolce e di inquinanti, e dell' intrusione salina nella parte nord della laguna di Venezia. IV A.I.O.L. Congress, Feb. 1981.

(5) APHA, AWWA, APCF, (1980). - Standard Methods for Examination of Water and Waste Water, New York, APHA.

(6) C.L. LUKE, (1968). - Determination of trace elements in inorganic and organic materials by x-ray fluorescence spectroscopy, Anal. Chim. Acta, 41, 237-250.

(7) G. APRILESI, R. CECCHI, G. GHERMANDI, G. MAGNONI and R. SANTANGELO, (1984). - Calibration and errors in the detection of heavy metals in fresh and sea waters in the ppb-ppm range, Nucl. Instr. Meth., 231 (B3), 172-176.

(8) Legge 16 Aprile 1973 n° 171, Interventi per la salvaguardia di Venezia.

(9) Decreto del Presidente della Republica del 20 settembre 1973, n° 962, Tutela della città di Venezia e del suo territorio dagli inquinanti delle acque.

INFLUENCE DE LA FLORE BACTERIENNE SUR LA FIXATION DU CADMIUM ET DU VANADIUM

par

M.J. GAUTHIER, G.N. FLATAU et J.Ph BREITTMAYER
I.N.S.E.R.M. Unité n° 40
1, avenue Jean Lorrain - 06300 NICE (France)

Abstract

Little has been done on the influence of bacteria on the uptake of heavy metals in water-sediment systems. The authors have studied the interactions between minerals, ionic matrix, organic matter and aerobic or anaerobic marine bacteria on the uptake of Cadmium and Vanadium by an artificial sediment. Some physiological properties of the bacterial strains were more specially examined : resistance to metallic ions and respiratory metabolism for heterotrophs, sulfate reduction for anaerobes.

Résumé

Il n'existe qu'un très petit nombre de travaux traitant de l'influence directe des micro-organismes sur le transfert des métaux lourds de l'eau vers les sédiments. Les auteurs ont étudié les interactions entre les minéraux sédimentaires, la matière organique et la flore bactérienne en utilisant expérimentalement un système eau-sédiment totalement contrôlé. Le rôle de la microflore bactérienne au cours du transfert a été analysé à l'aide de diverses souches pures. Une attention particulière a été portée à l'analyse des propriétés de fixation du Cadmium et du Vanadium en présence de germes sensibles ou résistants à ces ions métalliques, en aérobiose et en anaérobiose. Les résultats montrent que cette fixation dépend à la fois des conditions physico-chimiques et des capacités d'accumulation des métaux par les souches bactériennes. Certaines accélèrent le transfert en accumulant le métal, d'autres au contraire freinent cette fixation. Une certaine analogie est apparue dans le comportement des deux métaux, malgré leur charge électrique opposée.

*
* *

Introduction

Depuis plus de vingt ans, de nombreux travaux ont été réalisés dans le but de mieux connaître le cycle biogéochimique des métaux en milieux sédimentaires ou dans les systèmes eaux-sédiments telluri-

ques ou marins. Ils concernent avant tout les cations toxiques pour les êtres vivants (Hg, Cd, Pb, Cu, Zn...) ou ceux qui leur sont nécessaires (Na, K, Mg, Mn, Co...).

D'une manière paradoxalement assez cloisonnée, ces travaux ont été orientés soit vers l'analyse de l'évolution des éléments dans le milieu physique, soit vers l'étude de leur devenir dans les organismes. Ainsi, bien que de nombreuses données de détail restent à préciser, on connaît assez bien l'influence des facteurs physico-chimiques sur la spéciation et la mobilisation des métaux, et plus particulièrement du Cadmium, dans les milieux hydriques et sédimentaires grâce à de nombreuses études expérimentales, modélisations ou observations in situ (NRIAGU, 1980). Par ailleurs, l'accumulation des ions métalliques dans le milieu biologique et leur toxicité ont été largement étudiées, des procaryotes à l'homme (LEE, 1972; KRENKEL, 1975; NRIAGU, 1979, 1980). En ce qui concerne les micro-organismes, on sait qu'ils possèdent des propriétés d'adaptation. d'accumulation et de transformation tout à fait exceptionnelles par rapport aux autres éléments vivants, mais leur influence directe dans la mobilisation des métaux à l'interface eau-sédiment n'a que rarement été analysée d'une manière globale, et toujours dans des conditions non marines (KUREK et coll., 1976; RAMAMOORTHY et coll., 1977; TITUS et PFISTER, 1982).

Le but de ce travail était donc d'évaluer, en système expérimental contrôlé à l'aide d'un sédiment artificiel reconstitué et de souches bactériennes pures, le rôle respectif ou conjoint joué par les principaux éléments sédimentaires, les ions minéraux. certaines substances organiques métabolisables et diverses espèces bactériennes dans le transfert du Cadmium et du Vanadium de l'eau de mer vers les sédiments. Bien que son évolution dans l'environnement soit moins bien connue que celle du Cadmium, le Vanadium a été choisi du fait de son caractère anionique dans le milieu marin. Si cette méthode ne permet pas de connaître la valeur des transferts entre l'eau et le sédiment en dehors des conditions de l'expérience, elle semble suffisante pour évaluer qualitativement l'influence des variables biotiques ou abiotiques sélectionnées et comparer quantitativement l'importance de chacune d'entre elles.

Matériel et Méthodes

Sédiment artificiel. Dans le souci d'un bon contrôle des conditions expérimentales et de reproductibilité des tests, cette étude a été réalisée à l'aide d'un sédiment artificiel reconstitué (SAR), à partir de cinq substrats poudreux purs, dans les proportions suivantes : montmorillonite (SERVA Chemicals, particules de diamètre moyen 5 µm) : 2 Vol. - silice (Merck, 60 à 200 µm) : 1 Vol. - sable quartzeux naturel (0,5 à 1 mm) : 1 Vol. - CO_3Ca (Prolabo, 3 µm) : 1 Vol. - sable calcaire naturel (maerl broyé, 0,5 à 5 mm) : 1 Vol.

Principe expérimental. Les tests ont été systématiquement réalisés en flacons erlenmeyer de 250 ml, en pyrex (pour le Vanadium) ou en polycarbonate (pour le Cadmium) : des tests préalables ont montré que ces matériaux n'absorbent pas les métaux correspondants.

Toutes les expériences ont été menées en cinq exemplaires. La même réserve d'eau de mer a été utilisée pour l'ensemble des tests. Le schéma expérimental de base était le suivant : dans chaque flacon, 40 (ou 50) grammes de SAR ont été préalablement imprégnés par 28 (ou 35) ml d'eau de mer contenant ou non de la matière organique (eau d'imbibition). Après stérilisation (120°C, 20 minutes), ce sédiment a été recouvert par 135 (ou 160) ml d'eau de mer contenant le métal (Cd : 100 µg/l sous forme $CdCl_2$. 2 1/2 H_2O ; V : 1 mg/l sous forme VO_3Na) et, éventuellement, les cellules bactériennes (eau de recouvrement). Afin d'éviter toute turbulence au niveau des prélèvements, la capacité de fixation des métaux par ce SAR a été évaluée indirectement par la mesure de la décroissance du taux du métal dans l'eau de recouvrement, en analysant une série d'aliquotes de 2 ml prélevées après différentes périodes d'incubation. L'adsorption des métaux étant négligable sur les parois, nous avons admis que leur disparition de l'eau correspondait à une fixation par le SAR ou les bactéries.

Par cette technique, il a été possible d'analyser l'influence de différents substrats organiques (glucose, peptone, cystéine, ovalbumine, alginate de Na) à la concentration finale de 1 mg par gramme

de sédiment, ainsi que le rôle dans ce transfert de souches bactériennes hétérotrophes aérobies ou anaérobies facultatives et d'une souche anaérobie sulfato-réductrice.

Souches bactériennes. Les souches bactériennes utilisées au cours de ce travail ont été isolées d'un sédiment marin portuaire fortement contaminé (port de Villefranche-sur-Mer, Alpes-Maritimes, France) ou de la masse viscérale d'huîtres (littoral atlantique, Nantes, France).

Les souches hétérotrophes aérobies strictes ou facultatives ont été sélectionnées parmi les bacilles à Gram négatif, selon deux critères phénotypiques : leur résistance aux métaux (Cd, V) et leur propriété d'acidification du glucose (test de HUGH et LEIFSON, modifié par LEIFSON, 1963) (Tableau n° 1). Les souches oxydatives ou neutres appartenaient au groupe des pseudomonadacées, celles qui fermentaient le glucose à celui des vibrionacés. L'accumulation du Cadmium par les souches CdR et CdS ont été décrites antérieurement (FLATAU et GAUTHIER, 1983).

La souche anaérobie sulfato-réductrice a été isolée des mêmes sédiments (Villefranche-sur-Mer), en milieu de Starkey (RODINA, 1972).

N°souche	Provenance (a)	Concentrations minimales inhibitrices (exprimées en µg métal / ml)		Voie d'attaque du glucose
		Cd	V	
Cd^R_S	sédiment	28	/	oxydative
Cd^S	sédiment	0,8	/	oxydative
V^R	sédiment	/	600	neutre
V^S	sédiment	/	40	neutre
198	huître	5 - 10	/	fermentative
229	huître	10	/	fermentative
238	huître	1 - 5	/	fermentative
211	huître	5	/	oxydative
202	huître	80 - 100	/	oxydative
208	huître	40 - 50	/	fermentative
182	huître	80 - 100	/	fermentative
232	huître	80 - 100	/	fermentative
BA	sédiment	60 - 80	200	fermentative
BN	sédiment	60 - 80	200	neutre

Tableau n° 1 : Provenance et caractéristiques des souches bactériennes hétérotrophes utilisées. (a) sédiment : Port de Villefranche-sur-Mer; huître : claire ostréicole, littoral atlantique. /: tests non effectués.

Ces bactéries ont été inoculées dans l'eau de recouvrement du SAR à partir d'une pré-culture (24 h à 25° C) en milieu liquide (Marine Broth Difco pour les hétérotrophes, milieu de Starkey pour la souche réduisant les sulfates) à raison de 2 à 6.10^6 cellules par millilitre d'eau. La qualité des sources de carbone et d'azote ajoutées aux sédiments a été adaptée à la nature des souches ou du test: glucose et peptone (1 g/l) pour les hétérotrophes résistants ou sensibles aux métaux, glucose et NH_4Cl (1 g/l et 0,5 g/l respectivement) pour les souches acidifiant ou non le sucre, milieu de Starkey complet pour la souche réduisant les sulfates.

Le développement plus lent de cette souche sulfato-réductrice a nécessité une stabulation préalable des flacons pendant une journée avant ajout du métal et début des dosages. L'anaérobiose a été obtenue par le procédé Gaspak (Mérieux, France) sous atmosphère CO_2-H_2, en jarres de 5 litres ou en incubateur étanche.

Dosage des métaux. Le Cadmium a été dosé par spectrophotométrie d'absorption atomique à flamme (Varian Techtron 1100). Le Vanadium a été mesuré colorimétriquement par la méthode de BASSON et KEMPSTER (1980).

Analyse statistique des résultats. La significativité des effets des paramètres étudiés a été testée par analyse de la variance : les expériences ont été établies suivant un plan à deux facteurs, en blocs complets. Le premier facteur est le temps de contact eau-sédiment, le second étant la

condition expérimentale. La variable mesurée est le logarithme de la concentration résiduelle dans l'eau. Le passage au logarithme a été nécessaire pour normaliser les distributions et stabiliser les variances : on peut en effet considérer, en première approximation, que l'incertitude sur la mesure de la concentration est proportionnelle à cette concentration. Le facteur temps étant quantitatif, une analyse de covariance a d'abord été tentée, mais les tests ont conduit à rejeter. dans la plupart des cas, l'hypothèse de linéarité de la liaison entre le temps de contact et le log de la concentration. On a donc procédé à une analyse de variance et, si l'effet de traitement ou l'interaction temps-traitement était significatif, on a cherché à préciser les valeurs ou groupes de valeurs qui différaient entre eux par la méthode de SHEFFE (LELLOUCH et LAZAR, 1974).

Résultats - Discussion

Dans des conditions abiotiques, le SAR a montré un pouvoir d'adsorption linéaire en fonction du temps pour les deux métaux (Fig. 1). Cette fixation paraissait plus faible pour le Vanadium, en considérant le rapport entre le taux de métal fixé et celui de l'eau au début de l'expérience (pente 0,46 pour Cd, 0,37 pour V) : en valeur absolue, par contre, le transfert du Vanadium était beaucoup plus rapide (pente 3,75), sa concentration dans l'eau étant dix fois plus élevée que celle du Cd.

L'ajout des cinq substrats organiques au SAR n'a pas modifié significativement ses capacités de fixation du Cadmium et du Vanadium, à l'exception de l'alginate de Na qui a légèrement accéléré le transfert du Vanadium (Fig. 2). On relève ici une certaine divergence avec les données de la littérature (tout au moins pour Cd), qui montrent une proportionnalité entre la quantité de métal fixée par les sédiments naturels et leur contenu en matières organiques (MONACO, 1980 : RAPIN, 1980). On sait, en outre, que les milieux nutritifs utilisés pour la croissance des bactéries, qui contiennent en particulier de la peptone, des acides aminés et des sucres, complexent les métaux et réduisent ainsi la concentration en ions libres (RAMAMOORTHY et KUSHNER, 1975). Selon STUMM et MORGAN (1970), une forte concentration en cations (Ca, Mg) pourrait cependant conduire à une compétition avec Cd au niveau des sites de fixation de la matière organique ; la complexation elle-même pourrait être réduite par le pH de l'eau de mer (FRISTOE et NELSON, 1983). Quoiqu'il en soit, il est probable que le faible degré de perméabilité du SAR a limité cette complexation à la couche superficielle.

Les souches Cd^R et Cd^S, physiologiquement très voisines, ont toutes deux modifié l'élimination du Cadmium de l'eau, la souche R en le freinant et la souche S en l'accélérant (Fig. 3). Cette différence, statistiquement significative (seuil 5 %) pour les points 24 h et 72 h, est en partie explicable par les capacités d'accumulation très élevées des cellules S (FLATAU et GAUTHIER, 1983). L'influence opposée des cellules R pourrait être due à la formation d'un film bactérien à la surface du SAR, composé des cellules et de certains exopolymères ne liant par le métal, qui serait intervenu momentanément pour empêcher l'accès du métal au sédiment. BITTON et FREIHOFER (1978) ont montré que certaines bactéries sont capables de sécréter des polymères exocellulaires formant une couche protectrices à la surface des cellules, masquant passagèrement les sites de fixation possibles du Cadmium sur celles-ci.

L'extention de cette expérience à un nombre plus élevé de souches (incluant Cd^R et Cd^S) (Fig. 4) a montré qu'indépendamment de leurs autres propriétés physiologiques, seules les bactéries aérobies strictes oxydatives sensibles au Cadmium étaient capables d'augmenter significativement (seuil $<10^{-3}$) la vitesse d'élimination du métal de l'eau par rapport aux autres souches (oxydatives R et fermentatives S et R). L'analyse statistique a par ailleurs révélé une différence (seuil 5 %) entre les souches oxydatives R et les souches fermentatives (S et R), les premières ayant freiné plus fortement l'élimination du Cadmium de l'eau au cours des 96 premières heures d'incubation. Ces observations confirment donc les résultats précédents : elles suggèrent, en outre, une incidence plus ou moins directe de l'énergétique cellulaire des bactéries sur les interactions entre celles-ci et les minéraux sédimentaires pour la fixation du Cadmium. On sait en effet, que ce métal modifie l'activité respiratoire des bactéries (TYNECKA et coll., 1975 ; BABICH et STOTZKY, 1978) et son incorporation

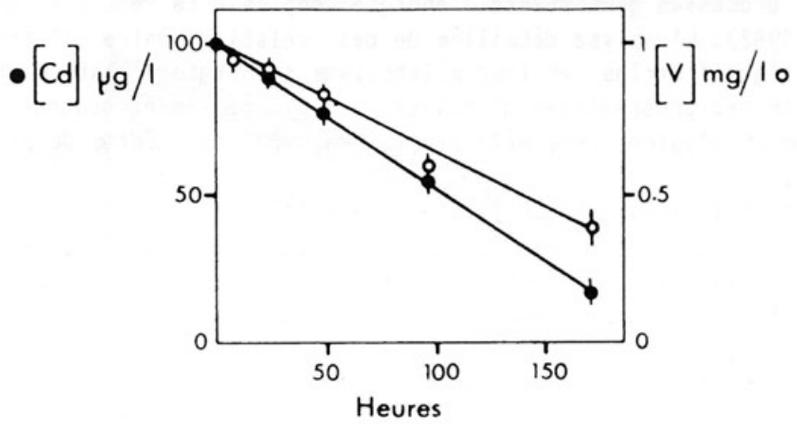


Fig.1 : Variations de la concentration en cadmium (•) et en vanadium (o) dans l'eau en présence de SAR. Le milieu ne contenait ni matières organiques, ni bactéries.

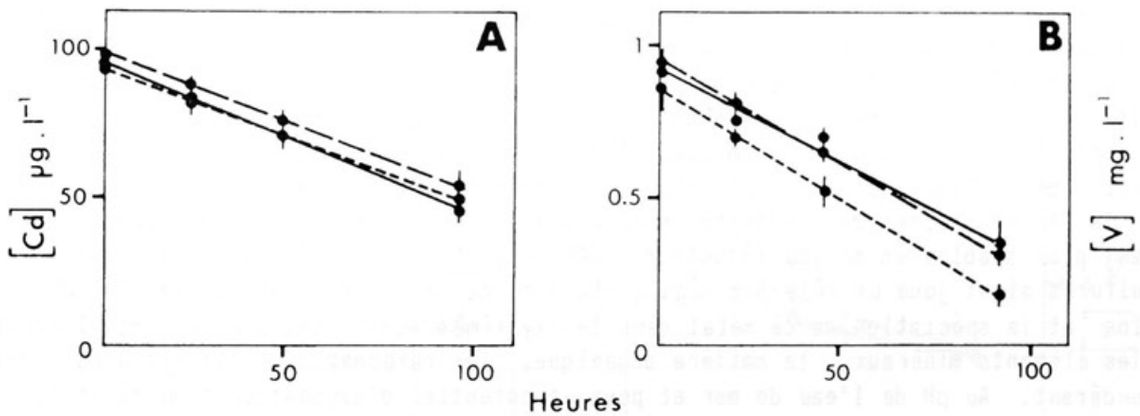


Fig.2 : Variations de la concentration en cadmium (A) et en vanadium (B) dans l'eau en présence de SAR, sans matières organiques (——) ou additionné de glucose (— — —) d'alginate de sodium (-----), d'ovalbumine ou de cystéine. Les deux derniers substrats ont donné des résultats très voisins de ceux obtenus avec le glucose.

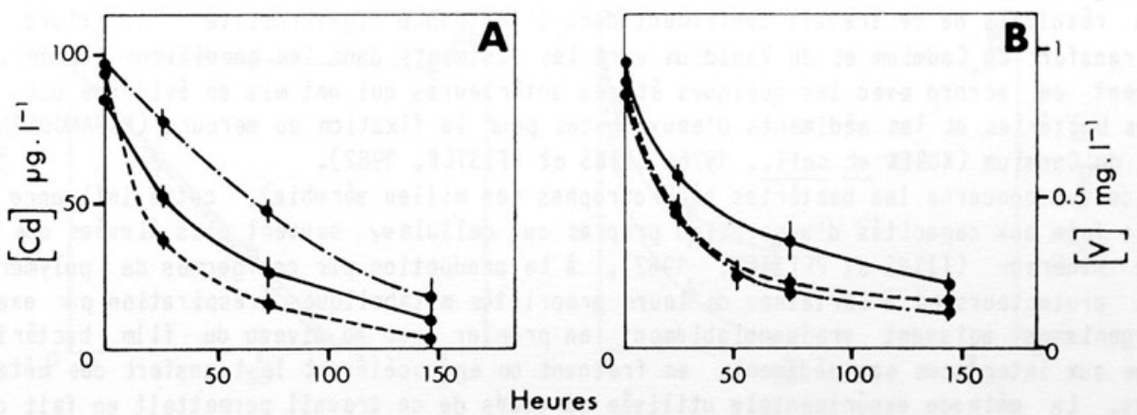


Fig.3 : Evolution de la concentration en cadmium (A) et en vanadium (B) dans l'eau de recouvrement du SAR, en l'absence (——) ou en présence d'une souche bactérienne hétérotrophe résistante (—·—·) ou sensible (-----) au métal. Le sédiment contenait 1gr de glucose et de peptone par Kg.

pourrait être liée aux processus générateurs d'énergie couplés à la respiration (TYNECKA et coll., 1981 ; PERRY et SILVER, 1982). L'analyse détaillée de ces relations entre l'accumulation du métal par les bacilles à Gram négatif marins et leur métabolisme respiratoire est en cours. Pour certaines espèces enfin, l'activité des phosphatases alcalines pourrait également concourir à la précipitation péricellulaire du Cadmium et d'autres ions métalliques (Pb, Zn) sous forme de phosphates insolubles (MACASKIE et DEAN, 1984).

En ce qui concerne le Vanadium (Fig. 3), aucune différence dans la fixation du métal n'est apparue en présence des deux souches V^R et V^S. On notera cependant qu'elles ont provoqué une même augmentation significative (seuil < 10^{-3}) de l'élimination du métal de l'eau par rapport aux témoins abactériens. Ceci résulte vraisemblablement d'une égale activité d'accumulation des deux souches, responsable d'une certaine compétition avec le SAR.

Les souches BA et BN ont, d'une manière analogue, significativement freiné la fixation du Cadmium par le SAR pendant plus de 50 heures (Fig. 5). Ces résultats voisins de ceux obtenus avec la souche CdR (Fig. 3), suggèrent que ces souches BA et BN ont modifié le transfert du métal vers le sédiment sans faire intervenir une modification du pH, ce qui a d'ailleurs été vérifié par la mesure de celui-ci. Dans le cas du Vanadium, la souche BA a passagèrement accéléré l'élimination du métal de l'eau (significatif au seuil 5 %).

Les tests réalisés en présence de bactéries sulfato-réductrices ont fait intervenir simultanément deux facteurs déterminants dans la mobilisation des métaux en milieu sédimentaire : un potentiel d'oxydo-réduction négatif (-300 mV environ) et la production d'H_2S par les micro-organismes.

Le SAR n'a fixé rapidement le Cadmium (100 % en 72 h) qu'en anaérobiose (Fig. 6) avec ou sans ajout des bactéries ; la réduction du milieu était donc seule responsable de la fixation du métal par le sédiment. Compte tenu du mode d'obtention de l'anaérobiose, on peut penser que le Cadmium a été fixé par le SAR sous forme de carbonate insoluble et/ou complexé à la matière organique, ces complexes étant plus stables en milieu réducteur (GRAMBRELL et coll., 1977). Il est cependant possible que les sulfures aient joué un rôle non négligable dans ce transfert. Selon KHALID (1978, 1980), la distribution et la spéciation de ce métal dans les systèmes aquatiques sont essentiellement contrôlées par les éléments minéraux, la matière organique, les carbonates et les sulfures , dont le rôle est prépondérant. Au pH de l'eau de mer et pour un potentiel d'oxydo-réduction négatif, le milieu était effectivement favorable à la stabilité de la forme CdS (HEM, 1972).

Dans le cas du Vanadium, par contre, la fixation du métal dans le sédiment a été à la fois importante dans le milieu abiotique, aérobie et anaérobie, et nettement accélérée par la production de sulfures par la souche sulfato-réductrice. Après 48 h d'incubation, on peut évaluer à 40 % le taux de Vanadium fixé par le SAR sous l'influence de l'activité bactérienne et à 60 % celui qui doit être expliqué par des processus physico-chimiques (adsorption sur les minéraux, complexation par la matière organique, précipitation, etc.).

Les résultats de ce travail confirment donc l'influence significative de la flore bactérienne sur le transfert du Cadmium et du Vanadium vers les sédiments dans les conditions marines. Ils sont globalement en accord avec les quelques études antérieures qui ont mis en évidence une compétition entre les bactéries et les sédiments d'eaux douces pour la fixation du mercure (RAMAMOORTHY et coll., 1977) ou du Cadmium (KUREK et coll., 1976; TITUS et PFISTER, 1982).

En ce qui concerne les bactéries hétérotrophes (en milieu aérobie), cette influence paraît être liée à la fois aux capacités d'adsorption propres aux cellules, souvent plus élevées que celles des éléments minéraux (TITUS et PFISTER, 1982), à la production par ces germes de polymères exocellulaires protecteurs et à certaines de leurs propriétés métaboliques (respiration par exemple). Les micro-organismes agissent vraisemblablement en premier lieu au niveau du film bactérien qui se développe aux interfaces eau-sédiment, en freinant ou en accélérant le transfert des métaux vers les sédiments. La méthode expérimentale utilisée au cours de ce travail permettait en fait d'évaluer le rôle de ce film plutôt que celui de la flore endogène des dépôts. Une influence analogue a été attribuée aux micro-organismes du "fouling" qui recouvre la surface des végétaux aquatiques, en ce qui concerne la contamination de ceux-ci par les ions métalliques (PATRICK et LOUTIT, 1977). Dans le cas des bactéries anaérobies sulfato-réductrices, en milieu fortement réduit, c'est la modification

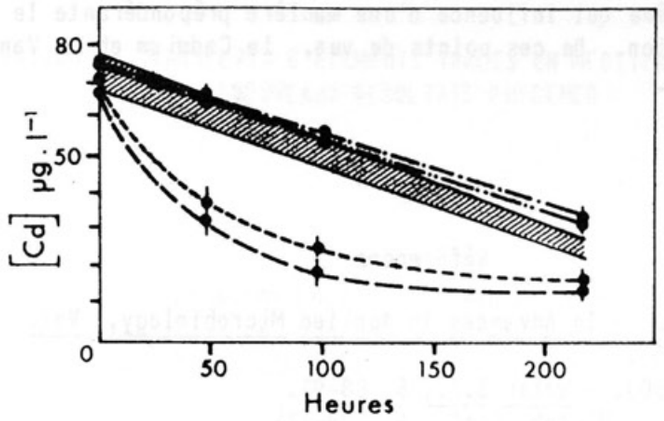


Fig.4 : Evolution de la concentration en cadmium dans l'eau de recouvrement du SAR, en présence de souches bactériennes hétérotrophes oxydatives sensibles au métal (CdS : ——— ;211:------), oxydatives résistantes (CdR:—·—·—;202:—·—·) et fermentatives sensibles ou résistantes (zone hachurée)(voir liste dans le Tableau n°1). Les souches fermentatives ont donné des résultats sans différence significative entre elles.

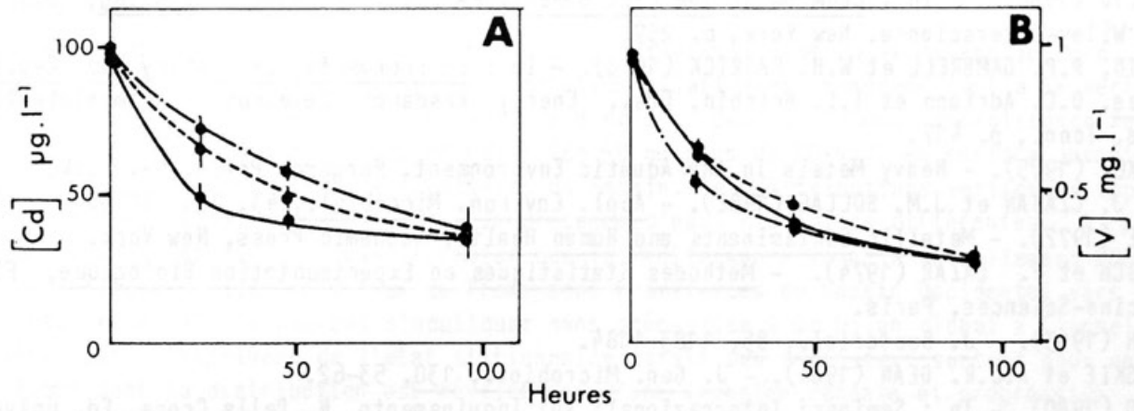


Fig.5 : Evolution de la concentration en cadmium (A) ou en vanadium (B) dans l'eau de recouvrement du SAR, en l'absence (———) ou en présence d'une souche bactérienne hétérotrophe acidifiant (—·—·) ou non (-----) le glucose. Le sédiment contenait 1 gr de glucose et de peptone par Kg.

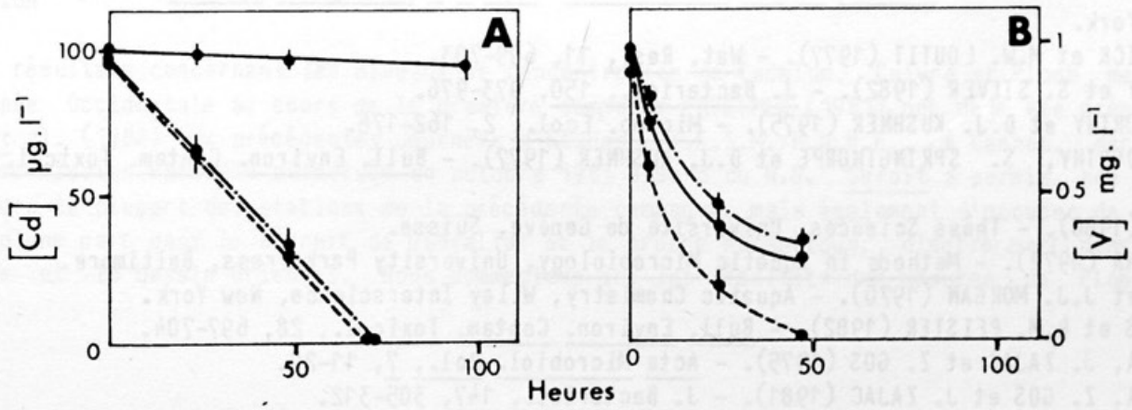


Fig.6 : Influence des bactéries sulfato-réductrices sur l'évolution de la concentration en cadmium (A) ou en vanadium (B) dans l'eau de recouvrement du SAR : témoins abactériens incubés en aérobiose (———) ou en anaérobiose (—·—·), milieu inoculé par la souche sulfato-réductrice (----) en anaérobiose. Le sédiment contenait du milieu de Starkey (28 ml pour 40 grammes).

physico-chimique du milieu lui-même qui influence d'une manière prépondérante le devenir du métal, en dehors des conditions de réduction. De ces points de vue, le Cadmium et le Vanadium ont montré un comportement assez peu différent.

Références

H. BABISH et G. STOTZKY (1978). - In Advances in Applied Microbiology, Vol. 23, D. Perlman, Ed., Academic Press, London, p. 55.

A.T. BASSON et P.L. KEMPSTER (1980). - Water S.A., 6, 88-91.

G. BITTON et V. FREIHOFER (1978). - Microb. Ecol., 4, 119-124.

G.N. FLATAU et M.J. GAUTHIER (1983). - Can. J. Microbiol., 29, 210-217.

R.B. FRISTOE et P.O. NELSON (1983). - Wat. Res., 17, 771-778.

R.P. GAMBRELL, R.A. KHALID et W.H. PATRICK (1976). - In : Dredging and its Environmental Effects, P.A. Krenkel, J. Harrison et J.C. Burdick, Eds., American Society of Civil Engineers, New York, p. 418.

J.D. HEM (1972). - Wat. Resources Res., 8, 661-679.

R.A. KHALID (1980). - In : Cadmium in the Environment. Part I : Ecological Cycling, J.O. Nriagu, Ed., Wiley-Interscience, New York, p. 257.

R.A. KHALID, R.P. GAMBRELL et W.H. PATRICK (1978). - In : Environmental Chemistery and Cycling Processes, D.C. Adriano et I.L. Brisbin, Eds., Energy Research Development Administration, Oak Ridge, Tenn., p. 417.

P.A. KRENKEL (1975). - Heavy Metals in the Aquatic Environment, Pergamon Press, New York.

E. KUREK, J. CZABAN et J.M. BOLLAG (1982). - Appl. Environ. Microbiol., 43, 1011-1015.

D.H.K. LEE (1972). - Metallic Contaminants and Human Health, Academic Press, New York.

J. LELLOUCH et P. LAZAR (1974). - Méthodes Statistiques en Expérimentation Biologique, Flammarion Médecine-Sciences, Paris.

E. LEIFSON (1963). - J. Bacteriol., 85, 1183-1184.

L.E. MACASKIE et A.C.R. DEAN (1984). - J. Gen. Microbiol., 130, 53-62.

A. MONACO (1980). - In : Seminari Internazionali sul'Inquinamento, N. Della Croce, Ed. Université de Gênes p. 75.

J.O. NRIAGU (1979). - The Biogeochemistry of Mercury in the Environment, Elsevier-North-Holland Biochemical Press.

J.O. NRIAGU (1980). - Cadmium in the Environment. Part I : Ecological Cycling, Wiley Interscience, New York.

F.M. PATRICK et M.W. LOUTIT (1977). - Wat. Res., 11, 699-703.

R.D. PERRY et S. SILVER (1982). - J. Bacteriol., 150, 973-976.

S. RAMAMOORTHY et D.J. KUSHNER (1975). - Microb. Ecol., 2, 162-176.

S. RAMAMOORTHY, S. SPRINGTHORPE et D.J. KUSHNER (1977). - Bull. Environ. Contam. Toxicol. 17, 505-511.

F. RAPIN (1980). - Thèse Sciences, Université de Genève, Suisse.

A.G. RODINA (1972). - Methods in Aquatic Microbiology, University Park Press, Baltimore.

W. STUMM et J.J. MORGAN (1970). - Aquatic Chemistry, Wiley Interscience, New York.

J.A. TITUS et R.M. PFISTER (1982). - Bull. Environ. Contam. Toxicol., 28, 697-704.

Z. TYNECKA, J. ZAJAC et Z. GOS (1975). - Acta Microbiol. Pol., 7, 11-20.

Z. TYNECKA, Z. GOS et J. ZAJAC (1981). - J. Bacteriol., 147, 305-312.

DISTRIBUTION ET TRANSFERTS D'ELEMENTS TRACES EN MEDITERRANEE OCCIDENTALE
NOUVEAUX RESULTATS PHYCEMED

par

G. COPIN-MONTEGUT, P. COURAU et E. NICOLAS
Laboratoire de Physique et de Chimie Marines, C.N.R.S.
B.P. n° 8 - 06230 Villefranche-sur-Mer (France)

Résumé

Nous présentons les résultats de mesures de Cadmium et de Plomb effectuées au cours de la deuxième campagne PHYCEMED (octobre 1983). Sept stations ont été échantillonnées dans le bassin Occidental méditerranéen, deux à Gibraltar et le proche Atlantique, deux dans le canal de Sicile et le bassin Oriental.

Pour les stations du bassin Occidental, les observations sont en excellent acccord avec les résultats de PHYCEMED 1 : pour le Cadmium, répartition relativement homogène tant à la verticale que d'une station à l'autre avec une valeur moyenne de 8 ng/l ; pour le Plomb, décroissance faible mais régulière des concentrations avec la profondeur (de 50 ou plus de 50 ng/l en surface jusqu'à 20 à 25 ng/l en profondeur). Par contre, aux frontières du bassin, les distributions verticales apparaissent contrastées, cette situation étant due à la présence d'eaux d'origines différentes. A partir de bilans hydrologiques connus, on peut calculer que la Méditerranée rejette actuellement 200 t/an de Cadmium en Atlantique et que 1.000 t/an de Plomb sont transférées du bassin Occidental vers le bassin Oriental. Ces résultats ne peuvent s'appliquer sans précaution à un bilan global à l'échelle de la Méditerranée, car l'hypothèse de l'état stationnaire paraît peu réaliste, surtout pour un élément comme le Plomb dont la distribution est révélatrice d'une invasion récente et progressive d'origine anthropique.

Introduction

Des résultats concernant les niveaux de concentration en Cadmium, Cuivre et Plomb mesurés en Méditerranée Occidentale au cours de la première campagne PHYCEMED (1981) ont déjà été présentés par LAUMOND et al. (1982) aux précédentes Journées d'Etudes C.I.E.S.M./P.N.U.E., à Cannes, décembre 82. La deuxième mission PHYCEMED effectuée en octobre 1983 à bord du N.O. Suroît a permis, non seulement de reprendre la plupart des stations de la précédente campagne, mais également d'occuper de nouvelles stations d'une part dans le détroit de Gibraltar et le proche Atlantique, d'autre part dans le canal de Sicile et le bassin Oriental. Nous commentons ici les résultats des mesures de Plomb et de Cadmium.

Prélèvements et analyses

Les positions des stations sont reportées sur la carte n° 1. Au total, une centaine d'échantillons a été prélevée à diverses profondeurs et analysée en double par deux techniques différentes :

voltampérométrie et absorption atomique. La première est effectuée directement à bord, une analyse nécessitant environ 45 mn. étalonnage compris. Pour la deuxième, les échantillons sont coprécipités à bord et conservés sous forme solide pour analyse ultérieure au laboratoire. Pour les détails analytiques, on se reportera à LAUMOND et al. (1983). Nous insistons toutefois sur le fait que la bonne qualité des mesures a été obtenue par l'emploi de dispositifs de prélèvements non contaminants et le travail analytique en conteneur laboratoire sous air filtré.

Distribution du Cadmium

Sur la figure 2(a) on a porté le profil des 7 stations du bassin Occidental. Ces résultats sont en parfait accord avec ce qui avait été observé au cours de la première mission PHYCEMED : une distribution assez uniforme tant à la verticale que d'une station à l'autre avec une concentration

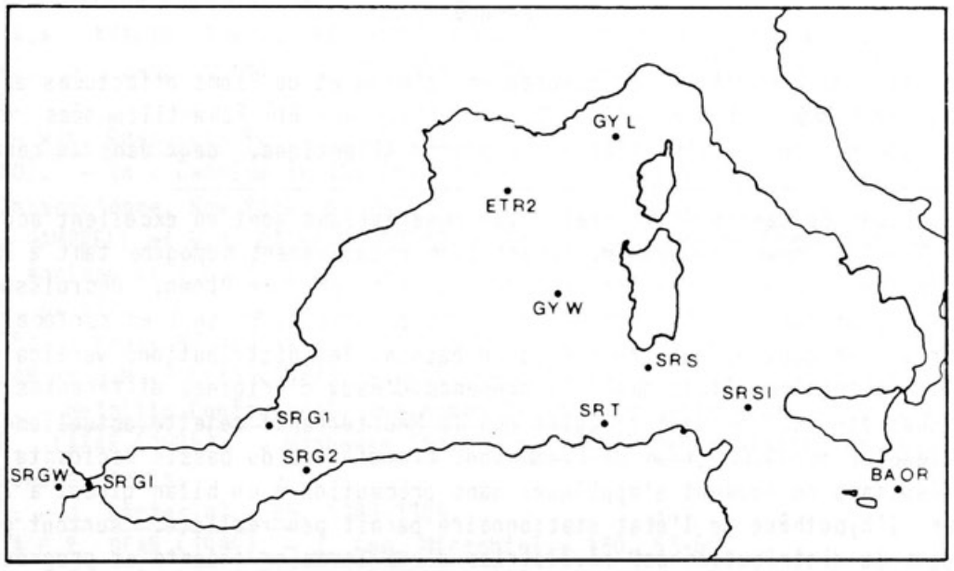


Figure 1. - Position des stations de la campagne PHYCEMED 1983.

moyenne de 8 ng/l (70 pmole/l). On n'observe pas de déficit en surface ni d'enrichissement important dans la zone profonde comme pour une station océanique. Ceci est probablement dû à deux phénomènes : un brassage rapide des eaux superficielles et un apport permanent de Cadmium par voie atmosphérique qui tendrait à empêcher l'apparition d'un déficit dans les eaux superficielles. On peut toutefois noter une tendance générale à l'augmentation des concentrations (de 10 à 15 %) dans la zone 100 à 300 m, où les teneurs en phosphore deviennent élevées.

La situation est toute différente pour les stations de Gibraltar présentées sur la figure 2(b). La station du proche Atlantique (SR GW) présente un profil très contrasté avec une zone 0-75 m appauvrie où les teneurs tombent à 1 ng/l. En dessous, les teneurs augmentent rapidement jusqu'à 10 ng/l à l'immersion 300 m près du fond. Cette valeur reste inférieur aux 20 ng/l observés dans l'Atlantique par BRULAND et FRANKS (1983) à des immersions plus importantes. A la station du détroit de Gibraltar (SR GI) on observe parfaitement le contraste entre eaux superficielles atlantiques de faibles teneurs en Cadmium et les eaux méditerranéennes dont le profil vertical se superpose à ceux des stations du bassin Occidental.

Les stations de l'est sont présentées sur la figure 2(c). Le profil vertical de la station du bassin Oriental (BA OR) est similaire à ceux du bassin Occidental avec des concentrations légèrement plus faibles à toutes profondeurs. Cette relative monotonie des distributions a pour effet qu'à la station du canal de Sicile (SR SI) on ne puisse différencier, à la précision des mesures, les eaux superficielles occidentales des eaux profondes levantines.

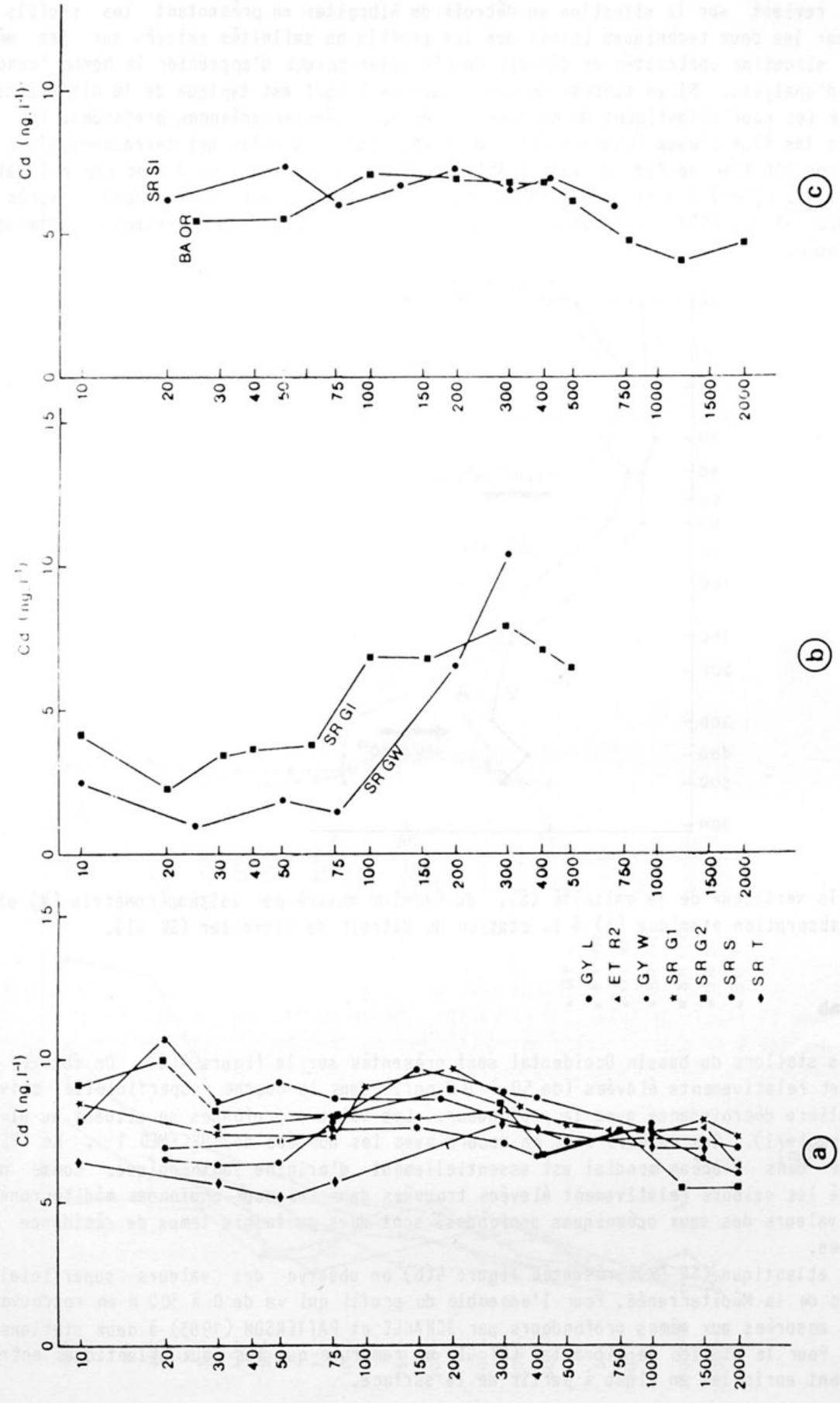


Figure 2. - Distribution verticale du Cadmium : (a) dans le bassin Occidental Méditerranéen - (b) cans le détroit de Gibraltar et le Proche Atlantique - (c) dans le canal de Sicile (SR SI) et le bassin Oriental (BA OR).

La figure 3 revient sur la situation au détroit de Gibraltar en présentant les profils en Cadmium mesurés par les deux techniques (ainsi que les profils de salinités relevés sur les mêmes échantillons). La situation contrastée du détroit de Gibraltar permet d'apprécier la bonne concordance des méthodes d'analyses. Si on suppose qu'une valeur de 4 ng/l est typique de la différence de concentrations entre les eaux atlantiques de surface et les eaux méditerranéennes profondes, et que l'on prend en compte les flux d'eaux échangés (voir BETHOUX, 1980), le bilan net correspondrait à une exportation d'environ 200 t/an de Cadmium vers l'Atlantique. Ceci est en accord avec une estimation faite par SPIVACK et al. (1983) à partir de données plus partielles (valeurs de surface). D'après les estimations d'ARNOLD et al. (1982) une bonne part de Cadmium proviendrait des retombées atmosphériques en Méditerrannée.

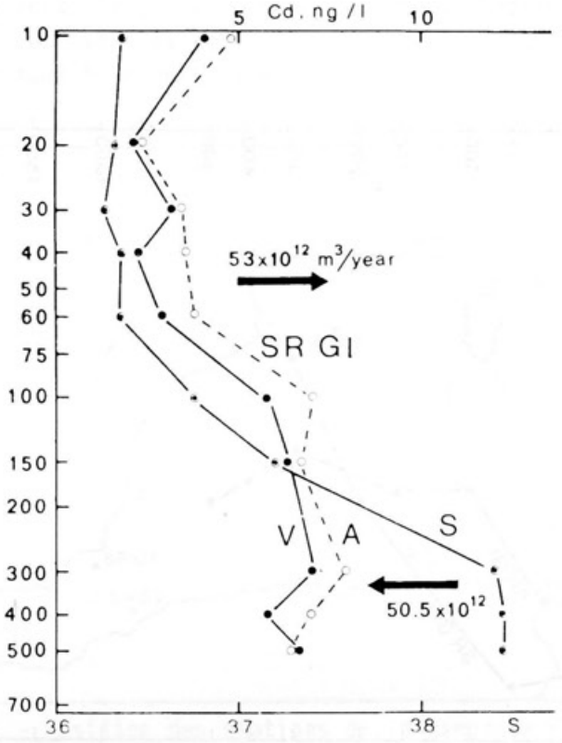


Figure 3. - Profils verticaux de la salinité (S), du Cadmium mesuré par voltampérométrie (V) et du Cadmium mesuré par absorption atomique (A) à la station du détroit de Gibraltar (SR GI).

Distribution du Plomb

Les profils des stations du bassin Occidental sont présentés sur la figure 4(a). On observe des valeurs variables et relativements élevées (de 50 à 140 ng/l) dans la couche superficielle suivies d'une lente et régulière décroissance avec la profondeur. Les valeurs profondes se situent au niveau de 25 ng/l (130 pmole/l). Ces valeurs sont en accord avec les données de PHYCEMED 1 . Le Plomb actuellement mesuré dans l'Océan mondial est essentiellement d'origine anthropique. Comme nous l'avons déjà signalé les valeurs relativement élevées trouvées dans les eaux profondes méditerranéennes (comparées aux valeurs des eaux océaniques profondes) sont dues au faible temps de résidence des eaux méditerranéennes.

A la station atlantique (SR GW) présentée figure 4(b) on observe des valeurs superficielles inférieures à celles de la Méditerranée. Pour l'ensemble du profil qui va de 0 à 300 m on retrouve le niveau des valeurs mesurées aux mêmes profondeurs par SCHAULE et PATTERSON (1983) à deux stations de l'Atlantique nord. Pour la station de Gibraltar (SR GI) on remarque que les eaux atlantiques entrantes sont immédiatement enrichies en Plomb à partir de la surface.

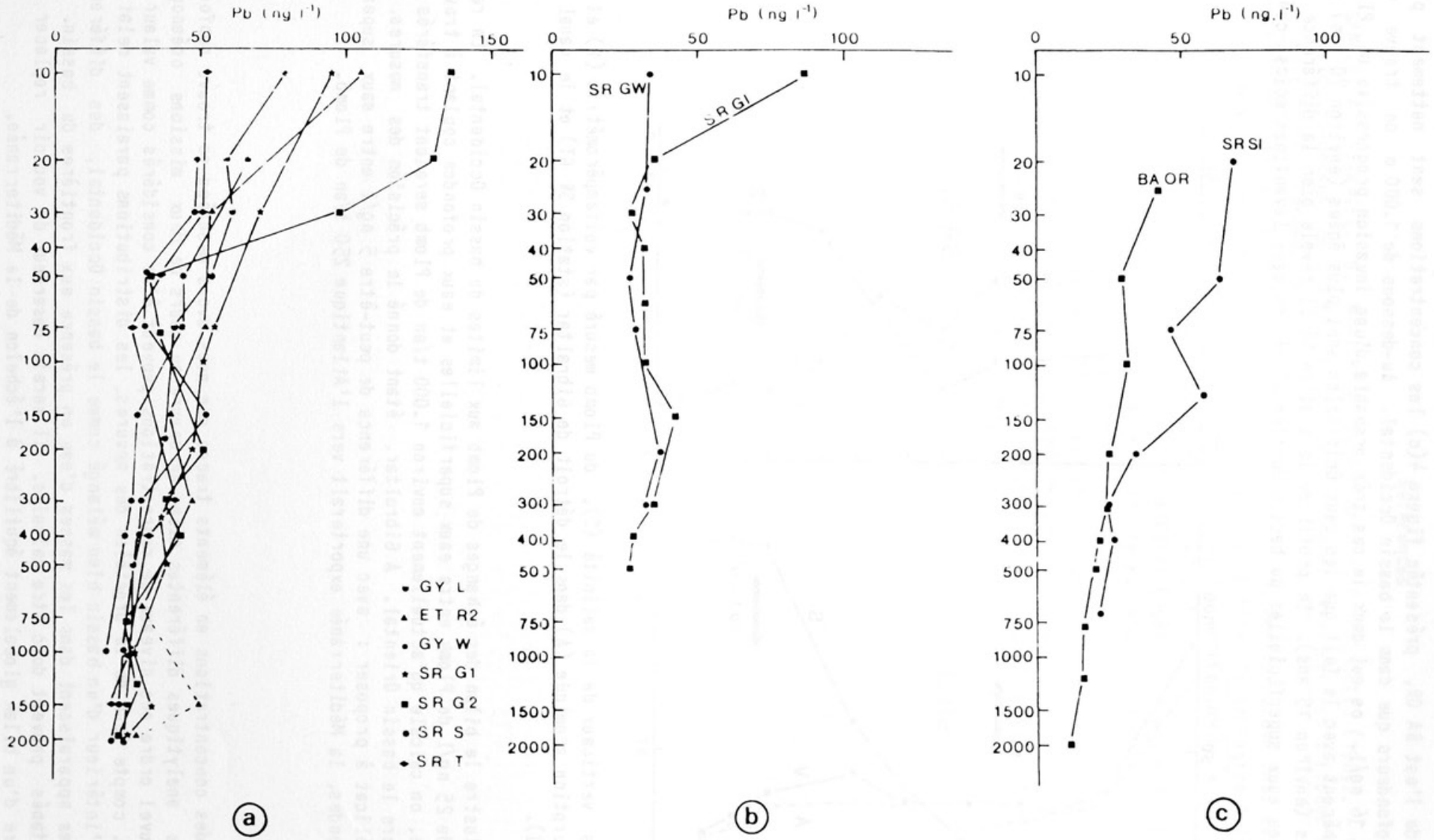


Figure 4. - Distribution verticale du Plomb : (a) dans le bassin Occidental Méditerranéen ; (b) dans le détroit de Gibraltar et le Proche Atlantique : (c) : dans le canal de Sicile (SR SI) et le bassin Oriental (BA OR).

A la station de l'est BA OR, présentée figure 4(c) les concentrations sont nettement plus faibles à toutes profondeurs que dans le bassin Occidental. Au-dessous de 1.000 m on trouve des valeurs inférieurs à 16 ng/l, ce qui dans le cas très probable d'une invasion progressive du Plomb atmosphérique est cohérent avec le fait que les eaux Orientales sont plus âgées (environ 70 ans) que les eaux Occidentales (environ 15 ans). Le profil de la station SR SI révèle bien la différence de concentration entre les eaux superficielles du bassin Occidental et les eaux levantines moins contaminées.

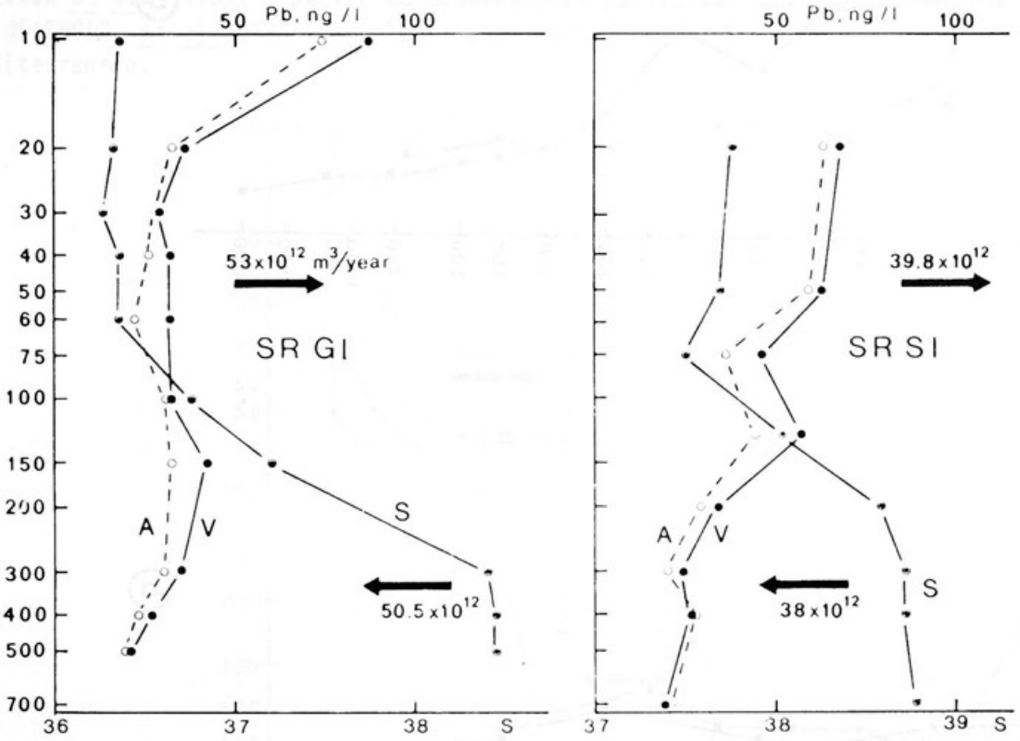


Figure 5. - Profils verticaux de la salinité (S), du Plomb mesuré par voltampérométrie (V) et du Plomb mesuré par absorption atomique (A) dans le détroit de Gibraltar (station SR GI) et le canal de Sicile (station SR SI).

La figure 5 illustre le bilan des échanges de Plomb aux limites du bassin Occidental. En retenant une différence de 25 ng/l de Plomb entre eaux superficielles et eaux profondes coulant à travers le détroit de Sicile, on calcule qu'actuellement environ 1.000 t/an de Plomb seraient transférés du bassin Occidental vers le bassin Oriental. A Gibraltar, étant donné la précision des mesures, un bilan net est plus délicat à proposer : avec une différence de peut-être 5 ng/l entre eaux superficielles et eaux profondes, la Méditerranée exporterait vers l'Atlantique 250 t/an de Plomb.

Conclusion

L'authenticité des concentrations en éléments traces que nous avons mesurées se trouve confortée par deux techniques analytiques différentes mises en oeuvre au cours de deux missions océanographiques. Jusqu'à nouvel ordre ces niveaux de concentrations peuvent être considérés comme valeur de référence. Bien que, compte tenu de la précision des mesures, les distributions paraissent relativement homogènes à l'intérieur d'un bassin bien mélangé comme le bassin Occidental, des différences tout à fait mesurables apparaissent dans les masses d'eau en présence aux frontières du bassin. Des bilans nets instantanés peuvent donc être calculés. Il serait hasardeux de vouloir replacer ces échanges dans le cadre d'un bilan globalement équilibré à l'échelon de la Méditerranée.

Références

M. ARNOLD, A. SEGHAIER, D. MARTIN, P. BUAT-MENARD et R. CHESSELET (1983). - Géochimie de l'aérosol marin au-dessus de la Méditerranée Occidentale. VIe Journées d'Etudes sur les Pollutions en Méditerranée, Cannes. déc. 82, 82. p 27-37.

J.P. BETHOUX (1980). - Mean fluxes across sections in the Mediterranean Sea, evaluated on the basis of the water and salt budgets and of observed salinities. Oceanologica Acta, 3, 1. p 79-83.

K.W. BRULAND and R.P. FRANKS (1983). - Mn, Ni, Cu, Zn, and Cd ir the Western North Atlantic. In : Trace Metals in Sea water, Plenum Press, p 395-412.

F. LAUMOND, G. COPIN-MONTEGUT, P. COURAU et E. NICOLAS (1983). - Niveaux de concentrations en métaux lourds dans les eaux de Méditerranée Occidentale. VIe Journées d'Etudes sur les Pollutions en Méditerranée. Cannes. déc. 82, p 115-123.

B.K. SCHAULE and C.C. PATTERSON (1983). - Pertubation of the natural lead depth profile in the Sargasso sea by industrial lead. In : Trace Metals in Sea water, Plenum Press, p 487-503.

A.J. SPIVACK, S.S. HUESTED and E.A. BOYLE (1983). - Copper nickel and Cadmium in the surface waters of the Mediterranean. In : Trace Metals in Sea water, Plenum Press p 505-512.

SUSPENDED ALUMINOSILICATES AND PARTICULATE RARE EARTH ELEMENTS IN WESTERN MEDITERRANEAN WATERS

by

N. RISLER, C.E. LAMBERT
P. BUAT-MENARD and R. CHESSELET
Centre des Faibles Radioactivités
Laboratoire Mixte C.N.R.S.-C.E.A.
BP 1, 91190 Gif-sur-Yvette (France)

Abstract

In the context of general study of particulate trace metal chemistry in Mediterranean waters, we present here data on the vertical distribution of suspended aluminosilicates and particulate Rare Earth Elements (R.E.E.) for four hydrographic stations in the western Mediterranean Sea (PHYCEMED I cruise).

It appears that in surface and intermediate waters, the concentrations of suspended aluminosilicates are about four times greater than values which we have observed in the North Atlantic Ocean : this reflects the higher level of the measured atmospheric input rate over the Mediterranean Sea. In bottom waters, most profiles show strong evidence of aluminosilicates resuspension, with a high degree of spatial and temporal variability.

Vertical concentration profiles of particulate R.E.E., normalized to aluminosilicates, show that these elements behave non-conservatively. These profiles enable us to identify some biochemical and/or physico-chemical processes which control the partitioning of these elements between the solid and the liquid phase : a) fast dissolution, in surface waters, of a significant fraction of the atmospheric input of R.E.E., b) for Ce, i) rapid scavenging of dissolved Ce in surface waters followed by slower and progressive dissolution in intermediate waters : ii) the effect of diagenetic processes at the water-sediment interface on the concentration of mobile Ce in resuspended sediments : iii) a further dissolution of Ce from the resuspended fraction is observed at Station GYW2.

As a wole, the behaviour of particulate rare earth elements appears potentially useful for assessing the fate of certain metallic pollutants in the Mediterranean Sea.

*
* *

Résumé

Dans le cadre de notre étude générale du cycle des métaux associés à la matière en suspension en Méditerranée, nous présentons ici un ensemble de résultats concernant la répartition verticale des aluminosilicates et des terres rares en suspension dans la colonne d'eau pour quatre stations de prélèvement en Méditerranée Occidentale (mission PHYCEMED 1).

Les concentrations d'aluminosilicates dans les eaux de surface et dans les eaux intermédaires sont en moyenne quatre fois plus élevées que ce que nous avons observé dans l'Atlantique Nord : ceci résulte d'apports atmosphériques plus intenses en Méditerranée. A l'approche du fond, presque tous les profils indiquent la présence d'une remise en suspension importante d'aluminosilicates. Ce phénomène se manifeste de façon très variable dans l'espace et dans le temps.

Comparé à celui des aluminosilicates, le caractère non-conservatif de la répartition verticale des terres rares en suspension permet de mettre en évidence une série de processus biochimiques et/ou physico-chimiques dans lesquels les échanges solide-liquide jouent un rôle prépondérant : a) dissolution rapide dans les eaux de surface d'une partie de l'apport atmosphérique, b) pour Ce, i) une adsorption rapide du Ce dissous sur la matière en suspension dans les eaux de surface suivie d'une dissolution plus lente et progressive du Ce particulaire dans les eaux intermédiaires : ii) la mise en évidence de l'origine diagénétique du Ce mobilisable dans le sédiment remis en suspension ; iii) une remise en solution supplémentaire du Ce à partir de la fraction remise en suspension est observée à la station GYW2.

La mise en évidence de ces processus souligne à quel point les terres rares sont utiles pour comprendre le comportement biogéochimique de certains polluants métalliques en Méditerranée.

*
* *

Introduction

Studies from our group in the Western Mediterranean Basin (CHESSELET et al., 1978 ; BUAT-MENARD et al., 1980 : ARNOLD et al., 1982) have stressed the potential importance of the atmospheric input as a source for particulate and dissolved trace metals and the variable influence of such an input on the trace metal chemistry of marine suspended matter. In this context, our current major objectives are :

i) to assess quantitatively the atmospheric input rate of metals from both natural and anthropogenic sources ;

ii) to understand the role of suspended matter in the cycling of trace elements throughout the water column ;

iii) to establish the importance of "early diagenetic" processes at the water-sediment interface in the remobilization of trace metals in the water column.

Our previous results (CHESSELET et al., 1978 ; BUAT-MENARD et al., 1980) have shown that many trace metals (and particularly heavy metals) are present in suspended matter from Mediterranean waters in concentrations at least an order of magnitude higher than can be accounted for by i) terrigenous particles of crustal composition (e.g. aluminosilicates) and ii) biogenic particles of planktonic composition. The enrichment of such elements probably results from active and passive scavenging by settling particles throughout the water column. In this paper, we attempt to get some insight into such processes by examining simultaneously the vertical distribution of "tracers" of the behaviour of particulate trace elements, both conservative (aluminosilicates) and non-conservative (rare earth elements). Aluminosilicates are the major abiogenic (terrigenous) component of marine suspended matter and act as scavengers of dissolved trace elements (LAMBERT, 1981). Rare Earth Elements (R.E.E.) have been chosen because of previous observations of fractionation of individual R.E.E. from one another in the marine environment (ELDERFIELD and GREAVES, 1982 ; DE BAAR et al., 1983). Their behaviour has been explained in terms of i) increasing ionic radius with atomic weight leading to a fractionation between light and heavy R.E.E. and ii) occurence of Ce (IV) and Eu (II) in addition to the trivalent oxidation state which generally characterizes the R.E.E. group. The study

of particulate R.E.E. in sea-water can therefore help to understand differences in the chemistry of some metallic pollutants such as heavy metals and transuranic elements (FUKAI et al., 1979 ; HOLM et al. 1980).

We report here data on the vertical distributions of suspended aluminosilicates (using scandium as an index) and some particulate R.E.E. (La, Ce, Sm, Eu, Tb, Yb, Lu) for four hydrographic stations (PHYCEMED 1 cruise ; see figure I). The observed distributions are discussed in order to evaluate the significance of atmospheric inputs, the role of biogeochemical processes in the water column and the effect of resuspension from sediments. To our knowledge, this is the first set of data on particulate R.E.E. in the Mediterranean Sea. Recent studies of dissolved R.E.E. in the oceanic water column have underlined that adsorption-desorption processes are the clue to the understanding of the pattern observed. ELDERFIELD and GREAVES (1982) have shown, in particular. that the concentrations of dissolved R.E.E. in the Mediterranean outflow are anomalous relative to waters above and below, higher for heavy R.E.E. and lower for light R.E.E. Our data set will be also used to provide evidence for some of the processes which could explain such an anomaly.

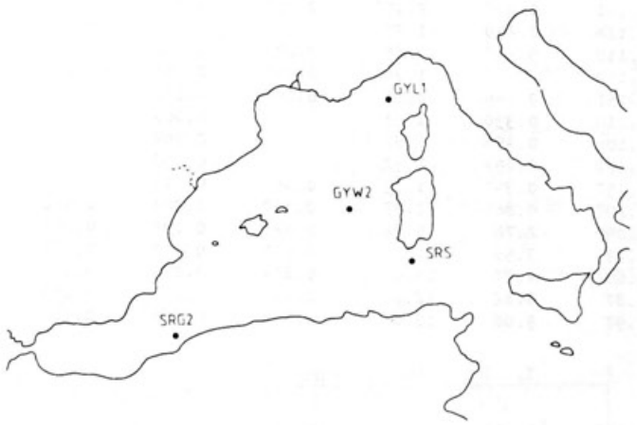


Figure I. - Location of the four hydrographic stations relevant to this work (PHYCEMED 1 cruise, April 1981) :

GYL1 : 43°06' N - 7°56' E	total depth : 2575 m
GYW2 : 40°37' N - 7°10' E	total depth : 2790 m
SRS : 38°30' N - 9°51' E	total depth : 1945 m
SRG2 : 36°17' N - 0°06' W	total depth : 2500 m

Sampling and analytical methods

Sampling and analytical methods have been extensively described elsewhere (BUAT-MENARD et al., 1980b; LAMBERT, 1981 : RISLER, 1984). Sea-water samples were collected with i) 10 l GO-FLO bottles in surface waters and ii) 30 l Niskin bottles, fitted with stainless-steel springs coated with Teflon, in deep waters. During the PHYCEMED 1 cruise, a new, grease-free hydrowire was used. Sea-water was filtered under pressure on acid-washed, pre-weighed, 0.4 um pore size, 47 mm diameter Nuclepore filters, using ultra-clean procedures (RISLER, 1984). The filters were analysed for elemental composition by Instrumental Neutron Activation Analysis. Following an irradiation of one hour under a thermal neutron flux of 1×10^{14} $n.cm^{-2}.s^{-1}$ (OSIRIS reactor, CEN Saclay) , the samples were counted on a 25 % efficiency Ge(Li) detector. The complete set of data as well as the discussion of analytical uncertainties can be found in RISLER (1984). The average precision (counting uncertainties plus the contribution from the procedural blank filter) is 3 % for Sc, 5 % for La and Ce, and 10 % for the other R.E.E.

DEPTH	TSM	Sc	La	Ce	Sm	Eu	Tb	Yb	Lu
m	$\mu g.l^{-1}$				$ng.l^{-1}$				
					GYL1				
10	259.0	0.269	0.800	3.73	0.141				
50	75.5	0.233	0.674	2.49	0.120	0.017			
100	26.5	0.156	0.403	1.45	0.065	0.012			
300	22.5	0.104	0.320	1.05	0.046	0.009		0.016	
500	32.0	0.140	0.384	1.74	0.062	0.001		0.030	
1000	18.3	0.103	0.282	1.34	0.049	0.008		0.012	
1150	19.7	0.101	0.317	1.22	0.049	0.011			
1500	22.7	0.115	0.397	1.46	0.059	0.008			
2000	29.0	0.234	0.664	2.39	0.122	0.019		0.040	
2200	50.3	0.253	0.912	3.37	0.184	0.029	0.048	0.066	
2500	61.0	0.374	1.04	3.82	0.142	0.030	0.034	0.073	
2560	289.0	3.03	7.92	23.5	1.24	0.225	0.125	0.543	0.080
2570	247.0	2.62	6.79	21.0	1.01	0.200	0.111	0.477	0.069
					GYW2				
10	157.3	0.390	0.750	4.99		0.039			
50	34.8	0.101	0.338	2.15	0.057	0.009			
120	32.3	0.114	0.430	1.93		0.011	0.008		
250	18.7	0.110	0.387	1.85	0.087	0.012	0.007		
400	16.7	0.159		1.94	0.045	0.015	0.002		
500	22.5	0.161	0.446	2.09	0.066	0.012		0.022	
800	16.0	0.110	0.330	1.36		0.009		0.015	
1200	13.0	0.106	0.458	1.87		0.009			
1650	12.1	0.092	0.254	1.32		0.008		0.008	
2200	12.8	0.157	0.347	1.73	0.068	0.013		0.020	
2600	26.5	0.297	0.864	2.97	0.080	0.025	0.018	0.028	
2690	81.6	1.09	2.78	8.98	0.445	0.095	0.055	0.189	
2740	106.3	1.39	3.59	11.5	0.575	0.117	0.088	0.229	
2770	122.1	1.60	4.07	13.6	0.674	0.128	0.114	0.278	0.044
2785	142.5	1.87	4.82	12.6	0.747	0.147	0.113	0.301	0.046
2789	155.4	1.97	5.08	16.8	0.838	0.168	0.109	0.359	0.048
					SRS				
10	42.7	0.082	0.261		0.041				
50	100.7	0.190	0.514	3.07	0.108	0.021			0.024
125	24.7	0.063	0.206	0.761	0.034	0.006		0.013	
250	29.0	0.109	0.359	1.73	0.057	0.010		0.025	
400	24.6	0.110	0.391	1.92	0.067	0.013	0.013	0.019	
500	24.1	0.127	0.353	1.70	0.063	0.011		0.027	
650	23.2	0.129	0.448	1.66	0.075	0.014		0.031	
900	12.6	0.100	0.327	1.16	0.057	0.009		0.020	0.003
1200	14.7	0.124	0.421	1.70	0.062	0.012		0.027	0.004
1600	18.5	0.192	0.611	2.20	0.102	0.018	0.015	0.040	
1845	20.7	0.197	0.644	2.19	0.104	0.019	0.014	0.037	0.006
1920	20.9	0.213	0.669	2.24	0.110	0.021	0.016	0.033	0.005
1925	20.9	0.243	0.742	2.37	0.077	0.023		0.038	
1940	21.6	0.205	0.631	2.18	0.098	0.018		0.029	0.005
1940	27.1	0.271	0.833	2.79	0.129		0.019	0.048	0.004
1944	23.0	0.248	0.735	2.66	0.116	0.022	0.017	0.048	0.007
					SRG2				
10	24.5	0.074	0.240	1.92	0.039	0.004			
20	29.0	0.098	0.247	1.60	0.033	0.008			
100	6.9	0.013	0.044	0.413	0.006				
150	27.3	0.101	0.317	1.76	0.052	0.009		0.033	
200	21.5	0.082	0.299	1.58	0.043	0.009		0.011	
250	26.1	0.125	0.574	2.59	0.060	0.011	0.008		
400	40.3	0.276	0.746	3.91	0.117	0.026	0.031	0.047	0.008
500	32.7	0.334	0.831	3.26	0.134	0.025	0.016	0.059	0.013
1000	24.1	0.235	0.623	2.00	0.089	0.023		0.028	
1600	25.4	0.142	0.389	1.55	0.061	0.011		0.016	
2000	16.1	0.161	0.409	1.34	0.068	0.014		0.031	0.006
2400	32.0	0.400	0.979	3.49	0.150	0.031	0.013	0.057	0.010
2480	33.1	0.384	0.930	3.29	0.140	0.029		0.057	0.012
2495	25.6	0.297	0.818	3.02	0.123	0.022	0.025	0.055	0.006
2499	31.0	0.326	0.769	3.03	0.133	0.024	0.015	0.057	0.009

No value : not determined

Table 1. - Total Suspended Matter (T.S.M.), Sc and R.E.E. concentrations.

Results and discussion

Results for individual samples are given in table 1. Total Suspended Matter (T.S.M.) concentrations range from 10 to 300 ug.l-1. The pattern of T.S.M. vertical distribution is similar to what has been observed previously in the Mediterranean Sea (COPIN-MONTEGUT, 1974 ; CHESSELET et al., 1978) as well as in the Atlantic Ocean (BISCAYE and ETTREIM, 1977 ; BREWER et al., 1976) : high concentrations in the top 100 m, reflecting biogenic sources ; lower concentrations in intermediate waters : increasing concentrations closer to the bottom due to resuspension from sediments. The influence of resuspension is variable from one station to another and has been shown to vary with time at station GYL1 (RISLER, 1984).

Behaviour of suspended aluminosilicates

Suspended aluminosilicate concentrations in ocean waters have been generally assessed through measurements of particulate aluminium (KRISHNASWAMI and SARIN, 1976: BUAT-MENARD and CHESSELET, 1979; LAMBERT et al., 1981). For technical reasons, such measurements have not yet been possible. Instead, we have used particulate Sc as an index of aluminosilicates. It has been shown by LAMBERT (1981) that the Al/Sc ratio in Atlantic particulates remains essentially unchanged with depth. For the Mediterranean Sea, we have verified previously for three stations (Nice-Calvi transect : BUAT-MENARD et al., 1980 ; RISLER, 1984) that there is no significant variation of the Al/Sc ratio with depth (mean value; 5×10^3). Although the Al/Sc ratio can vary by as much as 30 per cent from sample to sample, such a variability is the same as that observed for Mediterranean aerosols (ARNOLD et al., 1982; ARNOLD, 1984), pointing to the conclusion that no major scavenging of Sc occurs in the water column. We will therefore consider in the following discussion that particulate Sc is a good index of suspended aluminosilicate concentrations.

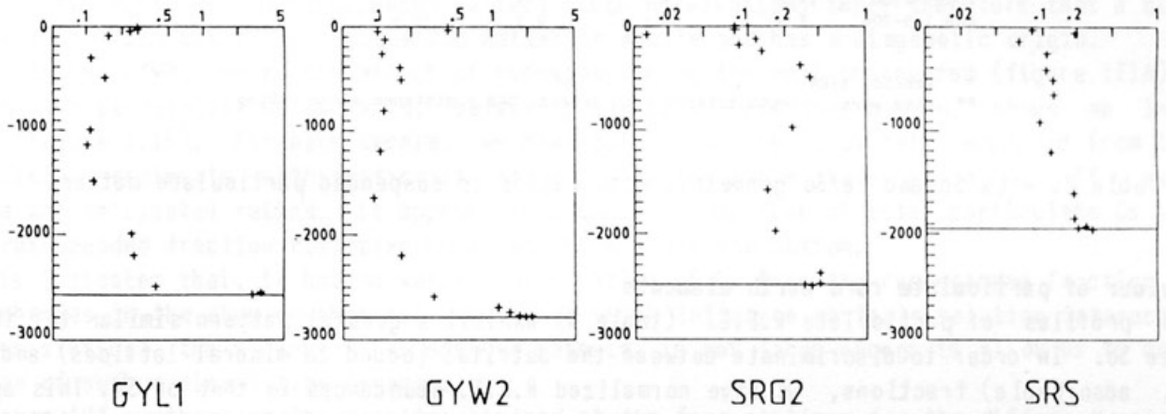


Figure II. - Vertical distributions of particulate Sc (ng.l-1). Depth is given in meters. Particulate Sc is used here as an index of aluminosilicates (see text).

The four profiles of particulate Sc are shown in figure II. The surface waters show an initial concentration of about 0.2 ng.l-1 corresponding to 10 ug.l-1 of aluminosilicates). The concentration then decreases in the first 100 m, reflecting the incorporation of aluminosilicates in large, fast-settling biogenic particles (WALLACE et al., 1981). On the average, particulate Sc concentrations in these Mediterranean surface waters are four times higher than in the North Atlantic (BUAT-MENARD and CHESSELET, 1979). This reflects most likely the higher level of the atmospheric input rate of aluminosilicates over the Western Mediterranean Sea (ARNOLD et al., 1982). Based on the model of BUAT-MENARD and CHESSELET (1979), we have calculated that the flux of aluminosilicates removed from surface Mediterranean waters can be explained, as is the case for the North Atlantic, by the input of aluminosilicate particles from the atmosphere.

Between 200 and 600 m depth, an increase in particulate Sc is observed at the four stations. The increase is most pronounced at SRG2 station. This general feature could be the result of either vertical or horizontal transport processes and, therefore, deserves further investigation.

At each station, in deep waters, particulate Sc starts to increase at a depth about 1000 m above the bottom. The increase with depth is more and more pronounced closer to the bottom. As shown for other oceanic regions (BISCAYE and EITREIM, 1977 ; LAMBERT, 1981) this is the result of resuspension from underlying sediments. The effect of resuspension on aluminosilicate concentrations is much more pronounced than for T.S.M. concentrations because of the mineralization of organic matter at the water-sediment interface (LAMBERT, 1981). At GYL1 and GYW2 stations, where resuspension is the highest, particulate Sc concentrations, 5 m above the bottom, are about 30 times higher than in the "clear water minimum", at 1000-1500 m depth.

La/Sc RATIOS

	GYL1	GYW2	SRG2	SRS
Atmosphere *	5 - 5.2	5 - 5.2	5 - 5.2	5 - 5.2
Ocean **				
10 - 50	2.9	2.5	2.9	3.0
100 - 700	2.8	3.3	3.4	3.3
800 -1600	3.1	3.3	2.7	3.3
2000 -2500	3.1	2.5	2.5	
DAB 1.5-100	2.6	2.6	2.5	2.8

Ce/Sc RATIOS

	GYL1	GYW2	SRG2	SRS
Atmosphere *	6.4- 8.5	6.4- 8.5	6.4- 8.5	6.4- 8.5
Ocean **				
10 - 50	12.2	17.7	21.2	16.1
100 - 700	10.5	14.6	17.5	14.2
800 -1600	12.6	14.6	9.1	12.2
2000 -2500	11.2	9.7	9.9	
DAB 1.5-100	7.9	8.5	9.3	10.5

* Arnold, 1984
** This work : depth intervals in meters; DAB = distance above bottom

Table 2. - La/Sc and Ce/Sc geometric mean ratios in suspended particulate matter.

Behaviour of particulate rare earth elements

Depth profiles of particulate R.E.E. (table 1) exhibit a general pattern similar to that of particulate Sc. In order to discriminate between the detrital (bound to mineral lattices) and mobile (biogenic, adsorbable) fractions, we have normalized R.E.E. abundances to that of Sc. This approach (RISLER, 1984) shows that the R.E.E. which we have measured behave similarly to La, with a major exception for Ce. In the following discussion, we will therefore examine only the depth distribution of La/Sc and Ce/Sc ratios.

La/Sc and Ce/Sc geometric mean ratios have been calculated for the different water compartments (table 2). It appears, first, that in surface waters, at the four stations, suspended matter is depleted in La by 40 to 50 per cent and enriched in Ce by a factor of 2 to 3, relative to Western Mediterranean aerosols. Since the source of particulate Sc in surface waters is most likely the atmospheric fall-out of aluminosilicates and since R.E.E. in Mediterranean aerosols are associated with such particles (ARNOLD, 1984), the observed depletion in particulate La in surface waters has its origin in a loss of La from atmospheric aluminosilicates. This could be due to fast dissolution processes occurring when aluminosilicate particles enter surface waters. Alternatively, if atmospheric fall-out occurs primarily by wet deposition (rain), La dissolution could take place in cloud and rain droplets, before reaching the sea-surface. As a consequence the difference between La/Sc ratios in aerosols and surface water particulates (table 2) could reflect the difference in composition between the dry and wet components of atmospheric particulate flux.

With respect to Ce, the explanation of the elevated Ce/Sc ratios in surface water particulates (table 2) is that dissolved Ce is readily adsorbed onto particles or incorporated into biogenic material (MURPHY and DYMOND, 1984). This implies a large source of dissolved Ce, possibly from rivers (MARTIN et al., 1976), from shelf injection (DE BAAR et al., 1983) or, as discussed for La, from dry and wet atmospheric deposition. It is not possible from our data to assess quantitatively the relative contribution of these various sources. In any event, scavenging of dissolved Ce in surface waters is considerably faster than it is for La and the other R.E.E. which we have measured (RISLER, 1984).

Deeper in the water column, we do not observe a significant trend in the La/Sc ratio (table 2). On the contrary, Ce/Sc ratio decreases with depth, especially at station SRG2. This trend is first observed between 100 and 700 m depth (table 2), far above the resuspension zone. This indicates that particulate Ce is significantly remobilized in intermediate waters (desorptive or regenerative processes), behaving similarly to nutrients (BRULAND, 1983).

In the resuspension zone, a simple explanation for low Ce/Sc ratio, which we observe in suspended matter (table 2), would be that such a low ratio reflects the mixing of downward settling particles (rich in Ce) with resuspended sediments deprived of mobile Ce (e.g. aluminosilicates of crustal composition). Indeed, the stations where resuspension is the highest (GYL1, GYW2) exhibit the lowest Ce/Sc ratio (table 2). However, this explanation is probably incorrect. It has been shown by BOUST et al. (1984) that a significant fraction of R.E.E. in surficial sediments is mobile. This mobile fraction is associated with Fe-Mn oxide coatings onto aluminosilicates, and is the result of early diagenetic processes involving precipitation of dissolved metals from pore waters For Mediterranean surficial sediments, we have shown that R.E.E./Sc ratios covary with the concentration of mobile iron, and reflect the variable redox conditions within the sediments (LAMBERT et al., 1984). Such a covariation is also observed in our samples of suspended matter collected close to the bottom (RISLER, 1984). Amongst the R.E.E. which we have measured, Ce shows the highest enrichment, both in surficial sediments and in resuspended matter. Such observations imply therefore that a substantial fraction of particulate Ce in resuspended matter is mobile and has a diagenetic origin.

At station GYW2, where the effect of resuspension is the most pronounced (figure IIIA), a close look at the particulate Ce/Sc ratio, between 5 and 200m above the bottom, shows an interesting feature (figure IIIb). For each sample, we have calculated the Ce/Sc ratio expected from the mixing of surficial sediments with particulate matter collected 200 m above the bottom. If we compare measured and calculated ratios, it appears that about 10 per cent of total particulate Ce is missing in the resuspended fraction collected 50 m and 100 m above the bottom.

This indicates that, in bottom waters dissolution of Ce from the resuspended fraction may occur due to changes in the physico-chemical conditions which influence particle-solution interactions. At the other stations. the amount of resuspended material is not large enough to allow us to confirm the existence of such a dissolution process.

Figure IVA synthesizes the results obtained at the four stations for the different water compartments and illustrates the similar depth-dependent behaviour of particulate rare earth elements. The most striking feature is the pronounced positive Ce anomaly. Interestingly, such a positive anomaly has not been observed in sediment trap samples from the Eastern Equatorial Pacific (MURPHY and DYMOND, 1984). This might indicate that different processes control particulate Ce levels in small particles and in large particles. The latter are not quantitatively collected by our sampling procedure. The analysis of R.E.E. on large particles collected more recently in Mediterranean waters (PHYCEMED 2 cruise) is currently under way and should allow testing of this hypothesis.

With respect to dissolved R.E.E., a strong negative Ce anomaly has been generally observed, particularly in the Mediterranean outflow in the Atlantic (ELDERFIELD and GREAVES, 1982 : see figure IVB). Our results support the hypothesis made by these authors that Ce behaves differently from the other R.E.E., because of preferential scavenging of Ce^{4+} onto particles. Clearly, much more work is needed to quantitatively assess the role of the processes observed here in controlling the internal cycle of R.E.E. (and metallic pollutants with similar chemistries) in the Mediterranean Sea.

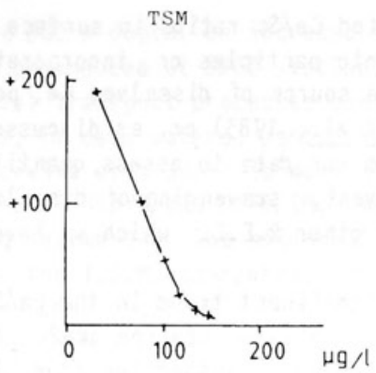


Figure 3A

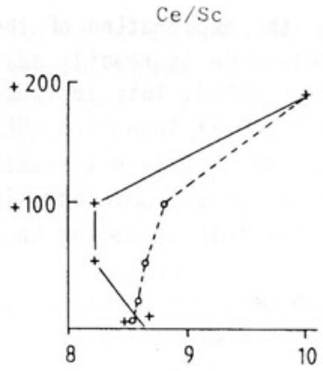


Figure 3B

Figure IIIA. - Total suspended matter concentration at station GYW2 versus distance above the bottom (in meters).

Figure IIIB. - Measured Ce/Sc ratio (+) ; Ce/Sc ratio (o) expected from the mixing of surficial sediments with suspended matter collected 200 m above the bottom.

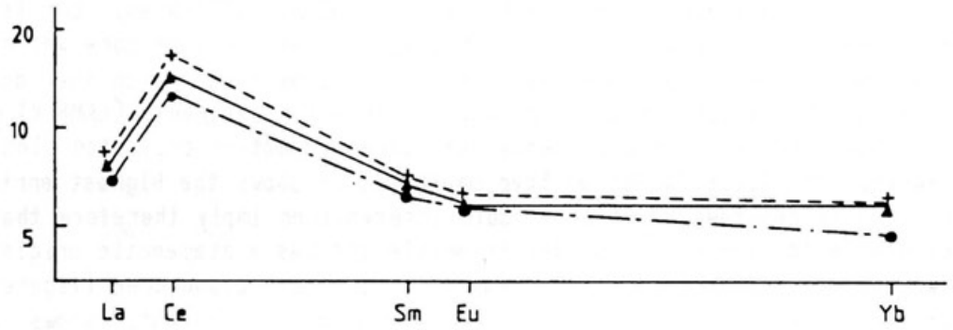


Figure IVA. - Shale-normalized R.E.E. pattern of suspended particulate matter, averaged for the four stations. R.E.E. concentrations have been normalized to Sc in order to eliminate the dilution by biogenic material. (+), surface waters : (▲), intermediate waters : (●) deep waters.

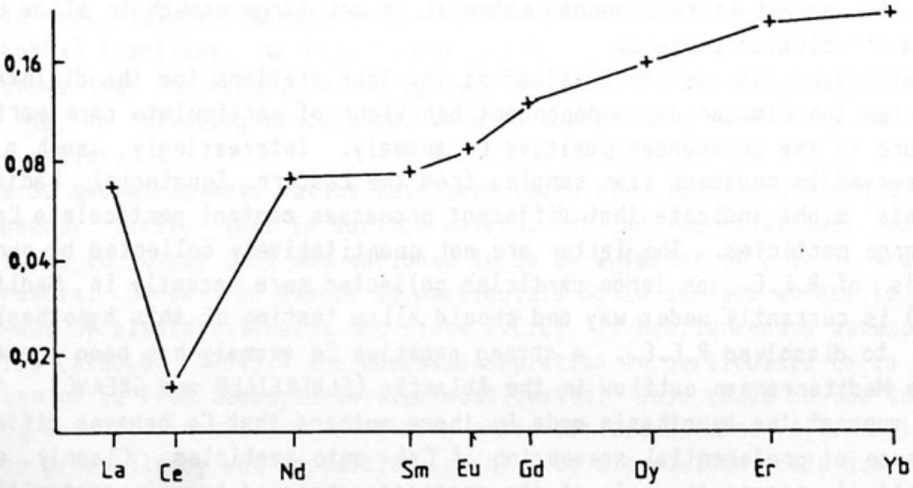


Figure IVB. - Shale-normalized R.E.E. pattern of Mediterranean outflow sea-water (adapted from ELDERFIELD and GREAVES, 1982).

Acknowledgements

We thank M. ARNOLD who collected the samples and the officers and crew of R.V Le Suroit for logistic support during the PHYCEMED 1 cruise. We are grateful to Pierre Sue Laboratory at Saclay, where we performed the neutron activation analyses. We acknowledge funding from C.N.E.X.O. (grants n° 80/2274 and 81/2415).

References

ARNOLD, M. (1984). - Géochimie et transport des aérosols métalliques au-dessus de la Méditerranée occidentale. Thèse de Doctorat ès Sciences, Université de Paris 7.

ARNOLD, M., A. SEGHAIER, D. MARTIN, P. BUAT-MENARD et R. CHESSELET (1982). - Géochimie de l'aérosol marin au-dessus de la Méditerranée Occidentale. VIth Workshop on Marine Pollution of the Mediterranean, Cannes, I.C.S.E.M./I.O.C./U.N.E.P.

BISCAYE, P.E. and S.L. ETTREIM (1977). - Suspended particulate loads and transport in the nepheloid layer of the abyssal Atlantic Ocean. Mar. Geol. 23, 155-172.

BOUST, D., J.L. JORON et P. PRIVE (1984). - Extraction séquentielle des terres rares dans les sédiments abyssaux : authigènèse et héritage. 10e Réunion Annuelle des Sciences de la Terre, Bordeaux, Soc. Géol. France Edit., Paris, p. 87.

BREWER, P.G., D.W. SPENCER, P.E. BISCAYE, A. HANLEY, P.L. SACHS, C.L. SMITH, S. KADAR and J. FREDERICKS (1976). - The distribution of particulate matter in the Atlantic Ocean. Earth Planet. Sci. Lett. 32, 393-402.

BRULAND, K.W. (1983). - Trace elements in sea-water. In Chemical Oceanography, 8, J.P. Riley and R. Chester eds, Academic Press, pp. 157-220.

BUAT-MENARD, P. and R. CHESSELET (1979). - Variable influence of atmospheric flux on the trace metal chemistry of oceanic suspended matter. Earth Planet. Sci. Lett. 42 399-411.

BUAT-MENARD, P., M. ARNOLD, D. BARON, H. BENARD, R. CHESSELET (1980 a). - Particulate trace metal chemistry in the open ocean and the Western Mediterranean : a comparative study. Vth Workshop on Marine Pollution of the Mediterranean, Cagliari, I.C.S.E.M./I.O.C./U.N.E.P., pp. 1005-1010.

BUAT-MENARD, P. C.E. LAMBERT, M. ARNOLD, R. CHESSELET (1980 b). - Multi-element neutron activation analysis : towards the geochemistry of particulate matter exchange between continent-atmosphere-ocean. J. Radioanal. Chem. 55, 445-452.

CHESSELET, R., D. BARON, H. BENARD, P. BUAT-MENARD (1978). - La chimie des métaux lourds dans l'aérosol de la Méditerranée occidentale et son influence sur la chimie des particules en suspension. IVth Workshop on Marine Pollution of the Mediterranean, Antalya, I.C.S.E.M./I.O.C./-U.N.E.P., pp. 81-90.

COPIN-MONTEGUT, C. (1974). - Contribution à l'étude des particules en suspension dans les eaux de mer. Thèse de Doctorat ès Sciences, Université de Paris 6, 147 p.

DE BAAR, H.J.W., M.P. BACON and P.G. BREWER (1983). - Rare-earth distribution with a positive Ce anomaly in the Western North Atlantic Ocean, Nature (Lond.), 301, 324-327.

ELDERFIELD, H. and M.J. GREAVES (1982). - The rare earth elements in sea-water, Nature (lond.), 296, 214-219.

FUKAI, R., E. HOLM and S. BALLESTRA (1979). - A note on vertical distribution of plutonium and americium in the Mediterranean sea, Oceanol. Acta 2 129-132.

HOLM, E., S. BALLESTRA, R. FUKAI and T.H. BEASLEY (1980). - Particulate plutonium and americium in Mediterranean surface waters, Oceanol. Acta, 3, 157-160.

KRISHNASWAMI S. and M.M. SARIN (1976). - Atlantic surface particulates : composition, settling rates and dissolution in the deep-sea, Earth Planet. Sci. Lett. 32, 430-448.

LAMBERT C.E. (1981). - Le cycle interne du fer et du manganèse et leurs interactions avec la matière organique dans l'océan. Thèse, Université de Picardie, France, 235 p.

LAMBERT C.E., C. JEHANNO, N. SILVERBERG, J.C. BRUN-COTTAN and R. CHESSELET (1981). - Log-normal distributions of suspended particles in the open ocean, J. Mar. Res. 39 77-98.

LAMBERT, C.E., N. RISLER, F DEHAIRS, Y. YOKOYAMA, H.V. NGUYEN and R. CHESSELET (1984). - Mise en place du sédiment récent dans deux zones pericontinentales. 10e Réunion Annuelle des Sciences de la Terre, Bordeaux, Soc. Géol. de France Ed., Paris, p. 331.

MARTIN, J.M., O. HOGDAHL and J.C. PHILIPPOT (1976). - Rare earth element supply to the ocean, J. Geophys. Res. 81, 3119-3124.

MURPHY, K. and J. DYMOND (1984). - Rare earth elements fluxes and geochemical budget in the eastern equatorial Pacific, Nature (lond.), 307, 444-447.

RISLER, N. (1984). - Les aluminosilicates et les terres rares dans les particules marines de la Méditerranée occidentale. Thèse de Spécialité, Université de Paris 7.

WALLACE, J.T. Jr, D.M. MAHONEY, R. DULMAGE, F. SORTI and N. DUDEK (1981). - First-order removal of particulate aluminium in oceanic surface layers, Nature (lond.), 293, 729-731.

SURFACE CURRENTS IN THE N.W AEGEAN SEA (GREECE) AS SHOWN FROM THE MOVEMENT OF DRIFTCARDS

by

E. TH. BALOPOULOS* and A.E. JAMES**
* Institute of Oceanographic and Fisheries Research
GR-166 04, Hellinikon (Greece)
** Department of Oceanography
University College, Singleton Park
Swansea SA2 8PP, S. Wales (U.K.)

Abstract

For the last thirty years, the Thermaikos Gulf, in the NW Aegean Sea, has been subjected to pollution by domestic waste and industrial effluents. To study the coastal water transport in the area, plastic driftcards were released at four different times, within a year, from 20 stations. A total of 4,000 driftcards was released and an average return rate of about 21 per cent was obtained. The results indicate that the surface water transport is dependent upon the prevailing wind conditions and the variable flow rate of large rivers discharging into the area. A relationship is established between the wind speed and the surface current velocity transporting the floating pollutants.

*
* *

Introduction

Although surface currents are known to be of great importance to the problem of coastal transport of pollutants, very little information is available on the surface circulation patterns of the Aegean Sea (NIELSEN, 1912 ; CARTER, 1956 ; SULTAN, 1981). This is particularly true of the Thermaikos Gulf, in the NW Aegean Sea, which is virtually unexplored (BALOPOULOS, 1977 ; KOUTITAS and O'CONNOR, 1980 ; GANOULIS and KOUTITAS, 1981 ; ROBLES et al., 1983), though it has been contaminated, within recent years, by a variety of pollutants, such as human sewage, industrial effluents, pesticides and organic matter (FRILIGOS, 1977 ; FRILIGOS and SATSMADJIS, 1977 ; VOUTSINOU and SATSMADJIS, 1983 ; CHESTER and VOUTSINOU, 1981).

An investigation of surface currents by driftcards has been carried out in the Thermaikos Gulf during the period 1976-1977, as part of the I.O.C./U.N.E.P. Pilot Project on the coastal transport of Pollutants (MED-VI). The objective of this paper is to provide information on the surface coastal currents in the NW Aegean Sea and an understanding of their seasonal variability, in relation to wind conditions.

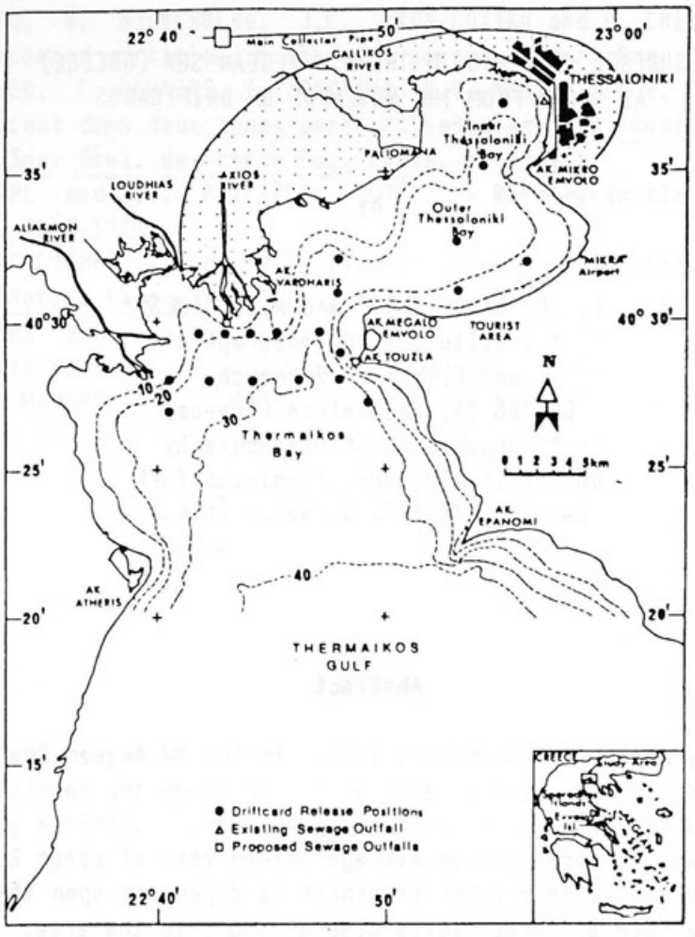


Figure I. - The study area, showing the tourist zone, the driftcard release positions, and the locations of the existing and proposed sewage outfalls. The most heavily industrialized coastal regions are indicated by the stippling .

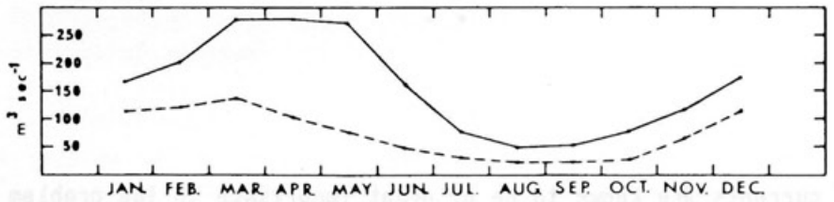


Figure II. - Mean monthly discharges (m3 s-1) from the rivers Axios (-----) (Years : 1926/27-30/31, 37/38-39/40, 41/42, 55/56-61/62, 64/65) and Aliakmon (- - -) (Years : 1962/63-70/71). Abstracted from Therianos (1974).

Area under investigation

The study area lies in the NW Aegean Sea and comprises three interconnecting shallow water embayments, with a total surface area of 320 km2 (figure I). It forms the inner part of a semi-enclosed, elongated continental shelf, which terminates in rather abrupt submarine escarpment, approximately 100 km to seaward (CONISPOLIATIS, 1979). Further seaward are situated the Sporadhes Islands and the Island Evvoia. The city of Thessaloniki, with a population of more than 1,200,000 inhabitants and its industrial zone are located to the north of the embayment.

River runoff is significant since four main rivers discharge into the area (Axios, Aliakmon, Loudhias, Gallikos). Of these, the rivers Axios (catchment area 22,450 km2) and Aliakmon (catchment area 6,075 km2), with mean annual discharges of respectively, 157 m3s-1 and 73 m3s-1 (THERIANOS, 1974), are the most important with regard to water supply. The flow rate of the River Axios in particular, which may reach 1000m3s-1 (according to the Greek Ministry for Public Works), plays a significant role in the formulation of the circulation patterns in the embayment (BALOPOULOS, 1982).

The annual variations, in the mean monthly discharge of the rivers Axios and Aliakmon are gradual and similarly distributed throughout the year, for both rivers (figure II). The amount of fresh water supplied from the rivers Loudhias and Gallikos is considerably lower and, although there is no information on the annual variations of their discharges, it is believed that they follow the trend of the rivers Axios and Aliakmon.

Rainfall is of the order of 48 cm yr-1 (ANGOURIDAKIS and MACHAIRAS, 1973). It is variable and frequently severe, primarily occurring between October and January and, also, between March and June. The land conditions are such that storm water for the most part runs directly and rapidly to the sea.

The northerly component winds have the highest frequency of occurrence during every month of the year (LIVADAS and SAHSAMANOGLOU, 1973). A special case is the local "Vardharis" which blows from the north-north-west quite often with a frequency equally distributed throughout the year. In summer (July-September), strong (10 m s-1) winds blowing from this quarter are the characteristic "Etesian winds" of the Greek and East Mediterranean region. These winds are common and, when they develop, they exhibit a diurnal character, blowing strongly during the day and abating at night. When the Etesian winds are not present, smaller scale "sea breezes" of 5 to 10 m s-1 generated by differential heating between land and sea develop almost every day : in the Thermaikos Gulf, they are often from the south and south-east.

The tidal range (30 cm at mean spring tides falling to 5 cm at mean neap tides) is very small (WILDING et al., 1980).

Methods

Driftcards similar to those described by OLSON (1951), but with modifications, were selected as Lagrangian indicators of surface currents. This method was feasible in terms of equipment expense and has been used by investigators in other regions of the Mediterranean Sea (PASQUAY, 1976 ; ILIC et al., 1978 ; HAVARD 1980). Each driftcard consisted of a yellow card 300 x 330 mm in size, folded twice to reduce its size to 150 x 165 mm and hermetically sealed with a mail envelope in a polyethylene transparent sheet of 0.1-0.2 mm thickness. On the card were printed a map of the study area, instructions to the finder and an identification number for the designation of the locality and time of release.

Four driftcard experiments, under the code name THEDRIFTEX-1, 2, 3 ,4 were carried out in the Thermaikos Gulf from December, 1976 to September, 1977. In these experiments, releases of driftcards were made at twenty stations and, in general, fifty driftcards were launched separately, but simultaneously at each station. Three-hourly wind data, for comparison with the driftcard movements, were collected at the Mikra airport and provised by the Greek Meteorological Service.

Results and discussion

a) Return rates

Of a total of 4,000 driftcards released, 850 (21.3 per cent) had been returned by April, 1978 (table 1). Driftcards were found only after coming ashore on beaches, although a small percentage of recovery (less than 2 per cent) was made at sea by fishing vessels and boats. About 3 percent of the

retrieved driftcards were reported with no information either about the area in which they were recovered, or about the time they were found, or both. In assessing the seasonal variation in the return rate of the driftcards, it was found that higher return rates were obtained in July and September. From this, it would seem that the return rate was much controlled by the number of people visiting the coasts, since this would clearly reach a maximum in summer, as by any other natural factors.

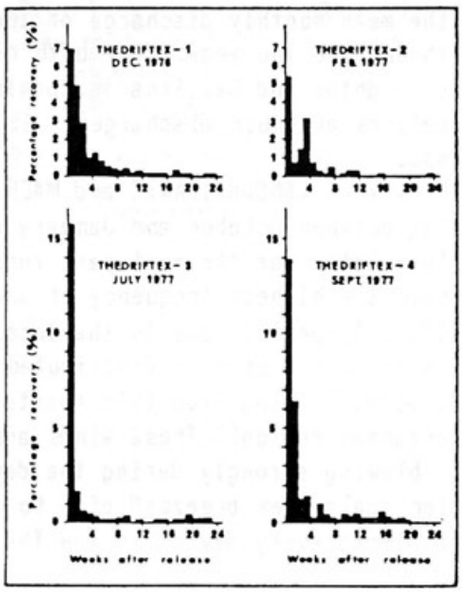


Figure III. - Recovery rates of driftcards.

b) Recovery frequency

The maximum rate of recovery in each experiment was obtained during the first week following the launching of the driftcards (figure III). In general, the recovery rate then started to decrease during the second week and even more during the rest of the first month, after which a very limited number of recoveries was recorded. The late recoveries were due to either a long period of lying on a beach before detection, or to really travelling long distances. The latter is especially true for the experiment THEDRIFTEX-2, for which the recovery rates during the third and fourth weeks were higher than that during the second week.

Table 1
Driftcards release and recovery information

Experiment	N°. of Driftcards released	N°. of Driftcards recovered	Percentage recovery
THEDRIFTEX-1 (Dec. 1976)	1000	209	20.9
THEDRIFTEX-2 (Feb. 1977)	1000	141	14.1
THEDRIFTEX-3 (July 1977)	1000	221	22.1
THEDRIFTEX-4 (Sept. 1977)	1000	279	27.9

c) Estimation of the direction and rate of transport

The interpretation of the movements of driftcards is invariably open to question. Only the position and the times of the release and recovery of the driftcards are known. The path and the time they lay on the beach before being sighted are unknown.

The beach of the study area is frequented by the public, especially in summer, when it is used for swimming and sporting activities. It may be considered, therefore, that the effect of the residence time of the driftcards on the beach is of little consequence, particularly in the experiments of July and September, when most recoveries were made within only one or two days of launching. Likewise, the general path (or paths) followed by each group of driftcards, in each experiment, may be satisfactorily deduced, since a relatively large number of driftcards originating from the same

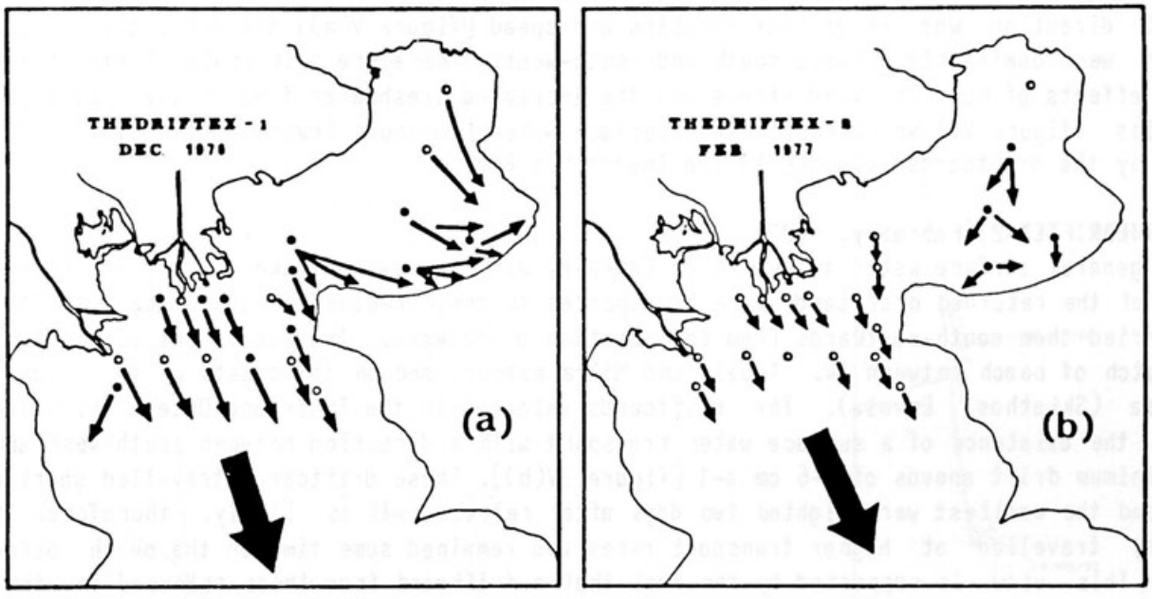


Figure IV. - General movements of driftcards for the experiments (a) THEDRIFTEX-1 and (b) THEDRIFTEX-2. o = stations from which at least ten driftcards were recovered within six months of release, • = other stations.

release position or from adjacent launching stations, was, in most cases, found on the same coastal area. These cases were assumed to be a group of landings and only the first detected driftcard was taken into consideration in determining the drift time and, consequently, the minimum drift speed. When an individual landing indicated a movement quite contrary to the general movement shown by the other driftcards, released from the same position during the same experiment, it was ignored.

A general description of the results obtained from the estimation of the direction and rate of transport is given below individually for each measuring session.

i) THEDRIFTEX-1 (December, 1976)

The driftcards released in the Inner and Outer Thessaloniki Bay travelled short distances (4-10 km) and the presumed routes they followed indicated the existence of a surface water transport towards the south-east [figure IV(a)] at minimum drift speeds of 5-10 cm s^{-1}, despite the daily residual wind (less than 1 m s^{-1}), which, at the begining of the survey, was to the north-west [figure V(a)]. This driftcard movement into the wind could be attributed to the strong northerly and north-westerly winds of about 7-15 m s^{-1} that prevailed over the study area during the three days preceding the driftcard releases and resulted in the formulation of a dominant south-eastward surface water flow pattern.

The driftcards dropped in the Thermaikos Bay travelled very long distances. Most of these returns were found on the west coast of the Thermaikos Gulf extending to the south of Ak. Atheris, and on the east coast of the Island Evvoia (figure I). Several driftcards were also recovered on the coasts of the Sporadhes Islands (Skiathos, Skopelos, Alonnisos). Generally, the inferred trajectories for the driftcard releases in the Thermaikos Bay suggested the existence of a surface water transport towards the south-east [figure IV(a)] at minimum drift speeds of 15-20 cm s-1, though four recoveries for the driftcards launched in the river discharge area indicated even higher transport rates, up to 35 cm s-1.

The existence of such a pattern of transport could be explained as being due to the wind action on the sea surface, during the time the driftcards were in the water. The wind had two prevailing directions. The main one from the north-west with speeds of 2-5 m s-1, occasionally reaching 14 m s-1 and the secondary one from the south-east with speeds of around 1-4 m s-1. However, the wind from the north-west direction was of greater duration and speed [figure V(a)] and hence the residual wind directions were dominantly between south and south-west. Therefore, it could be expected that the combined effects of both the wind stress and the increased freshwater flow of the discharge of the River Axios (figure VI) would result in a surface water transport towards the south, such as that indicated by the driftcards released in the Thermaikos Bay.

ii) THEDRIFTEX-2 (February, 1977)

The general surface water transport in February was broadly the same as that of December. The majority of the returned driftcards were transported to their recovery location by a surface drift, which carried them south-eastwards from the location of release. The recoveries were all made on a short stretch of beach between Ak. Touzla and Mikra airport and on the coasts of two islands of the Aegean Sea (Skiathos, Evvoia). The driftcards released in the Inner and Outer Thessaloniki Bay indicated the existence of a surface water transport with a direction between south-west and south-east at minimum drift speeds of 2-6 cm s-1 [figure IV(b)]. These driftcards travelled short distances (2-6 km) and the earliest were sighted two days after release. It is likely, therefore, that, in fact, they travelled at higher transport rates and remained some time on the beach before being detected. This view is supported by the fact that a driftcard from those released in the passage between Ak. Vardharis and Ak. Meg. Emvolon was recovered four hours after launching, suggesting a surface water transport of 23 cm s-1.

The driftcards released in the Thermaikos Bay travelled very long distances (150-300 km) to the south-east [figure IV(b)]; their inferred trajectories indicated the existence of a surface water transport at a drift speed of 15-18 cm s-1. Nevertheless, four recoveries from the vicinity of the River Axios showed even higher transport rates, in the order of 23-26 cm s-1.

The wind conditions during the experiment were characterized by a high percentage of occurrence of north-westerly component winds (which speeds of 2-4 m s-1, occasionally reaching 14 m s-1), which caused the residual wind directions to be consistently towards the south-east [figure V(b)]. The wind blowing from these directions and the additive effect of the increased flow of the fresh water discharged by the River Axios (figure VI) would result in the pattern of surface water transport indicated by the returned driftcards.

iii) THEDRIFTEX-3 (July, 1977)

During July , not one driftcard was returned from the islands of the Aegena Sea, where a significant number of them had been reported during the preceding experiments of February and December. In general, the driftcards were transported short distances and most recoveries were made on the coasts of the Inner and Outer Thassaloniki Bay and also on the western beach of the Thermaikos Bay.

The most striking feature of the surface water drift patterns was the existence of two distinct regions with opposite surface water transport characteristics. The driftcards released in the north-eastern part of the Thermaikos Bay initially moved to the north, drifted through the passage Ak. Vardharis and Ak. Meg. Emvolon, then travelled towards the north-east and, finally, were stranded in scattered places on the beaches in the Inner and Outer Thessaloniki Bay [figure VII(a)]. Early

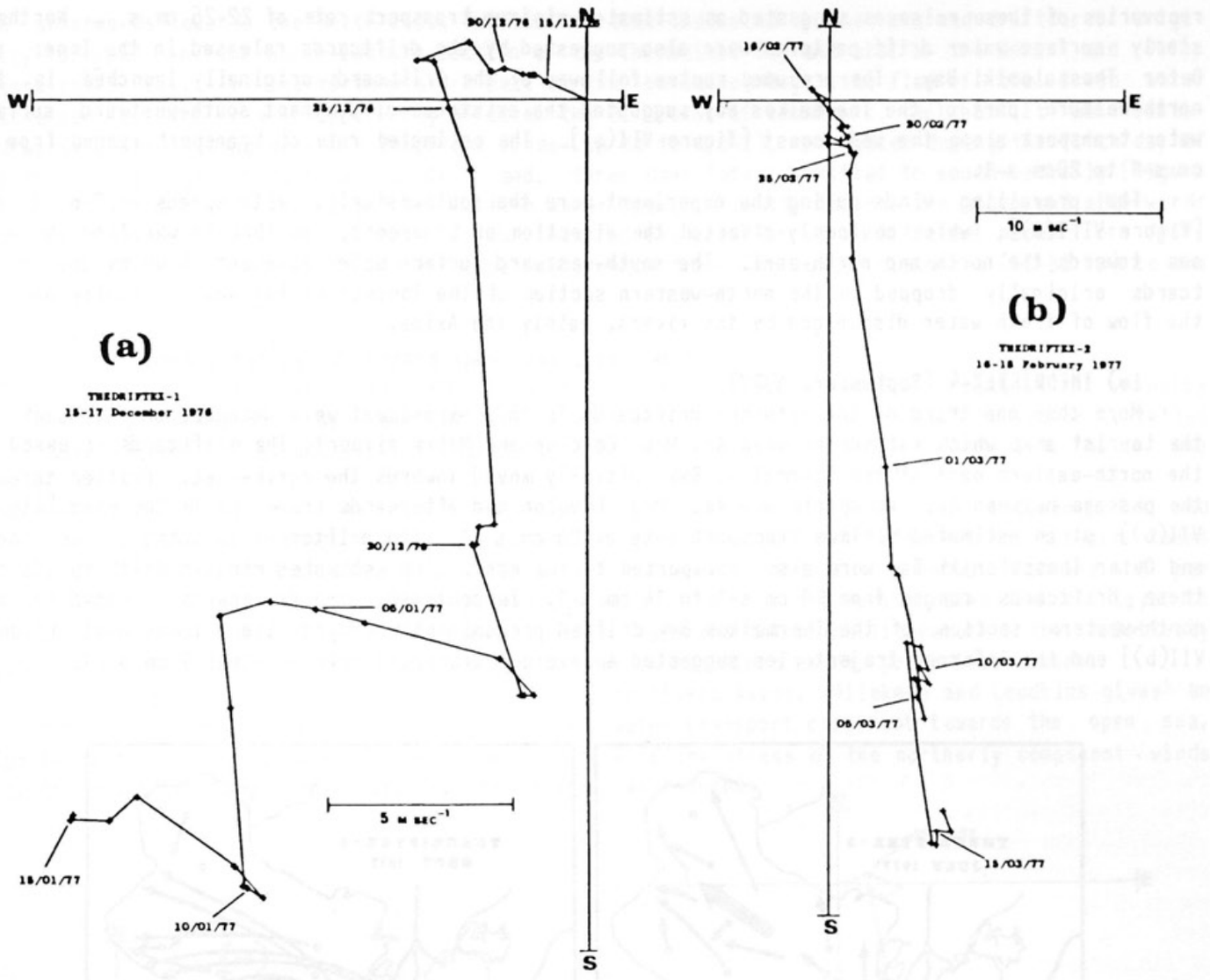


Figure V. - Wind progressive vector diagram (an indicator reference mark has been placed at 24:00 hours on each of the days) for the experiment (a) THEDRIFTEX-1 and (b) THEDRIFTEX-2.

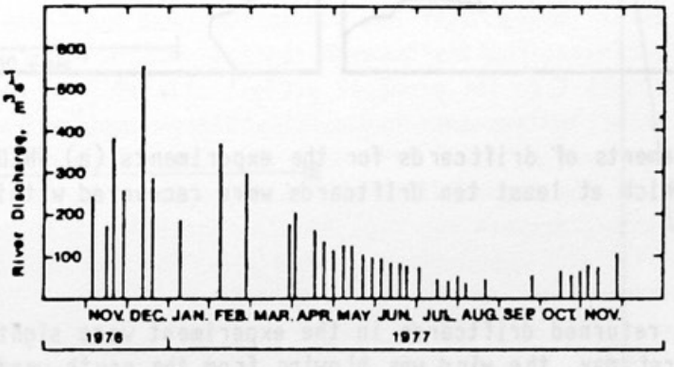


Figure VI. - Discharges (m3 s-1) from the River Axios, for the period of the driftcard experiments. Data obtained from the Greek Ministry for Public Works.

recoveries of these releases suggested an estimated minimum transport rate of 22-26 cm s^{-1}. Northeasterly surface water drift patterns were also suggested by the driftcards released in the Inner and Outer Thessaloniki Bay. The presumed routes followed by the driftcards originally launched in the north-western part of the Thermaikos Bay suggested the existence of dominant south-westward surface water transport along the west coast [figure VII(a)]. The estimated rate of transport ranged from 16 cm s^{-1} to 20cm s^{-1}.

The prevailing winds during the experiment were the southwesterly, with speeds of 2-6 m s^{-1} [figure VIII(a)], which obviously affected the direction of transport, so that it was from the open sea towards the north and north-east. The south-westward surface water movement shown by the driftcards originally dropped in the north-western section of the Thermaikos Bay was apparently due to the flow of fresh water discharged by the rivers, mainly the Axios.

iv) THEDRIFTEX-4 (September, 1977)

More than one third of the returned driftcards in this experiment were detected on the beach of the tourist area which extends between Ak. Meg. Emvolon and Mikra airport. The driftcards released in the north-eastern part of the Thermaikos Bay initially moved towards the north-east, drifted through the passage between Ak. Vardharis and Ak. Meg. Emvolon and afterwards travelled to the east [figure VII(b)] at an estimated minimum transport rate of 20 cm s^{-1}. The driftcards launched in the Inner and Outer Thessaloniki Bay were also transported to the east. The estimated minimum drift speeds for these driftcards ranged from 11 cm s^{-1} to 14 cm s^{-1}. In contrast, the driftcards released in the north-western section of the Thermaikos Bay drifted predominantly towards the south-west [figure VII(b)] and the inferred trajectories suggested an average transport rate of about 9 cm s^{-1}.

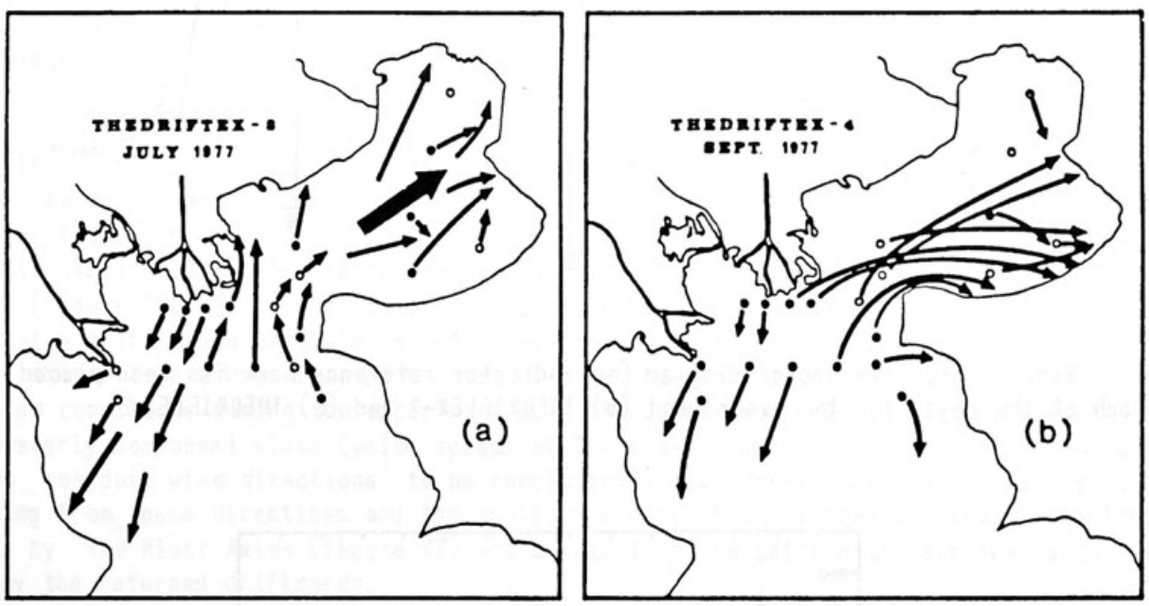


Figure VII. - General movements of driftcards for the experiments (a) THEDRIFTEX-3 and (b) THEDRIFTEX-4. o = stations from which at least ten driftcards were recovered within six months of release, • = other stations.

The majority of the returned driftcards in the experiment were sighted within only one or two days of release. On the first day, the wind was blowing from the south-west with speeds of 2-5 m s^{-1}, while on the second day it became north-westerly with speeds of 2-3 m s^{-1} [figure VIII(b)]. Wind stress from these directions would result in a surface water flow initially towards the north-east

and afterwards towards the south-east, similar to that indicated by the returned driftcards originally dropped in the north-eastern section of the Thermaikos Bay and also in the Inner and Outer Thessaloniki Bay. In the north-western part of the Thermaikos Bay, the flow of the fresh water discharged by the rivers apparently affected the direction of transport, so that it was southwestwards. The strong northely component winds (up to 10 m s-1), prevailed during the fifth day of the experiment, gradually became weaker, and, three days later, shifted to south-easterly [figure VIII(b)], resulting in a dominant sea surface water flow, initially towards the south and afterwards towards the west. This was reflected in the inferred trajectories of a sufficient number of driftcards (originally dropped in the Thermaikos Bay), which travelled longer distances (40-80 km) to the south-west.

d) Relationship between driftcard speed and wind speed

The theory of drift currents (EKMAN, 1905) predicts a linear relationship between the velocity of the wind above the sea, Vw, and the surface current, Vc, that is Vc = K Vw. The factor K, called the "wind factor" represents a percentage rate, Vc is given in cm s -1 and Vw in m S-1, as is the custom (HARVEY, 1968 ; TOMCZAK, 1964).

Average values of K in the various experiments in the Thermaikos Gulf ranged between 6.3 % and 9.2 per cent (table 2). TOMCZAK (1964), summarizing the determination of K by several authors. found that, for a thin surface layer of some 2 mm depth, K had been found to lie between 3.3 and 4.3 per cent. Also , GERGES (1980) estimated the average value of K for the Mediterranean Sea off Egypt to be 3.6 per cent. Compared with these values of K, referred to in the literature, the relatively higher values of K determined in our computations may be explained as due to various causes.

Thus, the flow of fresh water discharged by the rivers Axios, Aliakmon and Loudhias gives an additive, sometimes particularly strong, surface water transport component towards the open sea, superimposed on the surface transport component due to the stress of the northerly component winds and, therefore increases the values of K for a given wind spedd.

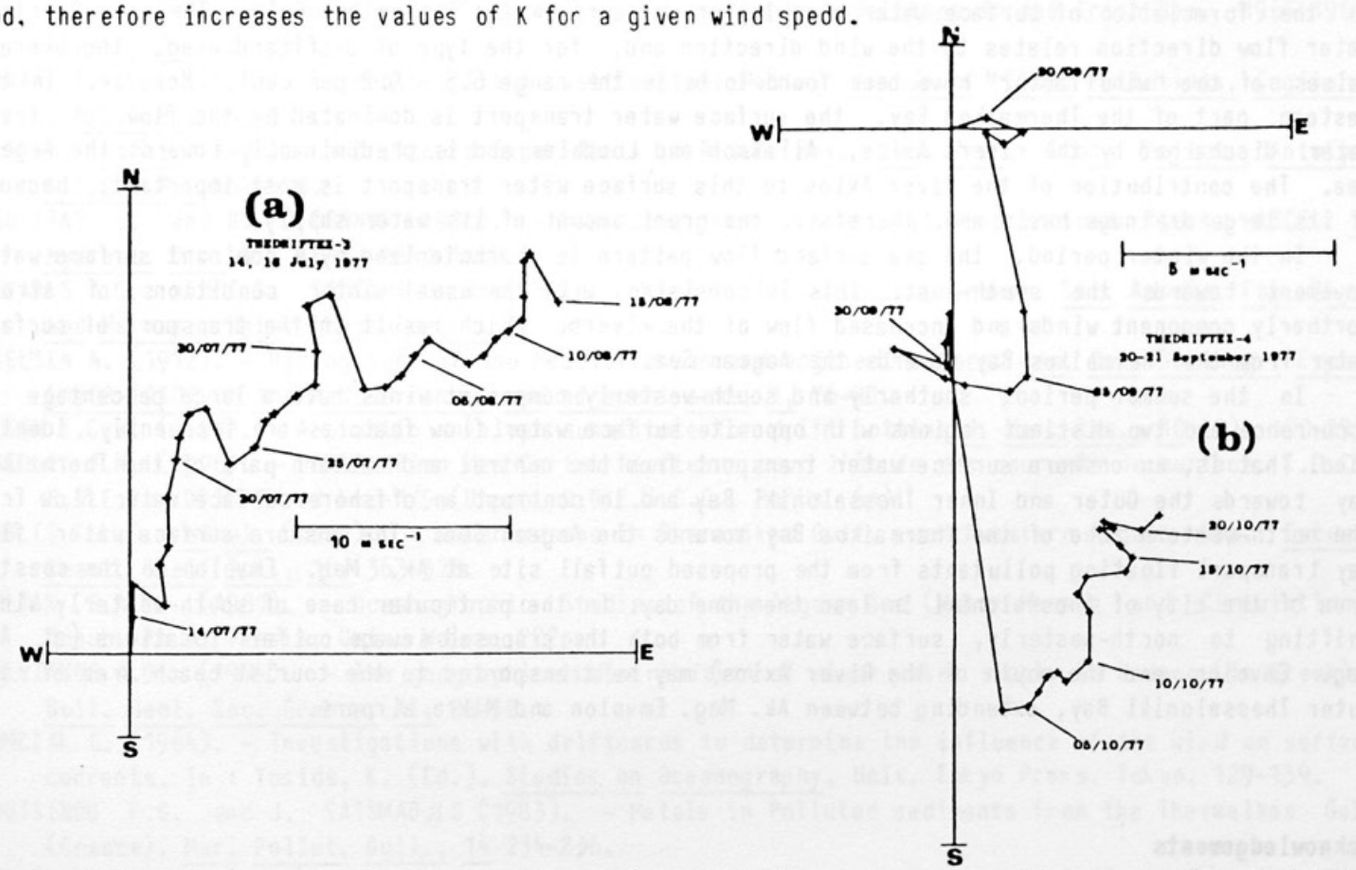


Figure VIII. - Wind progressive vector diagram (an indicator reference mark has been placed at 24:00 hours on each of the days) for the experiment (a) THEDRIFTEX-3 and (b) THEDRIFTEX-4.

Also, the wind speed observations have been made at the Mikra airport and, hence, they are almost invariably lower than the actual wind speeds on the sea surface in the open sea, which, in turn, means that the real values of K are lower than those calculated.

Moreover, the driftcards used are so light that they may be blown off the surface of water and be airborne for varying distances in strong winds. Therefore, the course of the drifcards and hence their speeds may not indicate the actual water speeds, whish may be considerably lower, meaning that the respective values of K may actually be lower.

Table 2
Values of the "Wind Factor", K,
for the various driftcard experiments

Experiment	"Wind Factor" K (%) Range of values	Average values
THEDRIFTEX-1	3.3 - 12.7	8.4
THEDRIFTEX-2	2.1 - 12.0	7.3
THEDRIFTEX-3	2.7 - 12.6	6.3
THEDRIFTEX-4	4.4 - 13.2	9.2

Conclusions

The investigation of surface currents by driftcards shows that the wind is an important factor in the formulation of surface water circulation patterns in the Thermaikos Gulf. The sea surface water flow direction relates to the wind direction and, for the type of driftcard used, the average values of the "wind factor" have been found to be in the range 6.3 - 9.2 per cent. However, in the western part of the Thermaikos Bay, the surface water transport is dominated by the flow of fresh water discharged by the rivers Axios, Aliakmon and Loudhias and is predominantly towards the Aegean Sea. The contribution of the River Axios to this surface water transport is most important, because of its large drainage basin and, therefore, the great amount of its water supply.

In the winter period, the sea surface flow pattern is characterized by a dominant surface water movement towards the south-east. This is consistent with the usual winter conditions of strong northerly component winds and increased flow of the rivers, which result in the transport of surface water from the Thermaikos Bay towards the Aegean Sea.

In the summer period, southerly and south-westerly component winds have a large percentage of occurrence and two distinct regions with opposite surface water flow features are frequently identified. That is, an onshore surface water transport from the central and eastern part of the Thermaikos Bay towards the Outer and Inner Thessaloniki Bay and in contrast an offshore surface water flow from the north-western zone of the Thermaikos Bay towards the Aegean Sea. The onshore surface water flow may transport floating pollutants from the proposed outfall site at Ak. Meg. Emvolon to the coastal area of the city of Thessaloniki in less than one day. In the particular case of south-westerly winds shifting to north-westerly, surface water from both the proposed sewage outfall locations (at Ak. Meg. Emvolon and the mouth of the River Axios) may be transported to the tourist beach area of the Outer Thessaloniki Bay, extending between Ak. Meg. Emvolon and Mikra airport.

Acknowledgements

The authors wish to thank Mrs Basel Marouda-Lambropoulou for drawing the figures.

References

ANGOURIDAKIS V.E. and P.C. MACHAIRAS (1973). - Precipitation in Thessaloniki (1). Sci. Annals, Fac. Phys. & Mathem., Univ. Thessaloniki, 13, 347-380.

BALOPOULOS E.TH. (1977). - Preliminary study on physical oceanographic data of period 1976. Technical Report. Thermaikos Systems Project., Instn. Ocean. Fish. Res., Athens, Greece, 144 p.

BALOPOULOS E.TH. (1982). - Circulation and Mixing in the Water Masses of the N.W. Aegean Sea (Noting Effects of Waste Disposal in Thermaikos Gulf). Unpub. Ph.D. Thesis, University of Wales, 755 p.

CARTER D.B. (1956). - The water balance of the Mediterranean and Black Seas. Drexel Instn. of Technology, Lab. of Climat IX, 3, Centerton, N. Jersey, U.S.A.

CHESTER R. and VOUTSINOU F.G. (1981). - The initial assessment of trace metal pollution in coastal sediments. Mar. Pollut. Bull., 12 (3), 84-91.

CONISPOLIATIS N. (1979). - Sedimentology and mineralogy of Thermaikos Bay, N.W. Aegean Sea. Unpub. M.Sc. Thesis, Univ. Wales, 133 p.

EKMAN W.V. (1905). - On the influence of the earth's rotation on ocean currents, Kgl. Svenska Vetenskapsakad. Arkiv. for Mal. Astron., Fysik., 2 (11), 32.

FRILIGOS N. (1977). - Seasonal variation of nutrient salts (N, P, Si), dissolved oxygen and Chlorophyll-a in Thermaikos Gulf (1975-76). Thalassia Jugos., 13 (3/4), 327-342.

FRILIGOS N. and J. SATSMADJIS (1977). - Nutrient distribution in the Gulf of Thermaikos (August, 1975). Thalassia Jugosl., 13 (1/2), 31-44.

GANOULIS J. and C. KOUTITAS (1981). - Utilisation de données hydrographiques et de modèles mathématiques pour l'étude hydrodynamique de Golfe de Thessaloniki (Greece). Rapp. Comm. int. Mer. Medit., 27 41-50, 6.

GERGES M.A. (1980). - Mixing and transport of some polluting effluents along the Egyptian Mediterranean Coast between Alexandria and Rosseta. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M., 917-928.

HARVEY J. (1968). - The movements of sea-bed and sea-surface drifters in the Irish Sea, 1965-1967. Sarsia, 34, 227-242.

HAVARD D.A. (1980). - Currents off the East Coast of Malta. Ves Jour. Etud. Pollutions, Cagliari, C.I.E.S.M., 945-948.

ILIC D. et al. (1978). - Driftcard Experiments in Rijeka Bay. IVes Jour. Etud. Pollut., Antalya, C.I.E.S.M., 567-571.

KOUTITAS C. and B. O'CONNOR (1980). - Modelling three-dimensional wind induced flows. ASCE, J. Hydraul. Div. N°. HY11, 1843-1865.

LIVADAS G.C. and C.S. SAHSAMANOGLOU (1973). - Wind in Thessaloniki, Greece. Sci. Annals, Fac. Phys. and Mathem., Univ. Thessaloniki, 13, 411-444.

NIELSEN N. (1912). - Hydrography of the Mediterranean and adjacent waters. Rep. Dan. Oceanogr. Exped. (1908-1910) to the Mediterranean and adjacent seas, 1, 76-91.

OLSON F.C.W. (1951). - A plastic envelope substitute for drift bottles. J. Mar. Res., 10 (2), 190-193.

PASQUAY J.N. (1976). - Utilisation des Cartes flotteurs pour l'étude des courants de surface. I.O.C.-U.N.E.P./ DRIFTEX-ad hoc-1/5 (Unpub. U.N.E.S.C.O., Paris. 6p).

ROBLES F.L.E. et al. (1983). - Water masses in Thermaikos Gulf, northwestern Aegean Sea. Estuar. Coast. Shelf Sci., 16, 363-378.

SULTAN S.A.R. (1981). - Oceanographic Studies of the Aegean Sea (Water Masses and Circulation). Unpub. Ph.D. Thesis, Univ. Wales, 222p.

THERIANOS A.D. (1974). - The geographical distribution of river water supply in Greece (In Greek). Bull. Geol. Soc. Greece, XI, 28-58.

TOMCZAK G. (1964). - Investigations with driftcards to determine the influence of the wind on surface currents. In : Yosida, K. (Ed.). Studies on Oceanography, Univ. Tokyo Press, Tokyo, 129-139.

VOUTSINOU F.G. and J. SATSMADJIS (1983). - Metals in Polluted sediments from the Thermaikos Gulf (Greece). Mar. Pollut. Bull., 14 234-236.

WILDING A. et al (1980). - Analyses of sea level fluctuations in Thermaikos Gulf and Salonica Bay, nothwestern Aegean Sea. Estuar. Coast. Mar. Sci. 10, 325-334.

ENVIRONMENTAL CONDITIONS IN MEX BAY, WEST OF ALEXANDRIA
IV. ROLE OF THE SEDIMENT WATER INTERFACE IN THE NITROGEN AND PHOSPHORUS DYNAMICS

by

M. EL-SAMRA*, Y. HALIM** and Th. MOUSTAFA*
* Alexandria Institute of Oceanography & Fisheries
Kayet Bey, Alexandria (Egypt)
** Faculty of Science, University of Alexandria
Moharrem Bey, Alexandria (Egypt)

Abstract

Sediment-bound nutrients and their fluxes, the rates of denitrification and nitrogen fixation were measured at the sediment-water interface in an area subjected to multiple pollution. About 146 to 256 ug at NH4 - Nm^{-2} day^{-1} and 3 to 135 ug at PO4 - P. m^{-2} day^{-1} are released, but no nitrate. Variability in the N/P flux ratio is related to sediment texture and differences in the denitrification rate. The latter is enhanced by organic pollution and accounts for 21 per cent of the agricultural run-off input of nitrate. The N_2 fixation rate is one order of magnitude lower and significantly correlated to sediment organic content.

*
* *

Introduction

The present work is part of a more comprehensive investigation of El Mex Bay west of Alexandria. In an attempt to assess the budget and dynamics of nutrient salts, it was deemed necessary to measure the processes taking place at the level of the sediment-water interface (MOUSTAFA, in preparation).

Numerous physical, chemical and biological activities are known to occur at the sediment-water interface which play an important part in the recycling of nutrients in the water body. In shallow coastal marine environments there is a good deal of evidence that the bottom sediment is a major nutrient supply source to the photic zone. In this work, the sediment-bound ammonia-nitrogen and phosphate, their flux and the rates of denitrification and nitrogen fixation were measured.

Aquatic Environmental Pollution Project, EGY/73/058.

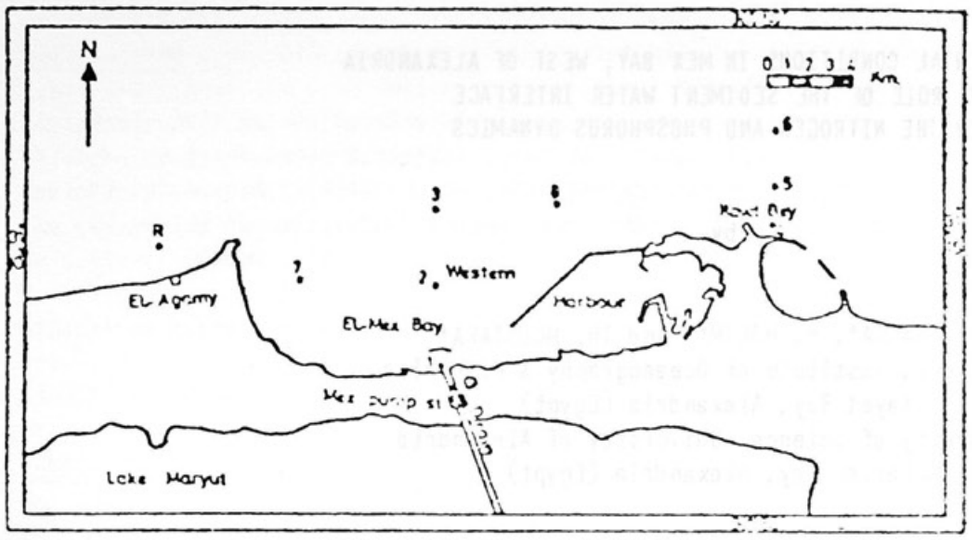


Fig. I
Location of stations, El Agamy, El Mex Bay and Kayet Bey.

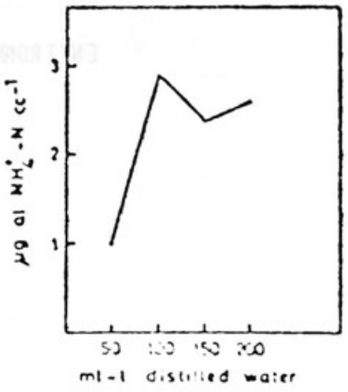


Fig. III
The effect at the amount of distilled water (ml) on the extraction of ammonium nitrogen from the sediment samples.

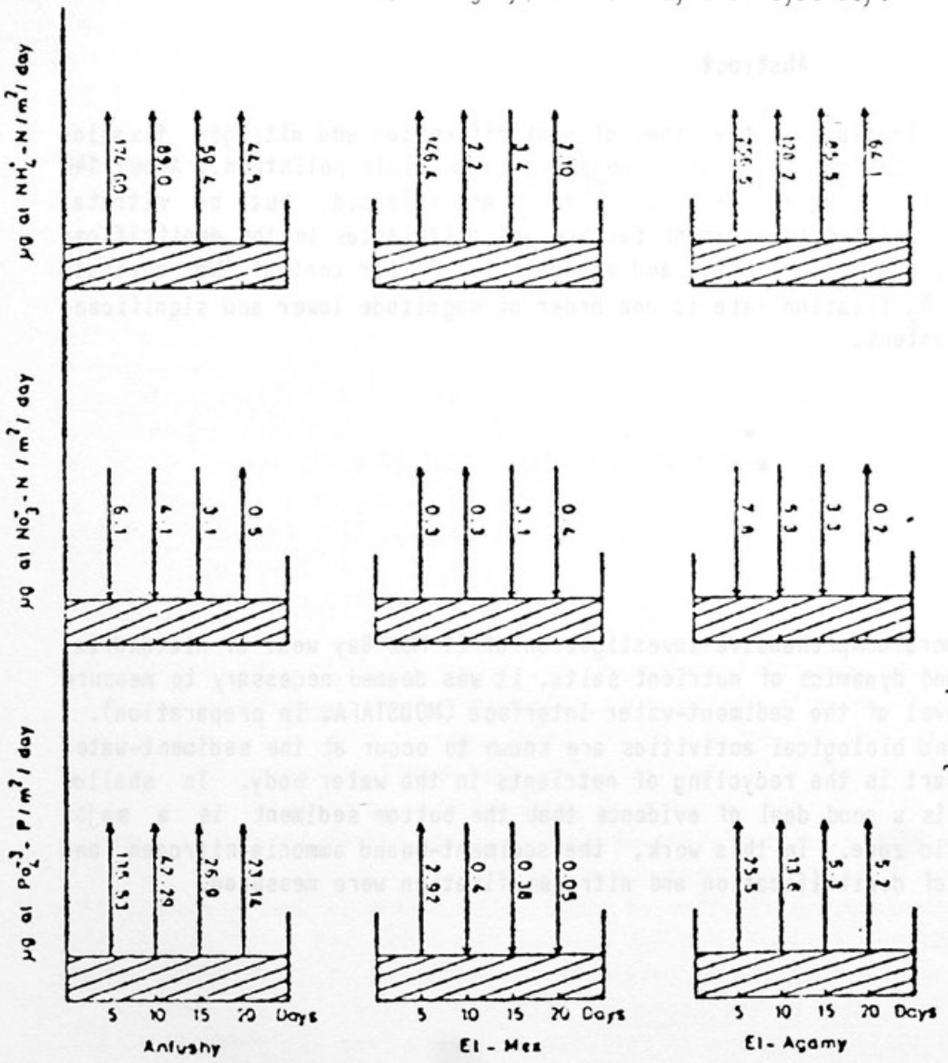


Fig. II
Nitrate ammonia and phosphate flux from sediments at El Mex, Kayet Bey and El Agamy (ug at m^{-2} day^{-1}).

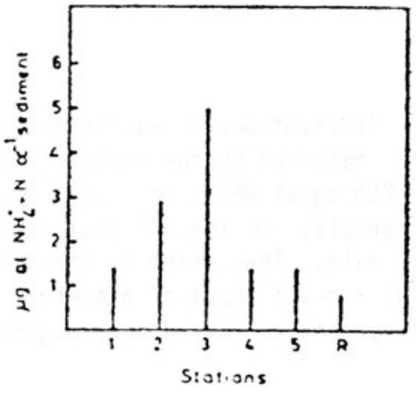


Fig. IV
The amount of ammonium nitrogen released from sediments at station 1-5 and R (ug at NH_4^+ - N/cc sediment).

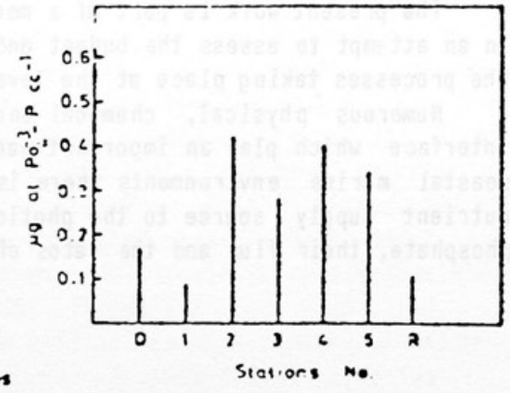


Fig. V
The amount of phosphate released from sediments at stations D 1-5 and R (ug at/cc sediment).

To understand the role of micro-organisms at the sediment-water interface, in situ measurements of their activities are imperative. The acetylene reduction assay technique has been extensively applied for detecting nitrogen activity in a variety of aquatic environments (PETERSON and BURRIES, 1976). Although the process of nitrogen fixation in sediments has been studied by several authors, only few ecological investigations provide information on the in situ rates.

The bottom sediments may also become the main sink for nitrogen through bacterial reduction of nitrate to nitrogen gas and its escape from the system (NIXON et al., 1980, KREMER and NIXON, 1978 and SEITZINGER et al., 1980). Denitrification in the sediment was measured to estimate the output of nitrogen from the investigated area. Quantification of this process is of importance to pollution scientists, hydrobiologists modelling nitrogen budgets and to agricultural scientists concerned about losses of nitrate fertilizers in the soil. Nothing is known about nitrogen fixation and denitrification rates in Egyptian waters and very little from the Mediterranean. Both processes appear to be correlated to organic content.

The site

The area investigated includes El Mex Bay west of Alexandria and extends for about 15 km between El Agamy and Kayet Bey headlands, and from the coast to a depth of about 15 metres. The bay receives a heavy load of untreated waste water both directly from a chlor-alkali plant and indirectly from Lake Maryut through El Mex Pumping station located at the outlet of El Umoum agricultural drain. The pumping station discharges about 2.86×10^9 m^3 $year^{-1}$. The main sewer of Alexandria at Kayet Bey headland discharges about 61.8×10^6 m^3 $year^{-1}$ of untreated domestic waste water. The current transport in this area being from west to east, El Agamy, located west of El Mex Bay, was taken as a reference station since it is almost free from the effect of the three effluents.

Material and methods

Sediment samples were collected using a Petersen grab sampler from seven sites (figure I) : Stations D, 1, 2, 3 are under the effect of the discharge of El Umum drain, stations 4, 5 and 6 are affected to various degrees by sewage discharge from the main outfall at Kayet-Bey. The reference station R, at El Agamy is affected by neither drain nor outfall.

The flux of nitrogen and phosphorus from the sediments was measured under static conditions in the laboratory, following the method of HARGRAVE and CONNOLLY (1978). A small portion of sea floor was cut off with some overlying water and incubated in a vessel without disturbance under controlled conditions and the flux measured at intervals of 5, 10, 15, 20 days. The flux per square metre into or out of undisturbed sediments was calculated :

$$F = \frac{V\,(C_o - C_t)}{A} \times \frac{10^4}{T}$$

where V is water volume over the sediments (litres), C_o, C_t, the dissolved concentrations before and after time T, A the sediment area (cm^2) enclosed.

The sediment-bound ammonium nitrogen was mechenically released by centrifuging a 1 cc wet sediment sample for ten minutes in 150 ml of ammonia-free distilled water (KAMIYAMA et al., 1977). The most suitable sediment to water ratio was determined experimentally (figure II) and found to be 1 cc sediment to 150 cc distilled water. Th concentration of ammonia in the supernatant distilled water measured (KOROLEF, 1969, in GRASSHOFF, 1976). Sediment-bound phosphate was measured by shaking

Table 1.- Nitrogen and phosphorus flux from sediments in different marine environment (ug at $m^{-2}h^{-1}$).

Location	Nutrient flux from sediments NH_4^+	NO_3 - N	PO_4^{-3}-P	Reference	Sediment type
Buzzards Bay (Massachusetts)		0.15		Rowe et al 1975	
Narragansett Bay (Rhode-Island)				Nixon et al 1976	Silty clay
Baltic	88.7	0.1	2.3	Balzer,1970	Mud
"	27.8				
Atlantic	0.55			Smith et al	Clayed silt
	0.61				Clayed silt
	33.8				Very fine sand
"Marine environment"	38.8			Pilson et al 1974	Mud
El Agamy	10.49	0.0	1.06	Present work	Sand
El Mex	6.10	0.013	0.12	Present work	Mud
El Anfushy	7.25	0.0	5.65	Present work	Sand

Table 2. - Ammonia released, type of sediment in situ concentration and salinity.

Station No.	Ammonia released, ug at NH_4^+-N/cc in 150 ml dist.	per liter	Type of sediment	Conc. ug at NH_4^+-N l^{-1} bottom water	S‰
1	1.51	10.07	fine sand	37.4	5.20
2	2.53	16.50	mud	3.81	38.23
3	5.01	33.37	mud	1.99	39.37
4	1.40	9.36	coarse sand	9.02	39.08
5	1.34	9.07	coarse sand	2.72	39.40
6	0.68	5.56	coarse sand	1.62	39.00

Table 3. - Phosphate released, in situ concentration sediment organic matter and salinity.

Station No.	ug at PO^{-3}_4-P/cc sediment in 150 ml dist.w.	per liter	ug at PO^{-3}_4-P/l^{-1} bottom water	% org. m sediment	PH	S‰
0	0.17	1.14	6.16	7.19	7.75	
1	0.09	0.62	4.22	3.14	7.65	
2	0.43	2.84	3.21	13.27	7.85	18.02
3	0.29	1.61	0.61	9.23	8.45	37.35
4	0.40	2.64	0.81	3.76	7.50	36.08
5	0.35	2.31	0.40	6.19	8.4	39.33
R	0.11	0.73	0.09	0.68	8.3	39.50

Table 4. - Denitrification rate (ug at $N_2 m^{-2}h^{-1}$), rate of nitrate, ammonia and phosphate flux (ug at $m^{-2}h^{-1}$) and % organic matter in sediment.

Station No.	Denitrification ug at N_2 m^{-2} h^{-1}		S organic matter		NO^{-2}_3-N NH^+_4-N PO^{-3}_4-P ug at m^{-2} h^{-1}		
	October 82	April 83	Oct. 82	April 83			
0	94.8	104.3	12.78	15.23	-	-	-
1	65.5	70.2	7.27	17.32	-	-	-
2	92.5	82.5	3.63	4.73	0.013	6.10	0.12
3	75.3	70.2	0.67	0.74	-	-	-
4	88.2	90.7	11.14	28.94	-	-	-
5	82.5	85.3	6.77	20.92	No	7.25	5.65
R	61.7	53.8	0.14	0.17	No	10.69	1.06

Table 5.
TN/TP ratio, April 1983.

Sation No.	TN/TP
1	8:1
2	10:1
3	8.5:1
4	11:1
5	10:1
R	17.5:1

Table 6. - Nitrogen fixation rates in sediments (ug at $N_2 m^{-2}h^{-1}$) (April and February 1982 and April 1983).

Station No.	N_2 fixed April 1982	N_2 fixed February 1982	N_2 fixed April 1983
0	2.85	9.71	4.51
1	9.57	3.0	31.79
2	9.38	4.19	9.24
3	1.50	30.21	3.86
4	37.53	3.0	9.0
5	37.53	11.43	13.54
6	-	15.0	7.3
R	0.47	8.21	0.0

a 1 cc sediment for one hour with 150 ml of distilled water in an electric shaker with a glass bead in the bottle. The phosphate released was measured (MURPHY and RILEY, 1962, in GRASSHOFF, 1976). The sorption processes of phosphate can be regarded as a very fast, almost momentaneous reaction (JACOBSEN, 1977).

The denitrification rate was measured by the acetylene inhibition technique of SORENSEN (1978). The sediment sample taken in a tube about 5 cm in diameter is sliced into 2 cm segments each of which is placed in another smaller tube. Acetylene saturated distilled water (2 ml of C_2H_2 per ml water) is then injected through a hole in the silicon-rubber stopper. One ml of the acetylene saturated water is injected into the sediment in five directions. The injection gives about 5 per cent saturation for the interstitial water. The core segment is then stoppered and 5 per cent (V/V) acetylene gas added to the air space through another hole. After a period of incubation of five hours, $Hg\ cl_2$ solution (0.1 M) is injected through the hole to block the reaction and N_2O measured by gas chromatography.

The rate of nitrogen fixation was measured according to the procedure of FLETT et al., (1976) developed and modified by EL SAMRA (1980). A bottomless bottle was pressed into the sediment, trapping the surface sediment together with a 1 mm layer of sea-water. The bottle was then sealed at the bottom whit a rubber cork and at the upper end with a rubber teat. The sediment was injected with 20 ml acetylene gas and incubated for three hours in situ after which a gas sample was taken from the gaseous phase of the reaction vessel for chromatographic analysis.

The organic matter content of the sediment was determined by ignition at 550 °C in a muffle furnace (UNGEMACH, 1960) % organic matter = % organic carbon X 1.8 (Trask factor).

Results and discussion

Nitrogen and phosphorus flux from the sediment

The direction and rates of ammonia, nitrate and phosphate fluxes at the interface are summarized in figure II. Sediments from three areas were investigated : El Agamy, El Mex and El Anfushy (Kayet Bey). Nitrogen flux from the bottom sediments was found to be mainly in the form of ammonia. Nitrate, on the other hand, was found to decrease gradually from both water and sediment. As expected. there was a very large flux of ammonia, namely, 146.4, 256.5 and 174.0 ug at. NH^+_4 - N m^{-2} day^{-1} at El Mex, El Agamy and El Anfushy respectively during the first five days of incubation. The greater diffusion rate of ammonia during this first phase results from both the initial higher sediment-water concentration gradient and from the unavoidable disturbance of the sediment. NIXON et al., (1976), KREMER and NIXON (1978) and BERNER (1976) also found the nitrogen flux from sediments to be mainly in the form of ammonia and the highest rate was measured during the first 17 hours of incubation. KREMER and NIXON (1978) found that nitrate and nitrite fluxes were usually small and the net flux roughly balanced to zero. The main nitrate sink is its reduction to nitrogen gas by the process of denitrification which is dealt with below. At El Mex Bay. the chlor-alkali waste discharge may inhibit bacterial reduction of nitrate and hence some nitrate flux could be measured in this area although at the slow rate of 0.3 ug at. NO_3 - N m^{-2} day^{-1}.

The rate of phosphate flux from the bottom sediments was smaller than for ammonia, namely, 135.5, 2.92, 25.5 ug at. Po_4^{-3}-Pm^{-2} day^{-1} at El Anfushy, El Mex and El Agamy respectively, in the first five days. The nitrogen to phosphorus flux was much lower at El Anfushy, where the denitrification rate is higher, than at El Agamy, namely, 1.3 : 1 and 10.1 : 1 respectively. The latter value is nearer to the expected ratio of 16 : 1 for organic matter. Differences in the rates of phosphate flux from the sediment are mainly due to differences in the sediment type (EL SAYED, 1977). This may explain the direction of flux in figure II. Sandy sediments at El Anfushy and El Agamy allow a greater phosphate flux from the sediments to the water column, while fine-grained muddy sediments (El Mex) favour phosphate adsorption rather than desorption. Measurement of nitrogen and phosphorus flux from sediments in different marine environments are shown in table 1. The rates of ammonia, nitrate and phosphate flux (ug at. $m^{-2}h^{-1}$) for the first five days fall within the range of values obtained by various authors by a similar method.

Release of sediment bound ammonium-nitrogen and phosphate-phosphorus

A large amount of ammonium-nitrogen is known to be bound on the sediment particles in adsorbed form in addition to the dissolved form in the interstitial water. In this experiment, ammonium-nitrogen in the sediment was mechanically extracted and the supernatant water decanted prior to determination. The amount of ammonium-nitrogen extracted increased by increasing the volume of distilled water up to 150 ml/cc sediment (figure III). The liberation of ammonium-nitrogen from the sediment sample may result from the disruption of the equilibrium between the dissolved form in the interstitial water and the adsorbed form on the particles by the addition of distilled water, and a new equilibrium reached. The results are given in table 2.

Except for station 1 the concentration in the bottom layer (in situ) was always lower than that released from the sediment. This negative sediment-water gradient favours the release of ammonia into the overlying water. At station 1, however, the gradient was reversed. This may be due to the high energy discharge of the pumping station at El Umoum Drain outlet maintaining the sediment in disturbance and resulting in the continuous release of ammonia nitrogen. According to KAMIYAMA et al., (1977) more of the adsorbed ammonium-nitrogen on sediment particles liberated to the supernatant by the increase in salinity and a new equilibrium between the two forms of ammonium-nitrogen in the suspension is attained. In addition to salinity, however, the type of sediment plays an important role. The results (table 2 and figure IV) show an increased release with increasing salinity at stations 1 to 3, confirming the results of KAMIYAMA et al., (1977). Less ammonium-nitrogen however was released at stations 4, 5 and R than at stations 1, 2, and 3. though salinity was higher at the former stations. This discrepancy may be related to the type of sediment. The retention capacity of coarse sand at stations 4, 5 and R is much lower than that of the fine sand and mud at stations 1, 2 & 3.

In the bottom sediments of neutral aquatic environments, dissolved ammonium-nitrogen diffuses from the interstitial to the overlying water. Its decrease from the interstitial water leads to desorption from the sediment particles into interstitial water and a new state cf equilibrium is reached between the two forms as observed in the experiments on ammonium flux. On the other hand, decomposition of organic nitrogenous material in the sediments contributes to raising the amount adsorbed on sediment. This may explain the continuous high flux of ammonia from the sediments into the water body in natural environments (figure II). For an accurate estimation of ammonium flux, the texture and type of sediments have to be considered together with the high energy water movements such as run-off, water currents, waves and other mechanical perturbations of the sediments. The length of the water columm over the sediments and the upward diffusion of ammonium ion should be taken into consideration when building a model for ammonia in the environment.

Sediment-bound phosphate : the amounts of phosphate released from the sediment were higher than the corresponding in situ values in the bottom water at the offshore stations (3, 5 and R) and lower at stations directly downstream from land run-off (D, 1, 2, and 4). As happens with ammonia, the energy of continuous run-off leads to continuous disturbance of bottom sediments maintaining the particulate phosphate in suspension. As for ammonia, there seems to be a state of equilibrium between phosphate adsorbed on the sediment particles and dissolved in the overlying water. Some other factors, however, affect phosphate diffusion from sediments. The oxidation state of the bottom sediment may be the main governing factor but it is not considered here. pH values were in the range reported by JITTS (1959) and EL SAYED (1977) to allow for maximum phosphate adsorption, namely between 6 and 8. EL SAYED (1977) found that an increase in salinity from 27 ‰ to 19 ‰ had no significant effect on the adsorption process.

Denitrification

No measurement of the rate of denitrification in Egyptian waters have been carried out before this work. The present work also constitutes the first attempt to correlate the denitrification process to nutrients in the overlying water and to organic matter in marine sediments. High rates of nitrogen gas release were measured in all collected samples. The average was about 80 ug at. N_2 m^{-2} h^{-1} and 79.57 ug at. N_2 m^{-1} h^{-1} during October 1982 and April 1983 respectively, a value which falls

within the ranges measured in similar coastal marine environments (SORENSEN, 1978 and SEITZINGER et al., 1980). The minima (61.7 and 53.8 ug at.N m^{-2} h^{-1}) were measured at St. R, and the maxima (94.8, 104.3 ug at. N_2 m^{-2} h^{-1}) in El Umum Drain (St.D). Higher rates were measured in the area affected by sewage water (St. 4 and 5) than in the area affected by industrial waste water in El Mex Bay. It is likely that the latter inhibits the activity of denitrifying bacteria. The nitrogen gas flux in October (1982) represents about 15 : 1 the net ammonia flux at St. 2 at similar temperature and 11 : 1 at St. 5. Denitrification accounts therefore for 92.7 per cent and 92 per cent of the remineralized nitrogen in the sediments at the two stations. At El Agamy (St. R) the nitrogen flux was lower, 61.7 ug at. N2 m^{-2} h^{-1}, representing about 6 : 1 the ammonia flux and accounting for about 85 per cent of the total nitrogen released from the sediments through remineralization. SEITZINGER et al., (1980) found a summer nitrogen flux of approximately 50 umol m^{-2} h^{-1}, 6 : 1 the net $N\bar{O}_3$ - $N\bar{O}_2$ flux and about half of the ammonia flux at similar temperature, acccounting for about 30 per cent of the total nitrogen released.

Sediment organic matter remains consistently low offshore (St.R, 2 and 3). St. 1, 4 and 5 showed an abrupt increase from October to April, reflecting the inhomogeneous condition at the effluent outlets. A significant organic content falls abruptly to 0.14 per cent and 0.17 per cent. A significant correlation (r = 0.6) was found between denitrification rate and organic matter content of the sediments. This correlation is not unexpected since the bacterial activity is primarily governed by the supply of reducible substrate. The process of denitrification therefore is enhanced by organic pollution and may be considered as a self-purification process in natural ecosystems.

The highly significant negative correlation (r -0.96) found between nitrogen gas release from the sediment and nitrate in the overlying bottom water indicates that denitrifiers obtain their energy for oxidation of organic matter from the reduction of nitrate to nitrogen gas according to the following equation (RICHARDS, 1965)

$$(CH_2O)_{106}(NH_3)16\ H_3PO_4 + 84.8\ HNO_3 \longrightarrow 106\ Co_2 + 16\ NH_3 + H_3Po_4 + 42.4\ N_2 + 148.4\ H2_0$$

The highly significant correlation (r = 0.98) also obtained between in situ phosphate concentrations and denitrification indicates a close dependence of the bacterial activity on phosphate in the environment. This correlation has not been reported before. It has been shown in vitro that the activity of the nitrogen fixers is also dependent on phosphate concentration (STEWART et al., 1970 and 1971). Further studies are required to clarify the interdependence of the two elements.

The ecological importance of this process in maintaining nitrogen as the major nutrient limiting productivity in marine coastal areas is obvious. Denitrification is a major factor lowering the N/P ratio (less than 16 : 1) in the coastal marine environment (table 5). From the table, we notice that TN/TP ratios are significantly lower than expected for the organic matter decomposition on the bottom under natural conditions, except for St. R (open sea) where the ratio (17.5 : 1) reached the accepted value. Measurements carried out by other workers from a variety of coastal environments have also indicated low N/P ratio in the benthic nutrient fluxes (ROWE et al., 1977, NIXON et al., 1975, PROPP et al., 1980 and others). Using the estimate of 20 km^2 for El Mex Bay area, a rate of denitrification of 77.7 ug -1 at. N_2 m^{-2} h^{-1} (St. 1 and 2) is reported, the nitrogen flux being of 190.83 tons year. This value represents 20.96 per cent of the total nitrate input through the drain to the Bay. Higher results have been reported for lake sediments, KEENEY et al., (1971) and CHEN et al., (1972) found about 63 per cent of the added nitrate was reduced in Lake Mendota sediments. CLINE and RICHARDS (1972) estimated 40 - 50 per cent of nitrate to be reduced through denitrification process in the eastern tropical north Pacific. The importance of the denitrification process in estimating the contribution of marine bottom sediments to the global nitrogen budget was estimated by SEITZINGER et al., (1980) to be 8.6 x 10^6 tons $year^{-1}$ for the world estuaries, which amounts to about 10 per cent of the rate estimated by HAHN and JUNG (1977) for the entire ocean.

Nitrogen fixation rate : the highest rate of nitrogen fixation activity in the sediment, 37.53 ug at. N_2 m^{-2} h^{-1}, as well as in bottom water was measured in the areas affected by sewage pollution (St. 4, 5) (MOUSTAFA, pers. comm) indicating that nitrogen fixation is bacterial rather than algal. The lowest nitrogen fixation activity in sediment, namely, 0.00 - 0.47 ug at. N_2 m^{-2} h^{-1} was measured at St. R. The higher rates of nitrogen fixation in sediment coincide with a higher organic matter

content especially at St. 4 and 5, while smaller rates occur in sediments much poorer in organic matter at St. R. A significant correlation ($r = 0.54$) was found between nitrogen fixation rate and organic matter content of the sediments. MULDER and BROTONEGONO (1974) and SCHICK (1971) suggested that in shallow lacustrine sediments and in unilluminated aquatic environments, in general, the intensity of nitrogen fixation, a process with a great energy demand, depends primarily on the quantity of organic matter available. KERIN and BREZONIK (1971) found that in Lake Moss Lee and Lake Tomahovk nitrogen fixation rates were of 6.3 and 85.0 ug at. N_2 m^{-2} h^{-1} respectively, while at Bivin Arm and Lake Kanapaha the rates were 77 and 206.7 ug at. N_2 m^{-2} h^{-1} respectively. OLAH et al. (1983) found rates of 40.2, 83, 5.8 and 4.1 mg N m^{-2} day^{-1} at Lake Balaton, Koros Backwater reservoir, polycultural fish pond and liquid manure fish pond respectively.

Conclusions

The present work is part of an experimental attempt to quantify the nutrient dynamics in a shallow coastal marine ecosystem subjected to multiple pollution. The processes taking place at the sediment-water interface are accounted for in this paper. Some general conclusions could be reached from the in situ and laboratory experiments.

1 - In shallow aquatic environments, the sediments represent a major nutrient source to the water column. Nitrogen flux from the bottom sediments was found to be mainly in the form of ammonia, and the rate of ammonia flux reaches about 0.75 tonne NH_4^+ - N Km^{-2} $year^{-1}$. This rate is affected mainly by sediment type, salinity, the length of the overlying water column and occasionnal disturbance of bottom sediments due to the condition of the sea. The release of sediment-bound nutrient salts results from the disruption of equilibrium between the salt content of overlying water, interstatial water and adsorbed salts on particles. Fine-grained sediments were more retentive than coarse sand. More ammonia than phosphate is fluxed, but no nitrate flux was detectable.

2 - The main nitrate sink is its reduction to nitrogen gas by the process of denitrification. This process prevents nitrate, and organic matter accumulation in coastal marine environments and can be described as a self-purification process. Estimation of the denitrification rate, therefore, can lead to an assessment of the trophic state of a marine environment and a tool for studying organic pollution. Denitrification was found to account for 21 per cent of the total nitrate input through land sources and for 92.7 per cent of the remineralized nutrient salts in the sediments. Denitrifying bacteria obtain their energy from oxidation of organic matter to reduce nitrate into nitrogen gas. The bacterial activity was found to be closely dependent on sediment phosphate. Its rate is therefore, significantly correlated to sediment organic matter and phosphate, resulting in a significantly lowered N/P ratio in the coastal marine environments.

3 - Nitrogen fixation rate in sediments was found to be negligible ranging from zero to about 15 ug at N_2 m^{-2} h^{-1}. However, in heavily organically polluted areas, it reaches 38 ug at N_2 m^{-2} h^{-1} indicating high activity of anaerobic bacteria, which obtain their energy to fix nitrogen from oxidation of organic matter.

4 - Experiments conducted in the present work, especially those on denitrification and nitrogen fixation measurements, are a new approach to the study of in situ rates of nutrient dynamics in the Mediterranean environment. It is recommended that in situ conditions be taken into consideration in any attempt to model a natural environment.

References

BALZAR (F.) & KELLER (I.), 1978. - Freiseteung von Eahrstoffer : pp. 24-28. In G. Wefer (ed.), Das Harrington Sound Projekt des SFB95/Reports Sonderforschungsbereich 95 - Wechselwirkung Meer - Meeresboden Kiel University.

BERNER (R.A.), 1976. - The benthic boundary layer from the viewpoint of a geochemist, pp. 33-35. In. I.N. Mc Cave (ed.), The Benthic Boundary layer, Plenum.

CHEN (R.L.), KEENEY (D.R.), GRAETZ (D.A.) & HOLDING (A.J.), 1972. - Denitrification and nitrate reduction in Wisconsin Lake sediments. J. Environ 1, pp. 158-162.

CLINE (J.D.) & RICHARDS (I.A.), 1972. - Oxygen deficient conditions and nitrate reduction in the tropical north Pacific Ocean. Limnol. Oceanogr. 17, pp. 885-900.

EL-SAMRA (I.M.), 1980. - Significance of nitrogen fixation and combined nitrogen uptake in shallow lakes. Ph. D. Thesis submitted to the Hungarian Academy of Science. pp. 122.

EL-SAYED (K.A.), 1977. - Geochemistry of Manganese and Iron in the recent shelf sediments off the Nile Delta in relation to phosphate adsorption. M. Sc. Thesis, Alexandria University, 122 p.

FLETT (R.J.), HAMILTON (E.D.) & CAMPBEL (N.E.R.), 1976. - Aquatic acetylene reduction techniques : solutions to several problems. Can. J. Microbiol., 22. pp. 43-51.

GRASSHOFF (E.), ed., 1976. - Methods of Sea Water Analysis. Erlag Chemis. Weinheim. New York, pp. 305.

HAHN (J.) & JUNGE (C.), 1977. - Atmospheric nitrous oxide : A critical review. 2. Naturforsch. 32, pp. 190-214.

HARGRAVE (B.T.) & CONNOLLY (G.F.), 1978. - Advice to collect supernatant water for Measurement of the flux of dissolved compounds across sediment surface. Limnol. oceanogr. 23, pp. 1005-1010.

JACOBSEN (O.S.), 1977. - Sorption of phosphate by Danish lake sediments. Publication N°. 282 from freshwater biological laboratory University of Copenhagen, Denmark.

JITTS (H.R.), 1959. - The adsorption of phosphate by estuarine bottom deposits. Aust. J. Mar. Freshw. Res., 10, pp. 7-21.

KAMIYAMA (K.), CKUDA (S.) & KAWAI (A.), 1977. - Studies on the release of ammonium nitrogen from the bottom sediments in Freshwater bodies - I. Jap. J. Limnol., 37, pp. 59-66.

KEENEY (D.R.), CHEN (R.L.) & GREATZ (D.A.), 1971. - Importance of denitrification and nitrate reduction in sediments to nitrogen budgets of lakes. Nature, Lond. 233, pp. 66-67.

KERIN (M.A.) & BREZONIK (P.L.), 1971. - Nitrogen fixation by bacteria in lake Mize, Florida, and in some lacustrine sediments. Limnol. Oceanogr. 16, pp. 720-731.

KREMER (J.E.) & NIXON (S.W.), 1978. - A Coastal Marine Ecosystem simulation and Analysis. Springer Verlag Berlin, pp. 217.

MULDER (E.G.) & BROTONEGORO (S.), 1974. - Free living heterotrophic nitrogen fixing bacteria. In : A Qispel (ed.). The biology of Nitrogen Fixation - North Holland Publishing Company, Amsterdam, pp. 37-85.

NIXON (S.W.), OVIATT (C.A.) & HALE (S.S.), 1976. - Nitrogen regeneration and the metabolism of coastal marine bottom communities, pp. 269-283. In J.. Anderson and A. Mac Fayden (eds.). The role of Terrestrial and Aquatic organisms in Decomposition processes. Blackwell.

NIXON (S.W), KELLY (J.R.), FURNAS (B.N.) & OVIATT (C.A.), 1980. - Phosphorus regeneration and the metabolism of coastal marine bottom communities. In Marine Benthic Dynamics. (eds. K.R. Tenore and B.C. Coull). pp. 219-242. Univ. of South Carolina Press.

OLAH (J.M.A.), ABDEL MONEIM & TOTH (L.), 1983. - Nitrogen fixation in the sediment of shallow lake Balaton, a reservoir and fishponds. Int. Revue Ges. Hydrobiol. 68 : 1, pp. 13-44.

PETERSON (R.B.) & BURRIAS (R.H.), 1976. - Conversion of acetylene-reduction rates to nitrogen fixation rates in natural populations of blue green algal. Anal Biochem. 73, pp. 404-410.

PILSON (W.E.Q.), OVIATT (C.A.) & DIXON (S.W.), 1979. - Annual nutrient cycles in a marine microcosm. Symposium on Microcosms in Ecological Research, Savannah River Ecological Laboratory, Nov. 18-10, 1978, August, Ca.

PROPP (M.V.), TARASOFF (V.G.), CHERBADGI (I.I.) & LOOTZIK (N.V.), 1980. - Benthic pelagic oxygen and nutrient exchange in a coastal region of the sea of Japan. In : Marine Benthic Dynamics (eds. F.R. Tenore and B.C. Coull), pp. 265-284. Univ. South Carolina Press.

RICHARDS (P.A.), 1965. - In Riley J.P. and Skirrow, G. (eds.), Chemical Oceanography, Vol. I Chap 13. Academic Press, London.

ROWE (G.T.), CLIFFORD (C.H.), SMITH (K.L. Jr) & HAMILTON, 1975. - Benthic Nutrient regeneration and its effect to primary productivity in coastal waters. Nature, Lond. 255, pp. 215-217.

ROWE (G.T.), CLIFFORD (C.H.) & SMITH (K.L. Jr), 1977. - Nutrient regeneration in sediments off cap Blano, Spanish Sahara. Deep-Sea Res. 24, pp. 57-63.

SOBIEK (H.J.), 1971. - Substrate and light dependent fixation of molecular nitrogen in Rhodospirillum rubrum. Arch. Microbiol. 75, pp. 89-101.

SEITZINGER (S.), NIXON (S.), PILSON (E.Q.) & BURKE (S.), 1980. - Geochimica et Cosmochimica Acta, Vol. 44, pp. 1853-1860. Pergamon Press.

SMITH (K.L. Jr), 1978. - Benthic community respiration in the N.W. Atlantic Ocean. In Situ measurements from 40-5200 m. Mar. Biol. 47, pp. 337-347.

SORENSEN (J.), 1978. - Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. Appl. Environ. Microbiol. 36, pp. 139-143.

STEWART (W.D.P.) & LEX (M.), 1970. - Nitrogenase activity in the blue green alga Plectonama boryanum strain 594. Arch. Microbiol. pp. 250-260.

STEWART (W.D.P.), MAGUE (T.), FITZGERALD (G.P.) & BURRIS (R.H.), 1971. - Nitrogenase activity in Wisconsin Lakes of differing degree of eutrophication. New Phytol., 70, pp. 497-509.

UNGEMACH (H.), 1960. - Sediment. Chemismus und Seine Besiehungen Zum stoff haushalt in 40 europeishen seen. Disseration. Kiel. pp. 1-420.

SEDIMENT SURFACE PROPERTIES AND ADSORPTION OF HEAVY METALS IN A TYPICAL KARST ESTUARY

by

Mladen JURACIC*, Esad PROHIC**, and Velimir PRAVDIC*
* Laboratory of Electrochemistry and Surface Phenomena
Center for Marine Research, The Rudjer Boskovic Institute
Zagreb, Croatia, (Yugoslavia)
** Institute of Geology, Zagreb, Croatia (Yugoslavia)

Abstract

The Krka River estuary is located in the typical karst region of the eastern Adriatic. It was chosen as a model for studies of the transfer mechanisms for pollutants from land to sea. Our interest concerned total suspended matter and sediments as scavengers for heavy metals as pollutants.

The Krka River is almost free of suspended matter due to the retention of its waters in the natural lakes formed by travertine barriers. The main supplier of suspended matter is a small River Guduca, which enters directly into the estuary, the Prokljan Lake. The mixing zone is within this lake, which exhibits characteristic stratification.

Sediments were collected in the estuarine zone by coring. Analyses on trace heavy metals (Pb, Zn, Cu, Ni) were made using emission spectrometry. The concentration of heavy metals was correlated with surface properties of the sediment particles, such as mineral composition, granulometric distribution, specific surface area, and organic coating.

It was found that the organics determine the adsorption of the trace heavy metals. Clay minerals show higher concentrations of trace metals, along with much larger specific surface areas.

*
* *

Introduction

Estuaries are precious coastal sites, subject to strong competition for various uses : as location of ports, human settlements, as recreational areas, and as highly bio-productive fishery grounds. Disposal of sewage and pollutants often results in a degradation of environmental quality. These areas are usually also characterized by high sedimentation rates. The scavenging by suspended particles results in large concentrations of pollutants retained in estuarine sediments.

This particular karst estuary has been chosen for studies because it is characterized by a low suspended matter load. Therefore it was interesting to investigate the presence of several trace heavy metals (Mn, Pb, Cu, Ni, Zn) in this estuary, and to study by the method of sequential leaching the nature of their bonding to sediments.

Topography, hydrography and geological background of the estuary

The estuary investigated is located in the Outer Dinaric karst region, in the so-called North Dalmatian erosional plane. It was developed in the Upper Cretaceous - Eocene limestones by lateral corrosional processes. In the catchment area of the Krka River there is also a subordinate quantity of klastic rocks (Eocene flysch and flysch-like deposits, and Quaternary marls and clays).

During glacial times the Krka River cut its valley into this erosional plane, and due to the low level of the sea surface this valley may be traced to the isobate of 100 m into the Adriatic sea. After the Holocene transgression the sea invaded the lower part of the valley and formed the recent estuary. In the upper part of the valley calc-tufa barriers started to build up, due to favourable temperature and geochemical conditions.

At present, the principal watercourse that enters the estuary is the Krka River. It has a mean freshwater inflow of approx. 55 m3/s, but with large seasonal and monthly variations (4-410 m3/s). Due to the formation of calc-tufa barriers and of lakes behind them, and a small extent of weathering of klastic particles, one can assume immediately that the input of suspended material to the estuary has to be relatively small.

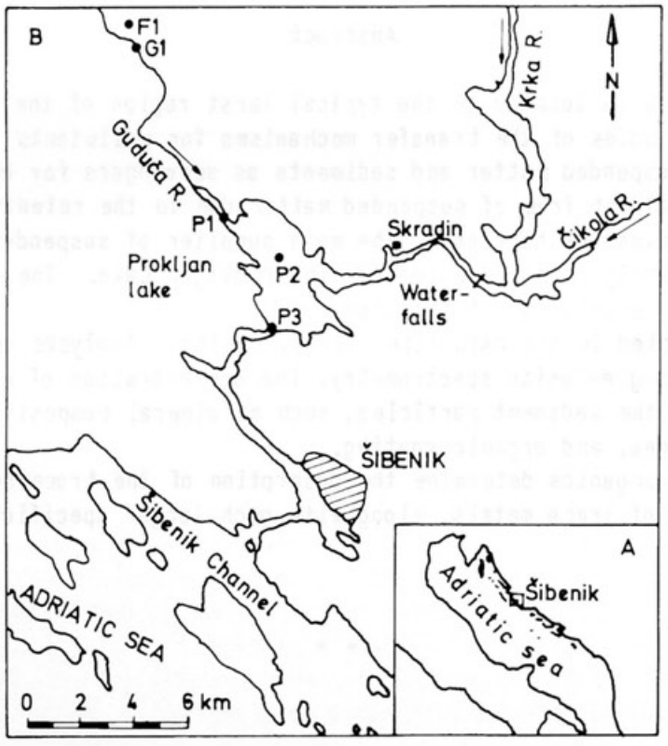


Figure I. - The map of the location of the Krka River estuary (A), and the location of sampling point (B).

Nowadays, the estuary proper is restricted to the part of the ancient River valley inbetween the last active calc-tufa barrier (Skradinski Buk waterfalls, height 46 m) which is the freshwater end-point, to the Sibenik Channel, to a total length of 22 km. The bottom depth in the estuary gradually deepens from 2m, below the waterfalls. to 42 m in the Sibenik Channel.

In recent hydrographic conditions (low tidal range of 20 to 50 cm) and due to a sheltered geography, the Krka River estuary belongs to the type of stratified estuaries (BULJAN, 1969). The brackish surface current is directed to the sea, whereas the sea-water bottom counter-current can be traced up to the Skradinski Buk waterfalls.

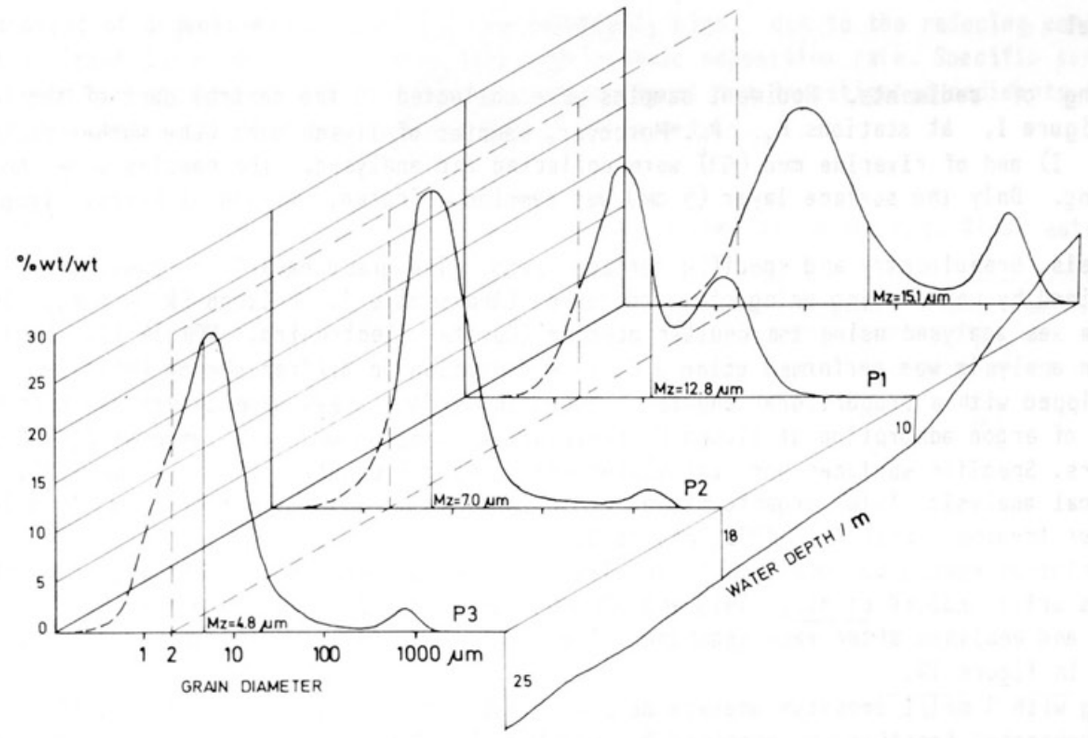


Figure II. - Granulometry of sediments. Sediment histograms along the profile from G1 to P3 (see figure I). Distances along the estuary are not in scale.

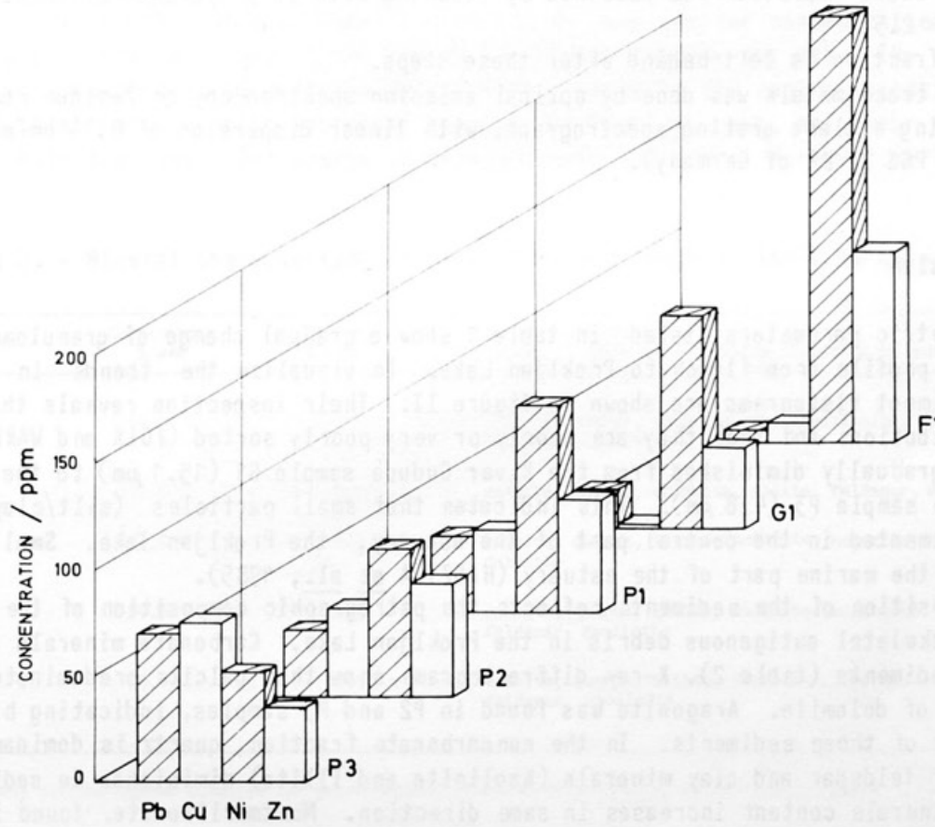


Figure III. - Trace heavy metals concentrations in sediments (fraction below 32 µm. except for F1 sample) for samples in the profile F1 to P3.

Experimental

Sampling of sediments. Sediment samples were collected in the central part of the estuary as shown in figure I, at stations P_1, P_2. Moreover, samples of flysch marl (the mother rock sample, F1 in figure I) and of riverine mud (G1) were collected and analysed. The samples were collected by Scuba-diving. Only the surface layer (5 cm) was sampled, frozen, within 12 hours, lyophilyzed and analysed.

Analysis. Granulometry and specific surface areas. The granulometric composition of the samples was determined by wet sieving using standard sieves (Analysette 3, Fritsch FR Germany). The fraction below 32 μm was analysed using the coulter counter (Coulter Electronics, MOD TA II, England). X-ray diffraction analysis was performed using a Cu K-∞ radiation in diffractometer (Philips, The Netherlands) equipped with a proportional counter. Specific surface areas were determined using volumetric techniques of argon adsorption at liquid N_2 temperature. Samples were activated at 200 °C and 10^{-4} Pa for 24 hours. Specific surfaces were calculated using the BET equation (PARFITT and SING, 1976).

Chemical analysis. Total organic matter was determined by weight loss of a 105 °C dried sediment sample after treatment with 15 % H_2O_2, and heating to 450 °C for 12 hours.

The original sample was analysed first. Next the samples were leached sequentially (PROHIC, 1984 ; modified after TESSIER et al., 1979 and SALOMONS and FORSTNER, 1980) in four steps, yielding 5 fractions and analysed after each leaching. The terms used below are for the purpose of identification shown in figure IV.

1. Leaching with 1 mol/l ammonium acetate at pH = 7, yielded the "exchangeable" fraction.
2. The "carbonate" fraction was obtained by leaching with 1 mol/l sodium acetate, and 1 mol/l acetic acid at pH = 5.
3. The "reducible" fraction was obtained using leaching with 1 mol/l hydroxylamine hydrochloride in 25 % v/v acetic acid at pH = 2.
4. The "organic bound" fraction was obtained by leaching with 30 % hydrogen/peroxide and 1 mol/l acetic acid at pH = 2.5.
5. The "residual" fraction is left behind after these steps.

Analysis on trace metals was done by optical emission spectroscopy on residue remaining after each extraction using a plane grating spectrograph, with linear dispersion of 0.74 nm/mm in the first order (Carl Zeiss, PGS 2, DR of Germany).

Results and discussion

The granulometric parameters listed in table 1 show a gradual change of granulometric distribution along the profile from flysch to Prokljan Lake. To visualize the trends in granulometric distribution, sediment histograms are shown in figure II. Their inspection reveals that all of them have bimodal distributions and that they are poorly or very poorly sorted (FOLK and WARD, 1957). The mean size, Mz, gradually diminishes from the River Guduca sample G1 (15.1 μm) to the lower part of the Prokljan lake sample P3 (4.8 μm). This indicates that small particles (silt/clay grade) are preferentially sedimented in the central part of the estuary, the Prokljan lake. Small grade particles are absent in the marine part of the estuary (HADZIJA et al., 1985).

Mineral composition of the sediments reflects the petrographic composition of the drainage area and the input of skeletal autigenous debris in the Prokljan Lake. Carbonate minerals are the major component in all sediments (table 2). X-ray diffractograms show that calcite predominates accompanied with small amounts of dolomite. Aragonite was found in P2 and P3 samples, indicating biogenic origin of the coarser part of those sediments. In the noncarbonate fraction, quartz is dominant and ubiquitous. The amount of feldspar and clay minerals (Kaolinite and illite) diminishes in sediments from G1 to P3. The iron minerals content increases in same direction. Montmorillonite, found in flysch, was found neither in the Guduca River, nor in the estuarine samples.

The content of organic matter (table 2) is relatively high, due to the reducing conditions in the surface sediment layer and to the relatively high organic deposition rate. Specific surface areas of the sediments investigated (table 1) show large capacity of the investigated sediments to accomodate both higher quantities of organic matter and of heavy metals.

Table 1. - Granulometric characterization of surface sediments in the Krka River estuary.

Sample	Median /um	Mean size /um	Sorting*	Skewness*	Kurtosis*	% fraction below 32 /um	Specific surface area, S_{BET} m^2/g
G1	12.5	15.1	2.68 very poorly sorted	-0.31 negatively skewed	1.42 leptokurtic	72.9	20.3
P1	8.9	12.8	2.19 very poorly sorted	-0.27 negatively skewed	0.82 platikurtic	70.4	39.4
P2	6.9	7.0	1.39 poorly sorted	-0.11 negatively skewed	1.36 leptokurtic	93.2	30.5
P3	4.8	4.8	1.59 poorly sorted	0.24 positively skewed	1.29 leptokurtic	93.6	35.2

* Granulometric parameters are in ø units, ø = $-\log_2 d$ (d = diameter in mm).
Definition of granulometric paarmeters after Folk and Ward (1957).

Trace element concentrations show two distinctive groups of heavy metals (figure III). Zinc and nickel are representatives of the first group (HIRST and NICHOLLS, 1958), which is supposed to be mainly of detritial origin. It was found that Zn and Ni have similar concentration ratios in flysch marl and along the profile. Their total amount diminishes towards the sea. The representatives of elements that are presumed to be anthropogenically influenced in the region investigated are lead and copper. Their total concentration increases towards the Prokljan Lake, and their mutual relationship might indicate more than one point source of anthropogenic influence. Moreover vertical distribution

Table 2. - Mineral characterization of surface sediments in the Krka River estuary.

Sample	Carbonate % w/w	Organic matter % w/w	Mineral composition Listed in order of decreasing incidence in sample*
F1	69.0	-	calcite, quartz, dolomite, montmorillonite, illite, feldspar, kaolinite
G1	59.6	5.3	calcite, quartz, dolomite, illite, feldspar, kaolinite
P1	37.9	8.7	calcite, quartz, illite, kaolinite, hematite, feldspar, dolomite
P2	57.5	4.9	calcite, quartz, illite, dolomite, aragonite, pyrite, feldspar, kaolinite
P3	55.1	5.5	calcite, quartz, illite, aragonite, dolomite, pyrite, feldspar, kaolinite

* X-ray diffractograms obtained for granulometric fraction below 32 /um, except for F1 sample.

of lead and copper in a sediment core taken at the P3 location (JURACIC and PROHIC, 1984) indicate their strong enrichment in only the surface layer. The supposed source of lead is the combustion of

leaded gasoline, and a probable source of copper could be antifouling copper paints, used for ships' bottoms (BELLINGER and BENHAM, 1978).

In an attempt to elucidate the possible bioavailability of these trace metals, the sequential leaching experiments were performed on P1 and P2 samples. Results of analyses are reported in figure IV. Manganese is mainly associated with the carbonate fraction. The second important quantity is in the exchangeable fraction (adsorbed cations + interstitial water), and that is correlated with the large surface area of sediments.

As supposed, Ni and Zn are mainly present in the residual and carbonate fractions. It means that they are incorporated in the crystal structure and thus not readily available to the benthic organisms. The nondetrital part of Ni and Zn is mainly bonded to the organic and reducible fraction. Lead and copper are mainly bonded to the reducible and organic fraction indicating that they are more bioavailable. Lead is preferentially in the reducible fraction, whereas copper is found in the organic fraction of sediments.

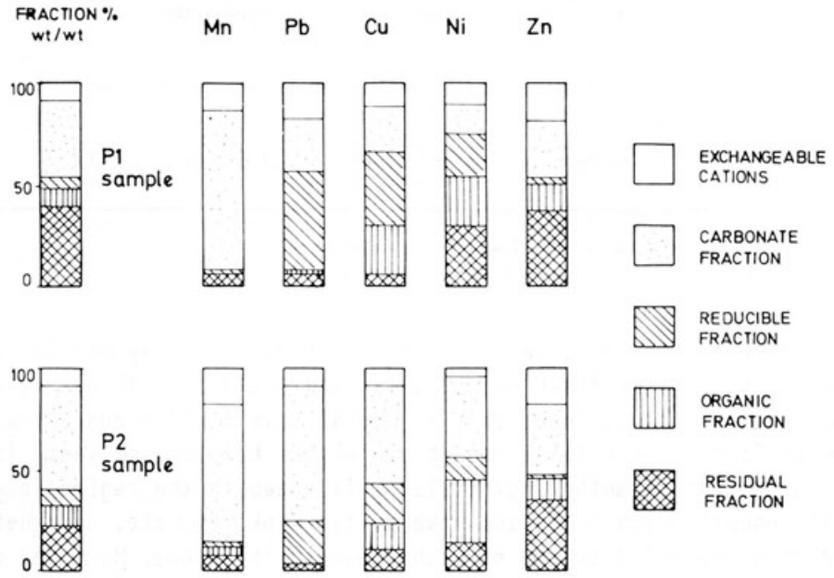


Figure IV. - Distribution of trace heavy metals in samples P1 and P2 obtained by sequential leaching.

Different distribution of heavy metals in P1 and P2 samples can be attributed to different mineral composition and environmental factors on two sampling sites.

Conclusions

From the results presented the following conclusions can be drawn for the Krka estuary sediments.

1. Most of the terrigenous fine-grained material that enters the estuary via the Guduca River is sedimented within it.
2. The sediments are mainly carbonates, both of terrigenous and authigeneous origin. Flysch and Quaternary sediments are the main source of noncarbonate minerals (quartz, feldspar, illite, kaolinite).
3. Two groups of trace heavy metals can be distinguished in sediments. Mn, Zn and Ni are representatives of detrital sources, while Pb and Cu are presumed to be anthropogenically influenced.

4. The sequential leaching experiment brings to the attention that different parts of each metal are bonded to exchangeable, reducible, and organic fractions of sediments. Metals bonded to these fractions are presumed to be more bioavailable and thus more harmfull to biota.

Acknowledgements

This work has been supported by grants from the Authority for Scientific Research of the SR of Croatia. It forms part of studies within the framework of MED POL activities, coordinated by U.N.E.P.'s Mediterranean Action Plan (Activity "L" of Phase II), and supported financially by the International Atomic Energy Agency (I.A.E.A.), Vienna.

References

BELLINGER, E.G. and B.R. BENHAM (1978). - The levels of metals in Borkyard sediments with particular reference to the contributions from ship-bottom paints, Environ. poll. 15, 71-81.

BULJAN, M. (1969). - Some hydrographic properties of the estuarine areas of the Krka and Zrmanja rivers (in Croatian, extented abstract in English), Krs Jugoslavije. 6, 303-330.

FOLK, R.L. and W.C. WARD, (1957). - Brazos River Bar : A study in the significance of grain size parameters, J. Sediment. Petrol. 27, 3-26

HADZIJA, O., M. JURACIC, M. LUIC, M. TONKOVIC and B. JERICEVIC (1984). - The carbohydrates in relation to mineralogic and granulometric composition of surface sediments in the karst estuary (River Krka estuary, Yugoslavia),Estuar. Coast. Shelf Sci. 21, in press.

HIRST, D.M. and G.D. NICHOLLS (1958). - Techniques in sedimentary geochemistry - 1. Separation of the detrital and non-detrital fractions of limestones, J. Sediment. Petrol. 28, 461-468.

JURACIC, M. and E. PROHIC (1984). - Sedimentation rate assessment in Krka River estuary from the sediment core analysis, Proc. of the 8th Chemistry of the Mediterranean, Primosten , Yugoslavia, May 16-24, 1984, 49-50.

PARFIT, G.D. and K.S.W. SING Eds. (1976). - Characterization of powder surfaces, Academic Press, London , 464 p.

PROHIC, E. (1984). - Trace element distribution in recent sediments of the Krka River estuary (in Croatian, abstract in English), Ph. D. Thesis, University of Zagreb.

SALOMONS, W. and U. FORSTNER (1984). - Trace metal analysis on polluted sediments, Part II : Evaluation of Environmental impact, Environ. Techn. Letters. 1, 506-517.

TESSIER, A., P.G.C. CAMPBELL and M. BISSON (1979). - Sequential extraction procedure for the speciation of particulate trace metals, Anal. Chem. 51, 844-851.

REMISE EN SUSPENSION DES PARTICULES A L'INTERFACE EAU-SEDIMENT EN MEDITERRANEE OCCIDENTALE CAMPAGNE PHYCEMED 83

par

Jean-Claude BRUN-COTTAN
Laboratoire de Physique et Chimie Marines
Université Pierre et Marie Curie, Paris (France)

Résumé

Au cours de la campagne PHYCEMED 81, des mesures de répartition granulométrique des particules en suspension ont été effectuées en pleine eau et de manière serrée dans les 100 premiers mètres au-dessus du fond. Ces mesures ont permis de caractériser les particules en suspension par leur concentration et par les paramètres de leur fonction de distribution granulométrique. Ces grandeurs caractéristiques présentent, à certaines stations, des variations significatives dans l'eau au-dessus du fond. Elles permettent la mise en évidence d'une variabilité temporelle des caractéristiques particulaires pouvant être de l'ordre de quelques heures. Cette variabilité et la forme des profils de concentration massique et des paramètres granulométriques, peuvent tout à fait correspondre à des phénomènes de remise en suspension discontinue, tels ceux rencontrés sur la pente continentale Est-Américaine.

*
* *

Introduction

En 1981 s'est déroulée la premmière campagne du programme PHYCEMED.

Pour l'ensemble des stations de la campagne PHYCEMED 81, une centatine de spectres de tailles des particules en suspension a été mesurée dans les premiers 100 mètres au-dessus du fond. Le système de mesure utilisé, un Coulter Counter lié à un analyseur 200 canaux par l'intermédiaire d'un amplificateur logarithmique (BRUN-COTTAN 76) permet une analyse très fine de la distribution granulométrique (fig. 2). L'ajustement au sens des moindres carrés de l'intégrale de cette distribution avec la loi Lognormale (BRUN-COTTAN 67, 75, LAMBERT et al. 81) montre (fig. 3) que cette loi décrit toujours très bien les échantillons analysés (coefficient de corrélation toujours supérieur à 0.92).

A partir des deux coefficients définissant la loi lognormale, moyenne et écart type (-9.17 et particules en fonction de leur taille (MAC CAVE 75, BRUN-COTTAN 76), il est aisé de calculer les paramètres relatifs aux particules mesurées (fig. 2).

Résultats

A partir des mesures effectuées et dans le cadre de cette campagne, les 2 paramètres qui paraissent les plus significatifs sont :

a) La concentration massique "C" (µg/kg), en masse humide, la division par 5 de cette valeur donne un ordre de grandeur de la concentration en masse sèche.

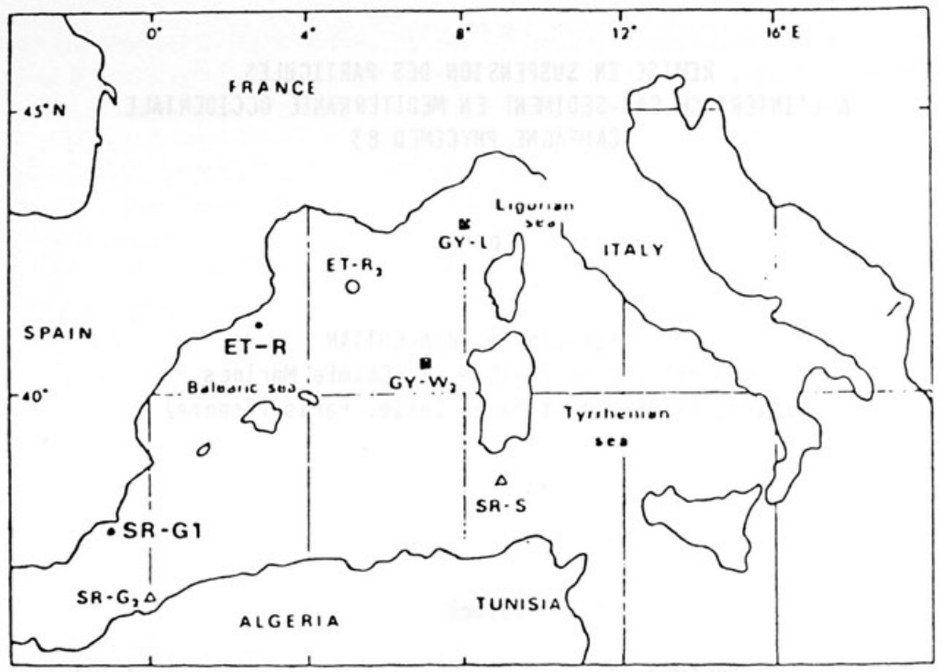


Figure 1. - Sampling sites, PHYCEMED cruise, April 1981. ○ estuary station : ET-R_2 Δ strait stations : SR-G_2 and SR-S ⊠ gyre circulation stations : GY-L and GY-W_2.

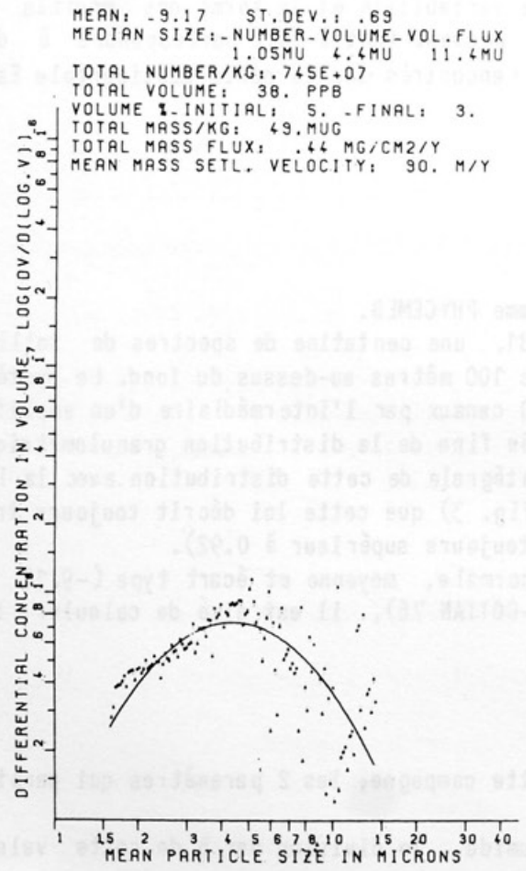


Figure 2.

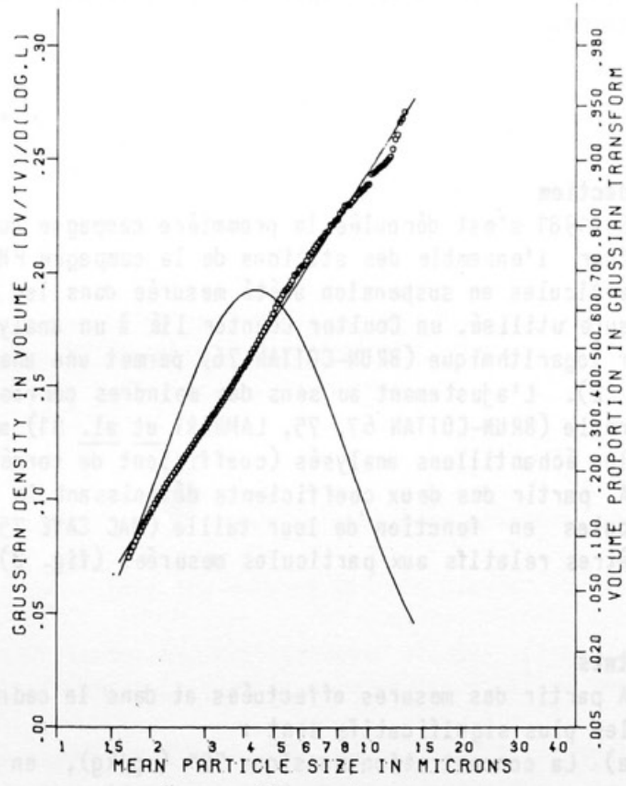


Figure 3.

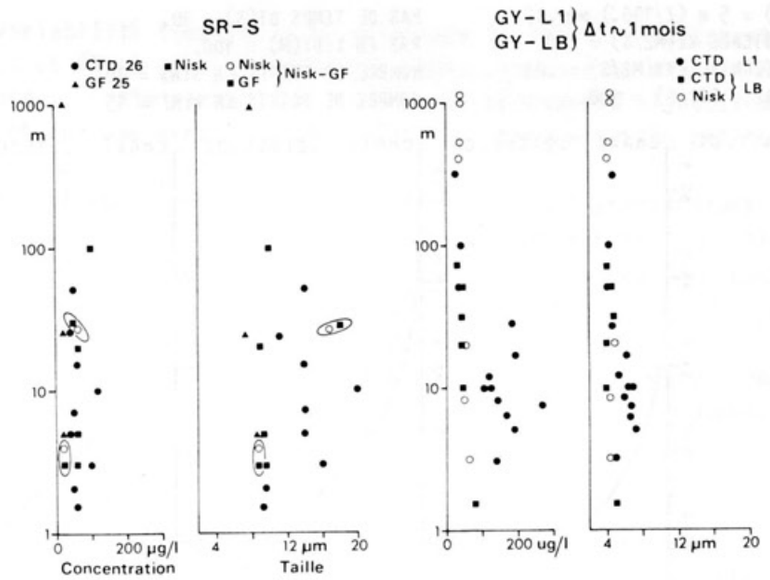


Figure 4.

b) La taille "l" (µm) de la particule de masse moyenne égale à $1/N \Sigma n_i m_i$ (4.4 dans la fig.2).

Les figures 4 et 5 montrent l'essentiel des résultats obtenus. Pour chaque station, sont représentées les variations de "C" et "l" en fonction de l'altitude en mètres au-dessus du fond. L'altitude est portée en échelle logarithmique : les valeurs de "C" sont dans la partie gauche et celles de "l" dans la partie droite.

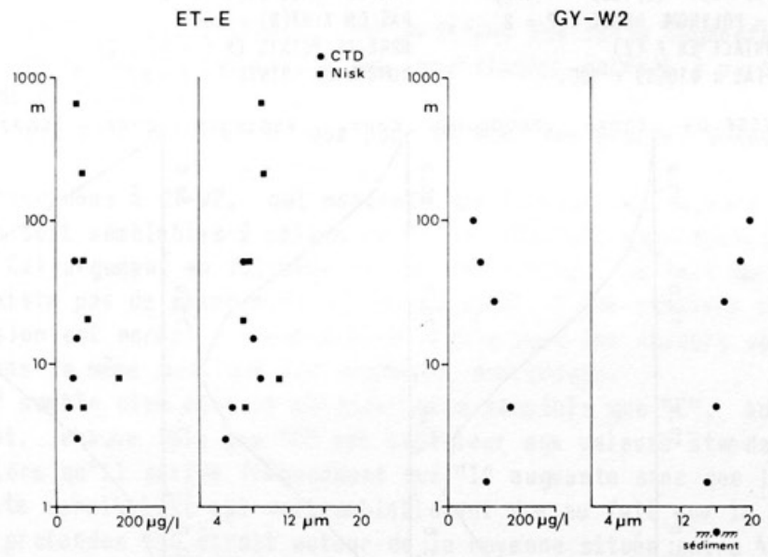
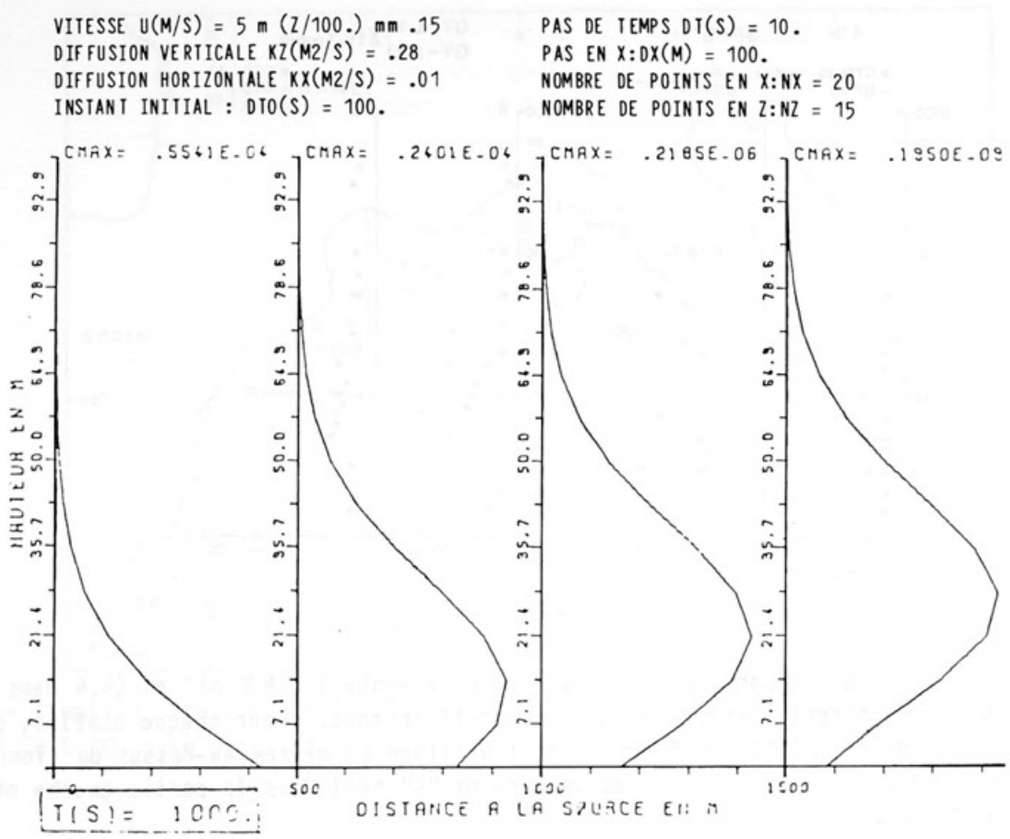


Figure 5.

Les faits majeurs observés sont :

1) la très bonne reproductibilité des résultats. En effet, à la station SR-S, une palanquée double de bouteilles Niskin ordinaires et go-flo, a été effectuée pour contrôler les éventuelles pollutions par la couche de surface, les valeurs entourées correspondent à des bouteilles séparées de 1 mètre.


VITESSE U(M/S) = 5 m (Z/100.) mm .15
DIFFUSION VERTICALE KZ(M2/S) = .28
DIFFUSION HORIZONTALE KX(M2/S) = .01
INSTANT INITIAL : DTO(S) = 100.
PAS DE TEMPS DT(S) = 10.
PAS EN X:DX(M) = 100.
NOMBRE DE POINTS EN X:NX = 20
NOMBRE DE POINTS EN Z:NZ = 15
CMAX= .5541E-04
CMAX= .2401E-04
CMAX= .2185E-06
CMAX= .1950E-09
HAUTEUR EN M
92.9
78.6
64.3
50.0
35.7
21.4
7.1
0
500
1000
1500
T(S)= 1000.
DISTANCE A LA SOURCE EN M

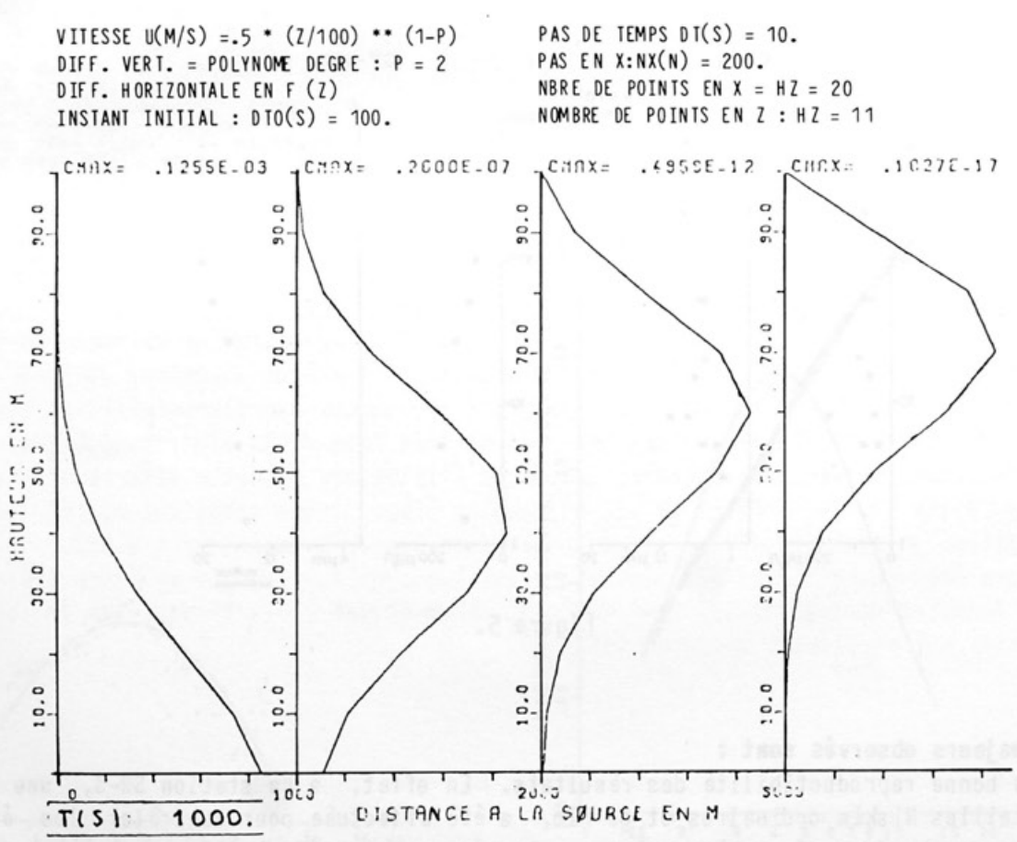

VITESSE U(M/S) =.5 * (Z/100) ** (1-P)
DIFF. VERT. = POLYNOME DEGRE : P = 2
DIFF. HORIZONTALE EN F (Z)
INSTANT INITIAL : DTO(S) = 100.
PAS DE TEMPS DT(S) = 10.
PAS EN X:NX(N) = 200.
NBRE DE POINTS EN X = HZ = 20
NOMBRE DE POINTS EN Z : HZ = 11
CMAX= .1255E-03
CMAX= .2000E-07
CMAX= .4955E-12
CMAX= .1027E-17
HAUTEUR EN M
90.0
70.0
50.0
30.0
10.0
0
1000
2000
3000
T(S)= 1000.
DISTANCE A LA SOURCE EN M

Figure 6.

2) La forte variabilité temporelle au voisinage du fond (et seulement là). Elle se voit à la station GY-L (GY-L1 et GY-LB sont séparées d'un mois),comme à la station SR-S où l'échelle de temps est de quelques heures. Cette variabilité temporelle correspond effectivement aux fluctuations naturelles du milieu et non aux erreurs expérimentales, toujours très petites devant l'échelle de ces fluctuations.

3) Les caractéristiques des particules, dans les 2 à 3 premiers mètres au-dessus du fond,sont voisines de celles des particules situées au-dessus de 100 mètres. Ce phénomène a déjà été observé lors de la campagne RACACA en 79, sur la pente continentale de New York Bight entre Long Island et le cap Hatteras. Cette campagne était entièrement dévolue à l'étude des particules en suspension dans la zone à faible taux de Radon 222 située dans cette région.

4) Aux valeurs les plus élevées de la concentration en particules. dans les 100 mètres au-dessus du fond, correspondent aussi les valeurs les plus fortes du diamètre moyen.

Discussion

La station GY-L1 présente des profils de "C" et "1", homogènes jusqu'au fond,typiques des suspensions situées normalement dans les eaux profondes. La station effectuée au même endroit, un mois plus tard (GY-LB), montre une augmentation notable de la concentration entre 5 et 30 mètres du fond, avec un glissement de la moyenne "1" vers les grandes tailles. Ceci paraît caractéristique d'une remise en suspension des particules sédimentées. La concentration en Scandium particulaire, caractéristique des argiles, mesurée dans le même temps par l'équipe de R. CHESSELET du CFR, augmente parallèlement et dans le même rapport, de .2 à .5 ng/kg. L'augmentation de la taille moyenne est caractéristique, elle aussi, de particules d'origine sédimentaire : elle dépend de la granulométrie de la surface du sédiment, on pourrait dire de sa peau, de la conservation partielle de cette granulométrie après la remise en suspension et du taux de dilution des particules remises en suspension par rapport à celui des particules "primitives" en place dans l'eau. Ce point important paraît assez bien établi par :

- Les résultats de la campagne RACACA, qui montrent une forte augmentation de "1", là où le coefficient de mélange turbulent est très élevé (coefficient calculé à partir du déficit en Ra222 mesuré par l'équipe de P. BISCAYE).
- Le fait que, tant pour PHYCEMED 81 que pour RACACA, les grandes valeurs de "1" n'apparaissent que près du fond.
- Les mesures effectuées à GY-W2, qui montrent que lorsque les valeurs de "1" de l'eau profonde sont élevées, elles sont semblables à celles du "1" du sédiment superficiel (eau surnagente dans un carottier Reineck). Cet argument en lui-même est un peu faible, du fait qu'il ne correspond qu'à un profil, et qu'il n'existe pas de mesures de "1" du sédiment, à des stations comme GY-L, où le "1" des particules en suspension est normal, c'est-à-dire faible avec des valeurs voisines de 4 à 6µm ; par contre, il va bien dans le même sens que les arguments précédents.

Le paramètre "1" semble bien être un marqueur plus sensible que "C", au phénomène de remise en suspension ; en effet, chaque fois que "C" est supérieur aux valeurs standard, "1" augmente dans le même temps (ET-E) alors qu'il arrive fréquemment que "1" augmente sans que la concentration varie de manière visible. Cette sensibilité est vraisemblablement due au fait que le spectre des tailles des particules marines profondes est étroit autour de la moyenne située entre 4 et 8 µm, c'est-à-dire très pauvre en grosses particules ; l'injection de particules ayant un spectre plus plat est centré sur des valeurs beaucoup plus élevées, de l'ordre de 20 à 100 µm, peut déplacer facilement la moyenne vers 15 à 20 µm, sans que l'effet sur la concentration sorte des fluctuations aléatoires autour du bruit de fond.

Tant à PHYCEMED 81 qu'à RACACA, on peut observer que les valeurs maximales de "1", et parfois de "C", sont rarement au ras du fond, mais le plus souvent à une hauteur située entre 3 et 30 mètres au-dessus du fond.

La forme de ces profils, liée à leur variabilité temporelle, nous a amenés à concevoir un modèle de remise en suspension en mode impulsionnel, par bouffées de sédiments, éparses dans le temps et

dans l'espace. Ce modèle numérique a été développé par C. PERIGAUD dans la partie de sa thèse de Docteur Ingénieur consacrée à la mission RACACA. Le modèle permet de retrouver la forme générale des profils observés à RACACA et donc à PHYCEMED 81 (PERIGAUD, BRUN-COTTAN 82, PERIGAUD 83). La figure 6 montre les profils de la concentration en sédiment remis en suspension 1.000 secondes après l'injection supposée instantanée et ponctuelle à l'échelle considérée : le coefficient de mélange vertical (calculé à partir des profils de Ra 222 par P. BISCAYE) est supposé constant dans la couche de mélange (fig. 6 haut) ou parabolique (fig. 6 bas). L'application de ce modèle à la Méditerranée exigerait la connaissance des valeurs du coefficient de mélange au voisinage du fond, ce qui n'est pas le cas actuellement. La similitude entre les profils "particules" profonds en Atlantique ouest et en Méditerranée permet cependant d'avancer que, qualitativement, les processus de remise en suspension en Méditerranée sont vraisemblablement aussi impulsionnels.

Références

J.C. BRUN-COTTAN, (1967). - Influence du marquage radioactif sur les paramètres dynamiques des sédiments pélitiques. Thèse de 3e cycle en océanographie.

J.C. BRUN-COTTAN, (1976). - Contribution à l'étude de la granulométrie et de la cinétique des particules marines. - Thèse de Doctorat d'Etat, Université Pierre et Marie Curie.

J.C. BRUN-COTTAN, (1976). - Stokes settling and dissolution rate model for marine particles as a function of size distribution. Journal of Geophisical Res. vol. 81 N° 9, p. 1601-1605.

C. LAMBERT, C. JEHANNO, N. SILVERBERG, J.C. BRUN-COTTAN and R. CHESSELET. - Lognormal distribution of suspended particles in the Ocean. Journal of Marine Research, vol. 30, 1, 1981, p. 77-98.

I.N. MAC CAVE. - Vertical flux of particles in the Ocean Deep-Sea Research, 1975, vol. 22, p. 491-502.

C. PERIGAUD, J.C. BRUN-COTTAN, P. BISCAYE and S. CARSON. - Resuspension process of sediment from the botton, in the New York Bight. EUROMECH 156, The mechanics of sediment transport, 12-14 juillet 1982.

C. PERIGAUD. - Mécanismes d'érosion des sédiments cohésifs sous l'action d'un courant. Thèse de Docteur-Ingénieur, Ecole Nationale des Ponts et Chaussées, 1982.

EFFETS DE LA DYNAMIQUE MEDITERRANEENNE SUR LE DEVENIR D'APPORTS ANTHROPIQUES

par

J.P. BETHOUX et G. COPIN-MONTEGUT
Laboratoire de Physique et Chimie Marines
Université Paris VI, U.A. C.N.R.S.
B.P. n° 8 - 06230 Villefranche-sur-Mer (France)

Résumé

Une modélisation simple, basée sur le schéma connu de la circulation des eaux et sur le devenir d'éléments chimiques dans le milieu marin, permet d'évaluer le temps nécessaire à la modification des concentrations marines profondes, à la suite d'apports telluriques ou atmosphériques. Malgré un temps de résidence des eaux relativement bref, notamment dans le bassin Occidental, le temps de réponse, pour un élément conservatif, comme pour un élément nutritif, est de plus d'un siècle. Les concentrations mesurées actuellement dans les eaux profondes ne reflètent donc que très partiellement la phase de progression des activités industrielles et agricoles des pays riverains de la Méditerranée, lors des années 60 et 70. La simulation d'une diminution des rejets montre également un retard de plusieurs décennies avant que les concentrations marines ne commencent à décroître.

*
* *

Introduction

Les principaux flux de la circulation en Méditerranée ont été précédemment évalués en utilisant notamment les variations géographiques des bilans en eau et en sel (BETHOUX, 1979, 1980 a). Les circulations horizontales superficielles et profondes sont intenses, très amplifiées par rapport à la cause initiale : le déficit en eau. Elles amènent à évaluer des temps de résidence des eaux (quotient du volume du bassin par les flux entrants ou sortants) d'environ 70 ans pour l'ensemble de la Méditerranée, de 60 ans pour le bassin Oriental, mais de seulement 15 ans pour le bassin Occidental. Un temps de résidence de 97 ans pour l'ensemble de la Méditerranée a, par ailleurs, été déduit de mesures directes de courant à Gilbraltar (LACOMBE et al., 1981). Ces échelles de temps sont faibles par rapport à celles des grands océans, elles devraient permettre d'étudier. a priori plus facilement, le devenir de certains éléments dont les rejets telluriques et atmosphériques ont fortement augmenté depuis le début de ce siècle, et notamment lors des années 1960-1980. Cependant, alors que le temps de résidence "hydrologique" ne fait intervenir que les flux horizontaux aux limites du bassin, le temps de réponse "bio-géochimique" englobe également les transferts verticaux entre couches superficielles et profondes qui dépendent d'une part du devenir de l'élément dans le milieu marin et, d'autre part, de la dynamique marine verticale. Les flux horizontaux ainsi que les processus de formation d'eaux profondes engendrent effectivement des mouvements verticaux qui mettent en relation les couches superficielles et profondes. Ainsi l'eau profonde sortant de la Méditerranée, constituée d'eau intermédiaire d'origine orientale et d'eau superficielle occidentale, entraîne rapidement une partie des apports anthropiques en direction de l'Atlantique.

Dans un précédent travail sur le cycle du phosphore (BETHOUX, 1980 b), l'évaluation quantitative et la localisation des rejets telluriques (U.N.E.P., 1977) permettaient d'expliquer d'une part les concentrations observées dans les eaux profondes, ainsi que leurs variations géographiques, et, d'autre part, le fort flux net de phospore de la Méditerranée vers l'Atlantique. Cette étude reposait sur l'hypothèse initiale d'un bilan équilibré en phosphore, ce qui ne peut constituer qu'une première approximation compte tenu de l'augmentation probable des rejets telluriques au cours des dernières décennies. Au cours des récentes missions océanographiques PHYCEMED 1 et 2 (en 1981 et 1983), les concentrations superficielles et profondes en différents éléments anthropiques ont été mesurées en Méditerranée. Elles pourraient servir à évaluer les apports telluriques et atmosphériques à la mer (dont l'estimation "terrestre" est délicate) et à prévoir le devenir marin de ces éléments, sous réserve qu'un temps de réponse ait été évalué.

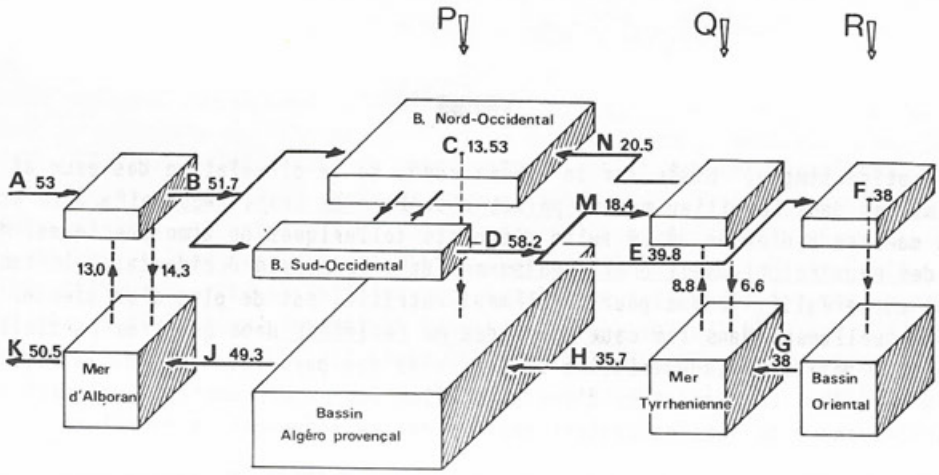


Figure 1 - Eléments de modélisation du cycle de traceurs chimiques en Méditerranée. Les flux horizontaux et verticaux retenus sont exprimés en $10^{12} m^3/an$. Les lettres A, B,...N caractérisent les concentrations en l'élément étudié. Les apports telluriques et atmosphériques sont notés P dans le bassin Algéro-provençal, Q en mer Tyrrhénienne et R dans le bassin Oriental

Méthode

Afin de simuler l'évolution dans le temps des concentrations marines, on a adopté un système à deux couches (0-125 m et 125-fond) dans quatre bassins : l'Oriental, la mer Tyrrhénienne, le bassin Algéro-provençal et la mer d'Alboran. La mer d'Alboran est considérée comme une entité à cause des intenses mouvements verticaux qui s'y produisent et qui ont une influence sur le contenu des eaux, tant méditerranéennes sortantes qu'atlantiques entrantes. Dans le bassin Oriental et le bassin Occidental, la couche superficielle n'est pas homogène par suite de multiples circulations et de l'hétérogénéité spatiale des apports telluriques. Le modèle simplifié proposé, par suite des hypothèses faites et de la fixation a priori de certaines concentrations superficielles, n'est pas représentatif de la couche de surface mais permet cependant de suivre l'évolution des concentrations profondes. Sur la figure 1, sont reportés les flux horizontaux et verticaux utilisés. Dans le bassin Occidental, on prend en compte les flux verticaux en mer Tyrrhénienne et la formation d'eaux profondes nord-occidentales dont l'évaluation, basée sur les variations de salinité aux limites du bassin Algéro-provençal, englobe également les diffusions verticales liées aux circulations cycloniques du golfe de Gênes, du golfe du Lion et de la mer Catalane. Dans le bassin Oriental, simplifié à l'extrême, on ne considère que les mouvements verticaux liés à la formation d'eaux denses dans le bassin Levantin et en mer Adriatique.

Les lettres A, B,... sont relatives aux différentes concentrations de l'élément chimique considéré. Les transferts entre la couche superficielle et la couche profonde sont liés d'une part aux mouvements verticaux des masses d'eaux (définis sur la fig. 1) et d'autre part à l'activité biogéochimique qui souvent se détermine à partir des concentrations observées. Les apports anthropiques sont notés P dans le bassin Algéro-provençal, Q en mer Tyrrhénienne et R dans le bassin Oriental. Ils sont supposés négligeables en mer d'Alboran vis-à-vis des flux atlantiques et méditerranéens.

Deux éléments, le sel et le phospore, l'un conservatif, l'autre très actif biologiquement, permettent de mieux comprendre le fonctionnement de la Méditerranée et d'évaluer les temps de réponse "bio-géochimiques" des grands bassins, consécutivement à une modification de l'état stationnaire initial. Un troisième élément, l'oxygène dissous, dont la concentration est directement liée à la formation des eaux profondes d'une part et, d'autre part, à la régénération de la matière organique, permet une approche complémentaire de l'évaluation des apports en éléments nutritifs.

Résultats

1. Cas d'un élément conservatif, le sel

Les variations géographiques de salinité ont déjà été utilisées pour établir le schéma de circulation proposé (figure 1). L'élément conservatif "sel" permet donc seulement de vérifier la cohérence du modèle et d'étudier le temps nécessaire pour atteindre des concentrations constantes, par exemple les salinités profondes actuelles, à la suite d'une modification initiale arbitraire de la salinité des eaux.

Les apports telluriques et atmosphériques en sel, P, Q et R, peuvent être négligés par rapport aux flux de sel à travers les détroits de Gibraltar et de Sicile. La circulation superficielle du bassin Occidental est complexe par suite des diverses communications entre la mer Tyrrhénienne, le golfe du Lion et le bassin Algéro-provençal. Dans ce travail, pour simplifier, on relie les salinités superficielles, C dans la zone de formation d'eau profonde, D au détroit de Sardaigne, E au détroit de Sicile et N au canal de Corse, à la salinité B en mer d'Alboran, en respectant l'équilibre actuel (BETHOUX, 1980 a). Les volumes des eaux superficielles et profondes du bassin Oriental sont (exprimés en 10^{14} m^3) respectivement de 2 et 21. Ceux des eaux profondes tyrrhéniennes et algéro-provençales sont respectivement de 4 et 9. La couche superficielle est supposée à une densité de 1,027 et celle de la couche profonde est de 1,029. Le système d'équations suivantes permet de décrire l'évolution des salinités en Méditerranée, sous l'effet des bilans en eau (bassins de concentration), à l'échelle de temps, I, annuelle, à partir d'une salinité initiale supposée, par exemple égale à celle de l'eau atlantique entrante, A = 36,18.

$$P = Q = R = 0$$
$$A = 36{,}18$$
$$B(I) = 0{,}779\ A + 0{,}111\ J(I-1) + 0{,}111\ K(I-1)$$
$$C(I) = 1{,}038\ B(I) \quad \text{(donnée)}$$
$$D(I) = 1{,}013\ B(I) \quad \text{(donnée)}$$
$$E(I) = 1{,}011\ B(I) \quad \text{(donnée)}$$
$$F(I) = 0{,}810\ F(I-1) + 0{,}201\ B(I-1)$$
$$G(I) = 0{,}982\ G(I-1) + 0{,}018\ F(I-1)$$
$$M(I) = 1{,}016\ B(I)$$
$$N(I) = 1{,}037\ B(I) \quad \text{(donnée)}$$
$$H(I) = 0{,}899\ H(I-1) + 0{,}084\ G(I-1) + 0{,}017\ B(I-1)$$
$$J(I) = 0{,}945\ J(I-1) + 0{,}040\ H(I-1) + 0{,}016\ B(I-1)$$
$$K(I) = 0{,}750\ J(I) + 0{,}125\ A + 0{,}125\ B(I)$$

Sur la figure 2, les valeurs actuelles de salinité des eaux profondes G (au détroit de Sicile), J (dans le bassin Algéro-provençal) et K (à Gibraltar) sont atteintes en près de 400 ans, soit entre 4 et 6 fois le temps de résidence des eaux de l'ensemble de la Méditerranée. Les 9/10e de l'évolution de la salinité sont couverts en environ 175 ans, tant dans le bassin Occidental que dans l'Oriental. Ce calcul s'applique à tout élément conservatif n'ayant pas d'incidence notable sur la densité des

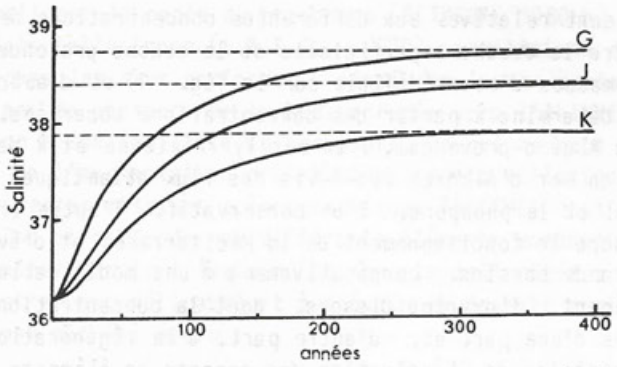


Figure 2 - Evolutions dans le temps de la salinité des eaux profondes G (B.Oriental), J (B. Algéro-provençal) et K (M.d'Alboran), vers les valeurs actuelles (respectivement 38,74- 38,40 et 37,90) sous l'effet du seul déficit en eau de la Méditerranée, en supposant des salinités initiales partout égales à la salinité de l'eau atlantique entrante, soit 36,18 .

eaux (élément trace). Il ne peut donc décrire réellement l'évolution de la salinité, dont toute modification, par son effet sur la densité, entraîne un changement des courants et rend caduc le modèle ici proposé, basé sur la stationnarité de la circulation. Bien que la Méditerranée réagisse plus rapidement que les autres océans, la concentration finale est relativement longue à atteindre. A notre échelle de temps, un élément conservatif d'origine anthrophique ne peut donc avoir une concentration stationnaire, compte tenu de l'évolution probable des apports au cours des dernières décennies.

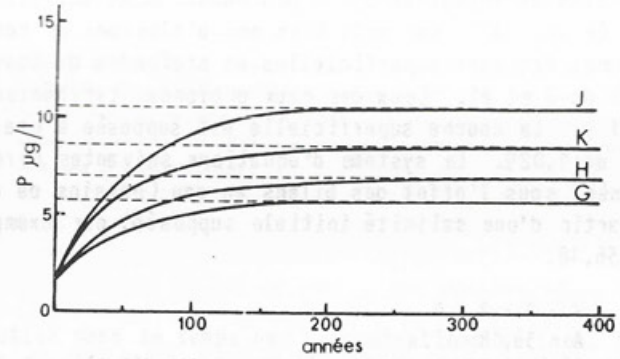


Figure 3 - Evolutions dans le temps des concentrations des eaux profondes en phosphore G (B.Oriental), H (M.Tyrrhénienne), J (B.Algéro-provençal) et K (M.d'Alboran) vers des valeurs actuelles, sous l'effet des apports telluriques, en supposant des concentrations initiales partout égales à la teneur des eaux atlantiques entrantes, soit environ 1,55 10^{-4}kg/m^3.

2. Cas d'un élément nutritif, le phosphore

Les apports telluriques en phosphore sont importants (P + Q + R = 3,8 10^8 kg/an, U.N.E.P., 1977, 1984) et les apports atlantiques superficiels sont faibles, bien qu'un possible enrichissement par des eaux sub-superficielles ait été évoqué à la suite des mesures de la mission MEDIPROD 4 (COPIN-MONTEGUT et al., 1982). Le phosphore n'est pas conservatif dans la couche de surface puisqu'il est biologiquement utilisé et entraîné avec les particules (détritus biologiques, pelottes fécales...) dans la couche profonde où il est reminéralisé et conservatif. Ce processus d'utilisation est relativement rapide puisque, dans les eaux superficielles aux détroits de Sardaigne et de Sicile, les concentrations en phosphate D et E ne montrent pas trace des enrichissements subis en mer d'Alboran

et dans le bassin Nord-Occidental, par mécanismes dynamiques et par rejets telluriques. Un premier essai de modélisation, en répartissant les apports P, Q et R sur toute la colonne d'eau, s'est traduit par des concentrations trop fortes dans la couche de surface (et trop faibles en profondeur). En conséquence, compte tenu de cet essai et de l'échelle de temps annuelle. on suppose un transfert direct de P, Q, et R dans les couches profondes. Les bilans de la Méditerranée, du bassin Oriental et de la mer Tyrrhénienne nous font adopter les conditions initiales suivantes pour les concentrations superficielles en phosphore :

$A = 1{,}55\ 10^{-6}$ kg/m^3 : $E = 0{,}6\ A$; $D(I) = 0{,}25\ A + 0{,}25\ B(I)$: $M(I) = N(I) = C(I)$:

Le système d'équations est alors :

$P = 1{,}4\ 10^{8}$ kg/an · $Q = 0{,}3\ 10^{8}$ kg/an · $R = 1{,}9\ 10^{8}$ kg/an (données U.N.E.P., 1984)
$B(I) = 0{,}779\ A \quad 0{,}111\ J(I-1) \quad 0{,}111\ K(I-1)$
$M(I) = 3{,}163\ D(I) - 1{,}298\ A$
$G(I) = 0{,}982\ G(I-1) \quad 0{,}011\ A \quad 0{,}476\ R$
$H(I) = 0{,}911\ H(I-1) + 0{,}095\ G(I-1) - 0{,}005\ M(I-1) + 0{,}25\ Q$
$J(I) = 0{,}945\ J(I-1) \quad 0{,}040\ H(I-1) \quad 0{,}111\ P \quad 0{,}058\ B(I-1) - 0{,}065\ D(I-1) \quad 0{,}023\ M(I-1)$
$K(I) = 0{,}750\ J(I) + 0{,}125\ A + 0{,}125\ B(I)$

Les évolutions des concentrations G, H, J, et K des eaux profondes en fonction du temps, sous l'effet des apports en phosphore des eaux atlantiques et des rejets telluriques P, Q et R, sont tracées sur la figure 3. Des valeurs proches des concentrations actuelles (respectivement 5,9 - 7 - 10,8 - et 8,7 10^{6} kg/m^3) sont atteintes en environ 300 ans, tandis que les $9/10^{e}$ de l'augmentation sont acquis en 120 ans. Pour un élément nutritif, de même que pour un élément conservatif, les concentrations mesurées ne peuvent correspondre à un état stationnaire.

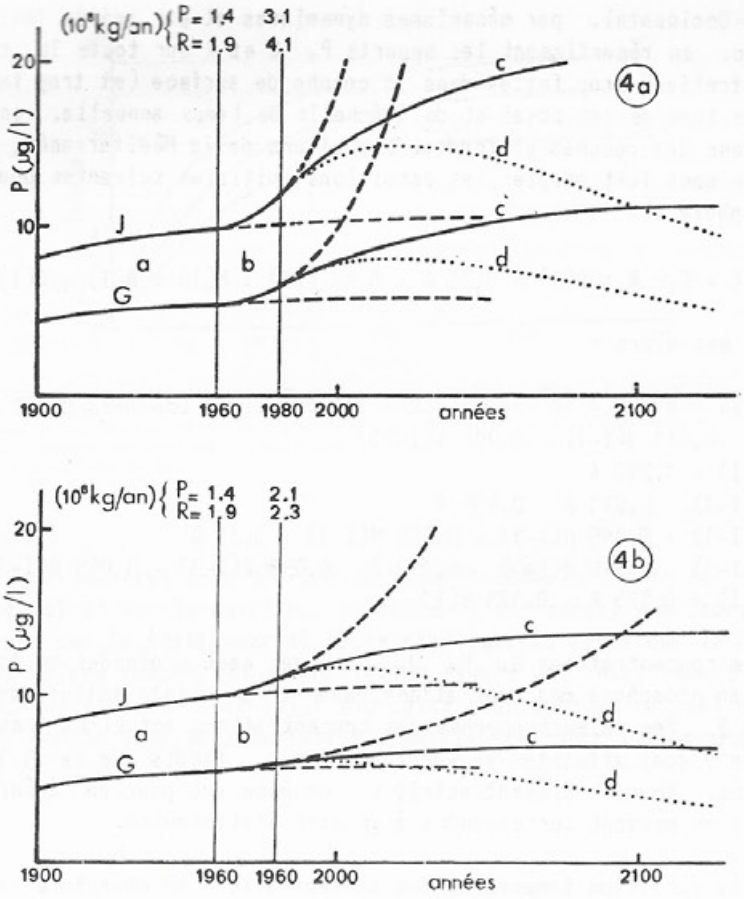
3. Simulation de la variation temporelle des concentrations en phosphore liée à l'activité humaine

Le modèle simple proposé permet de simuler les variations temporelles des concentrations marines, par exemple en phosphore, sous réserve de connaître un schéma d'évolution des apports telluriques, dont les seules données disponibles sont celles de l'U.N.E.P. (1984). Les enquêtes internationales nécessitant souvent un délai important, et les rejets pouvant être minimisés, on peut supposer que les données U.N.E.P. se rapportent aux rejets effectués vers 1960. début de la période de forte croissance industrielle, agricole et urbaine.

A titre d'exemple. sur les figures 4a et 4b. ont été portées les évolutions des concentrations en phosphore des eaux profondes orientales, G, et occidentales, J, calculées à partir des hypothèses suivantes :

- les rejets telluriques annuels en phosphore, P, Q, et R, sont considérés comme constants de 1900 à 1960 et égaux aux évaluations de l'U.N.E.P. En conséquence. dans l'hypothèse d'un apport atlantique inchangé, les concentrations G et J en 1900 sont prises égales aux 3/4 des valeurs actuelles.
- de 1960 à 1980, P, Q et R subissent une augmentation continue, soit de 4 % par an, (figure 4a, hypothèse maximaliste), soit de 2 % par an pour P et Q, et de 1 % par an pour R (figure 4b, hypothèse minimaliste).
- après 1980 les rejets P, Q et R soit restent constants, à leur niveau de 1980, soit diminuent de 1% par an.

Entre les années 1900 et 1960, les courbes "a" décrivent l'augmentation des concentrations (initialement supposées égales aux 3/4 des valeurs actuelles), sous l'effet d'apports telluriques constants. Après 1960, lors de la phase supposée de progression des rejets, l'augmentation des concentrations (courbes "b") est tout d'abord modérée puis s'amplifie fortement. Dans le cas de l'augmentation des apports de 4 % par an, P et R ont plus que doublé au cours de la période 1960-1980, tandis que le doublement prévisible des concentrations (courbes "c") ne devrait être observé



Figures 4 a et 4 b - Evolutions dans le temps des concentrations des eaux profondes G (orientale) et J (occidentale) en phosphore, en fonction des apports telluriques P,Q et R. Courbes "a": les apports sont constants jusqu'en 1960 et égaux aux estimations (UNEP,1984). Courbes "b": les apports augmentent de 4% par an (fig. 4a) ou P et Q de 2% et R de 1% par an (fig. 4b). Courbes "c": les apports restent constants, aux valeurs atteintes en 1980. Courbes "d": les apports décroissent de 1% par an à partir de 1980.

que plus d'un siècle plus tard, dans l'hypothèse où les rejets seraient maintenus à leur niveau de 1980. Si l'on admet que la phase de croissance économique des années 1960-1980 s'est traduite par une augmentation notable des rejets telluriques, il y a un déphasage entre les concentrations mesurées actuellement dans les eaux profondes de la Méditerranée et celles qui correspondront à l'atteinte ultérieure d'un hypothétique état stationnaire. Cette constatation peut expliquer l'accord relatif d'un bilan supposé à l'équilibre (BETHOUX, 1980 b), calculé avec les concentrations mesurées principalement entre 1965 et 1975 d'une part, et des rejets qui, bien que seulement connus en 1977, doivent relever d'une période antérieure. Les courbes "d" correspondent à l'hypothèse optimiste d'une diminution des rejets de 1 % par an à partir de l'année 1980. On peut remarquer que les concentrations dans les eaux profondes continuent d'augmenter pendant encore près de 40 ans (fig. 4a) et 25 ans (fig. 4b), avant l'amorce d'une décroissance. Seule la connaissance socio-économique des activités industrielles, agricoles et urbaines des pays riverains permettrait de fixer l'évolution des rejets anthropiques en Méditerranée, et donc de prévoir la variation des concentrations marines en différents éléments. Les mesures marines, ponctuelles dans le temps et l'espace, pourraient alors confirmer les tendances calculées sur une échelle de temps supérieure à la décennie.

	Bassin Oriental	Bassin Occidental
1 zones	b. Levantin, Adriatique	b. Nord-Occidental
2 ml O_2/l	6 6,5	6,4
3 ml O_2/l	(6,3) 5,9	5 6,4
4 ml O_2/l	G = 4,4	J = 4,3
5 consommation ml O_2/l	1,5	0,7 2,1
6 productivité gr C/m^2/an	14,3	16,9 50,7
7 fertilité potentielle gr C/m^2/an	6	36
8 consommation en P 10^6 kg P/an	583	1060
9 apports P Unep 1977 10^6 kg P/an	122	235

Tableau 1

4. L'oxygène dissous

La diminution de la concentration en oxygène des eaux profondes, liée à la reminéralisation de la matière organique, permet également une évaluation du phosphore utilisé, dont le montant résulte des apports et recyclages hydrologiques et biologiques. Les données concernant le bassin Oriental et le bassin Occidental sont résumées sur le tableau 1. A la ligne 1, sont rappelées les zones de formation d'eaux denses et, ligne 2, les concentrations atteintes en oxygène, compte tenu des caractéristiques (T, S) des eaux (MILLER et al., 1970) et de l'hypothèse d'une sursaturation de 10 %. A la ligne 3 figure la concentration en oxygène des eaux profondes néo-formées. Dans le bassin Oriental, la concentration théorique serait de 6,3 ml/l, mais, par suite de la diminution de l'oxygène de la couche d'eau levantine lors de son cheminement sub-superficiel vers l'ouest, de 6 à environ 5 ml/l, la concentration résultante en mer Ionienne n'est que de 5,9 ml/l. Dans le bassin Occidental, la concentration des eaux néo-formées est de 6,4 ml/l si toutes les eaux ont été sursaturées en surface, et de seulement 5 ml/l si les eaux intermédiaires ne passent pas en surface avant de plonger. A la ligne 4 sont reportées les concentrations G et J des eaux profondes. La différence des concentrations des lignes 3 et 4 donne la consommation en oxygène (ligne 5) qui, compte tenu des flux annuels, du rapport de Redfield et de la superficie des bassins, permet de passer à la productivité (ligne 6). A la ligne 7 sont rappelées les fertilités potentielles, basées sur l'utilisation de tout le phosphore disponible dans chaque bassin (apports et recyclage hydrologiques). La différence des lignes 6 et 7 donne une estimation de la production liée au recyclage biologique qui représente respectivement 58 et 29 % de la productivité totale dans le bassin Oriental et dans le bassin Occidental. Selon la synthèse de SOURNIA (1973), le recyclage biologique peut effectivement représenter entre 28 et 77 % de la productivité totale. Dans le bassin Occidental, la productivité déduite de la valeur minimale (5 ml/l) de la concentration en oxygène des eaux profondes néo-formées constitue une sous évaluation inférieure à la fertilité potentielle du bassin. A la ligne 8 du tableau sont portées les quantités de phosphore correspondant aux productivités calculées (ligne 6), et, à la ligne 9, les apports définis par l'U.N.E.P. (1977). La fertilité potentielle présumait l'utilisation de plus de 3 fois les apports telluriques, par suite du recyclage hydrologique (BETHOUX, 1980 b). La productivité déduite de la consommation en oxygène introduit un facteur supplémentaire lié au recyclage biologique, et l'utilisation de près de cinq fois les apports de l'U.N.E.P. Dans la mesure où le phosphore du recyclage hydrologique n'est certainement pas utilisé en totalité (période hivernale et turbulence forte), notamment dans le bassin Occidental, les taux actuels de productivité permettraient de supposer des apports telluriques en phosphore plus importants. L'oxygène dépend, pour son renouvellement, de la formation des eaux profondes. Sa variation de concentration en profondeur, liée à une variation des rejets telluriques et de la productivité, serait peut-être plus facile à mesurer que celle des concentrations profondes en phosphore.

Conclusion

Les flux horizontaux et verticaux sont intenses en Méditerranée, et, malgré une profondeur moyenne importante des différents bassins, le temps de résidence des eaux y est relativement bref (entre 15 et 70 ans environ). Une modélisation simplifiée de la circulation permet de montrer que toute modification d'apport en élément conservatif nécessite près de 175 ans pour que les 9/10e de la variation attendue des concentrations en cet élément soient atteints. Dans le cas d'un élément nutritif, non conservatif dans la couche de surface, le temps de réponse équivalent est de 120 ans, dans l'hypothèse d'apports constants. Un essai de simulation des conséquences d'une augmentation continue d'apports telluriques (ou atmosphériques) montre un temps de retard d'environ une décennie, avant que les concentrations profondes ne commencent à augmenter nettement. Dans l'hypothèse où les apports auraient doublé entre les années 1960-1980, par suite de l'industrialisation des pays riverains de la Méditerranée, puis auraient été stabilisés, le doublement de concentration dans les eaux profondes ne devrait être approché que dans plus d'un siècle pour le phosphore. De même, si les rejets diminuaient, il faudrait attendre plusieurs décennies pour observer une diminution de concentration des eaux profondes en phosphore. Compte tenu de l'augmentation probable des rejets anthropiques au cours des dernières décennies, les concentrations marines actuelles dans les eaux profondes ne correspondent donc pas à un état stationnaire. Elles permettent cependant un premier bilan global des apports, quelquefois méconnus à l'échelle d'une région telle la Méditerranée. Par ailleurs dans l'hypothèse où un schéma d'évolution des rejets anthropiques serait établi, la connaissance des concentrations actuelles permettrait la prévision à plus long terme du niveau de pollution marine.

L'oxygène dont la concentration en profondeur est liée d'une part aux apports superficiels, lors de la formation d'eau profonde, et. d'autre part, à sa consommation lors de la reminéralisation de la matière organique, pourrait être un traceur intéressant en Méditerranée.

Références

J.P. BETHOUX, 1979. - Budgets of the Mediterranean Sea. Their dependance on the local climate and on the characteristics of the Atlantic waters. Oceanologica Acta, 2, 157-163.

J.P. BETHOUX, 1980. - Mean water fluxes across section in the Mediterranean Sea. evaluated on the basis of water and salt budgets and of observed salinities. Oceanologica Acta, 3, 79-88.

J.P. BETHOUX, 1980. - Le cycle du phosphore dans le bassin Occidental de la mer Méditerranée, modèle d'étude du devenir de rejets telluriques. Ves Journées Etud. Pollution C.I.E.S.M., 997-1004.

G. COPIN-MONTEGUT, B. COSTE, J.C. GASCARD, J. GOSTAN, P. LECORRE, H.J. MINAS, T. PACKARD, A. POISSON, 1982. - Nutrient regeneration and circulation patterns in the Strait of Gibraltar and in the Western Mediterranean Sea, E.O.S., 63.

H. LACOMBE, J.C. GASCARD, J. GONELLA, J.P. BETHOUX, 1981. - Response of the Mediterranean to the water and energy fluxes across its surface, on seasonal and interannual scales. Oceanologica Acta, 4, 247-255.

A.R. MILLER, P. TCHERNIA, H. CHARNOCK, D.A. McGILL, 1970. - Mediterranean Sea Atlas, Woods Hole Oceanographic Institution, Massachusetts, pp 190

A. SOURNIA, 1973. - La production primaire planctonique en Méditerranée, Essai de mise à jour. Bull. Etude en Commun de la Méditerranée, 5, pp 128.

U.N.E.P., 1977. - Preliminary report on the state of pollution of the Mediterranean Sea. U.N.E.P. Prelim. Rep. I.G. 11/I.N.F. 4, pp 209.

U.N.E.P., 1984. - Pollutants from land-based sources in the Mediterranean. U.N.E.P. Regional Seas Reports and Studies N° 32, pp 97.

ROLE OF ORGANIC AGGREGATES IN THE TRANSPORT OF POLLUTANTS IN MEDITERRANEAN ESTUARIES

by

Vera ZUTIC, Gustave CAUWET and André MONACO
Center for Marine Research
"Rudjer Boskovic" Institute
Zagreb (Yugoslavia)
Laboratoire de Sédimentologie et Géochimie Marines
Université de Perpignan
Perpignan (France)

Abstract

The general understanding of mechanisms and rates of pollutant removal and resuspension in estuaries is still rudimentary. In particular, the course of the predominantly heterogeneous reactions cannot be predicted owing to our ignorance of the extent of organic coatings on the particles and the role of organic rich particles.

A considerable fraction of dissolved trace constituents, inorganic and organic pollutants, will bind to the organic aggregates, a highly reactive fraction produced when river and sea-water mix by flocculation of riverine organic matter and in situ primary production.

In this investigation we present a new methodological approach and direct evidence on the non-conservative behaviour and transformation of dissolved organic matter during estuarine mixing in a Mediterranean estuary. The results obtained are in agreement with previous mineralogical and geochemical studies of West-Mediterranean estuaries (MONACO, 1975, ALOISI et al., 1979) which indicated the importance of organic colloids and organo-mineral particles in the vertical transport of pollutants.

*
* *

Introduction

Estuaries are extremely reactive sites in the transport pathways of land-derived materials towards the ocean. The distribution patterns in estuaries as well as their temporal and spatial variations are usually very complex as result of various simultaneous and independent physical, chemical and biological processes. These processes affect not only the natural constituents but also the various pollutants transported by the rivers and can decide the behaviour of these toxic substances (heavy metals and organics).

Starting with the MED-POL PHASE II Monitoring Programme of the Krka Estuary (Adriatic Sea)1 we have been engaged not only in measurement of analytical concentrations of pollutants and natural micro-constituents in the Mediterranean estuaries but also in a characterization of mechanisms of processes taking place at river/sea-water (2) and particle/water interface (3-5).

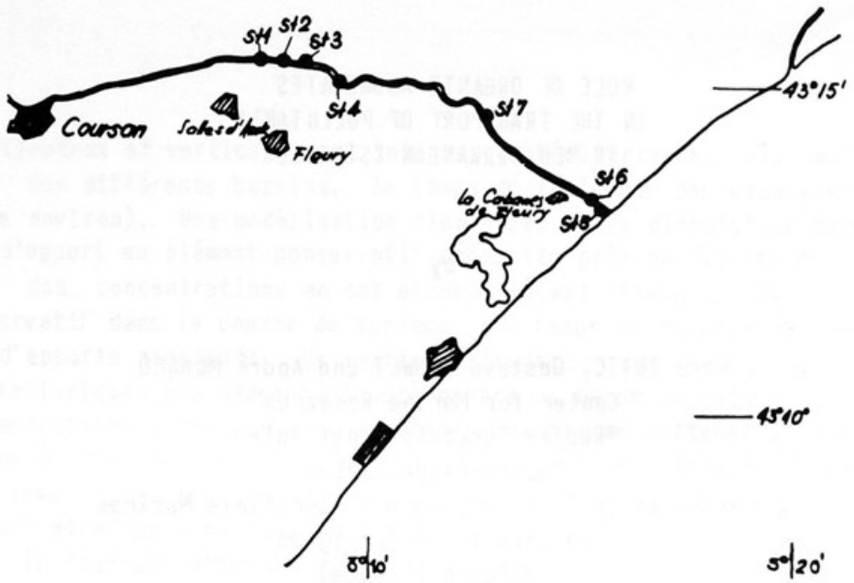


Fig. I - Sampling stations at the estuary of the river Aude, Gulf of Lion.

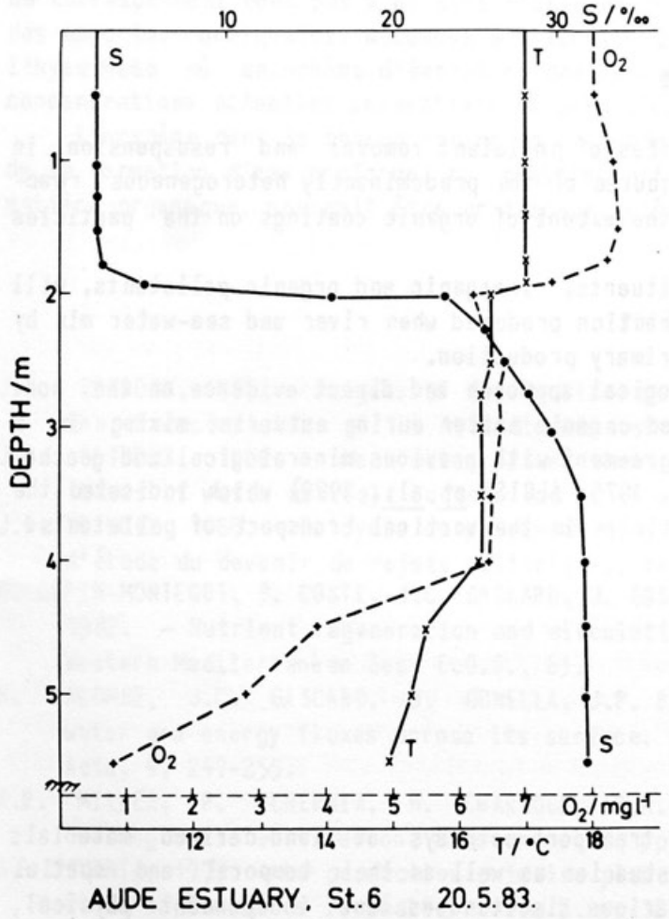


Fig. II - Typical vertical profile for salinity, temperature and oxygen (Station 6) in the period 16-20 May 1983.

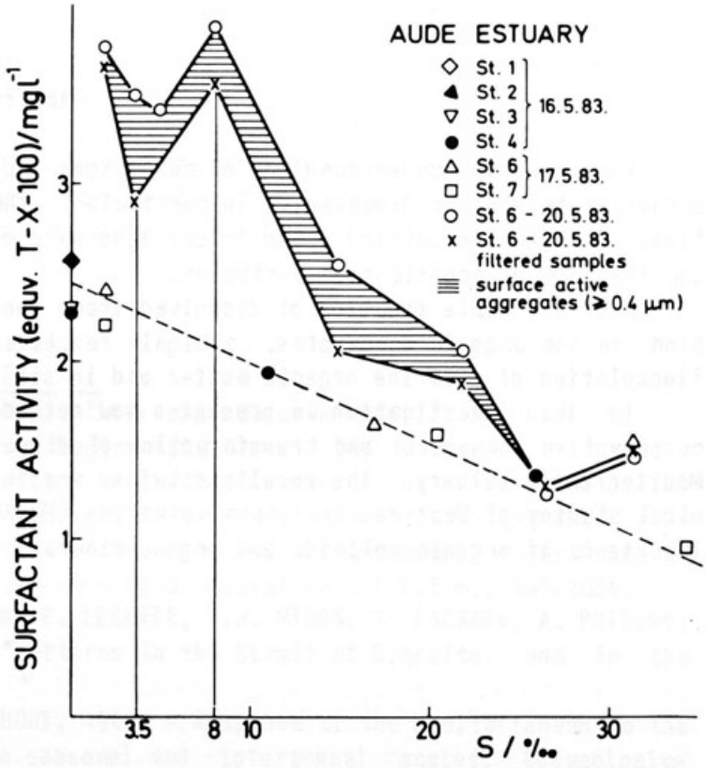


Fig. III - Relationship between surfactant activity values and salinity of the samples. Samples of 16 and 17 May were taken with 30 l Niskin-bottles and measured within > 10 hours delay. Filtration no effectued. Samples of 20 May were taken with I l bottle at precise depth and salinity and analysed directly (o) and after filtration (x) within < 5 hours.

Selective and sensitive electroanalytical techniques developed for qualitative and quantitative characterization of microconstituents since our engagement in the UNDP-assisted Adriatic III Project (6) have ben applied in the characterization of estuarine processes with simultaneous interest in the laboratory work on the selected model systems and field work in two Mediterranean estuaries (river Krka Adriatic and river Aude - West Mediterranean).

Carefully designated data collection schemes are necessary to distinguish how individual processes affect the behaviour of any particular chemical in a given estuary. These efforts are however justified by the fundamental role that these systems play as a boundary between continents and ocean and because of their economic and social importance in the development of man's activities. The capacity of the estuaries to react properly to the impact of these activities has often been overestimated and they are actually amongst the most disturbed ecosystems in the developed countries (note the example of an increasing eutrophication in the northern Adriatic (8).

In recent years the major chemical reactions occurring in the estuarine environment have been identified (9-11). Although a wide range of trace and major elements has been studied, the general understanding of mechanisms and rates of pollutant removal and resuspension in estuaries in still rudimentary.

In particular, the course of the predominantly heterogeneous reactions cannot be predicted owing to our ignorance of the extent of organic coatings on the particles and the role of organic rich particles (12).

Regarding the organic coatings and organic rich particulates most attention is paid to the resistant terrigenous humic material (13) and very little is known on recent. in situ produced aquatic dissolved and particulate organic matter in the estuarine mixing layers.

Recent controlled laboratory experiments have indicated the importance of incorporation and deposition of trace metals by flocculent organic matter (14). Evidence from direct field studies is equivocal because of the limited number of systematic investigations, analytical methodology problems (in qualitative and quantitative characterization of dissolved and particulate matter), fluctuating river water concentrations and possible removal and input mechanisms, and uncertainties in specifying the actual fresh and sea-water end-members of an estuary.

The aim of the present communication is the characterization of the transformation of organic matter and its reactivity during estuarine mixing in a defined natural system.

Materials and methods

Investigated area

Aude estuary (figure I) has been selected for this study in a low water season (May, 1983) because of its optimally defined conditions :

1 negligible tidal fluctuations
2 simple geometry
3 no complicated current regime
4 no significant pollution inputs throughout its flow
5 already well documented geochemistry during the investigations of pro-delta formation mechanisms (15-16) in which mineralogical and geochemical analysis indicated that flocculation was the main mechanism of the deposition.

In May 1983, this estuary was characterized by a very sharp halocline (figure II), the salinity changing from 2 ‰ to 25 ‰ in less than 20 cm. The temperature and oxygen vertical profiles show the same sharp decrease around two meters (17.2 °C to 16.5 °C and 8.5 to 6.5 mg/l O_2). From 4 m to the bottom (5.5 m) an important decrease in dissolved oxygen (6.5 to 1 mg/l), and in temperature (16.5 °C to 15 °C) is apparent, probably correlated with the biological activity at the sediment/water interface.

Sampling

Sampling was performed with a standard 30 l Niskin sampler and 1 l glass bottle that allowed sampling along the halocline with a sufficient vertical resolution (< 10 cm). Vertical profiles were sampled along the longitudinal transect to the estuary (figure I).

Filtration and electrochemical analysis were performed within five hours after sampling.

Methodology

Organic material available for adsorption on the surfaces of suspended particles was examined by simultaneous measurement of dissolved (D.O.C.) and particulate organic carbon (P.O.C.) (17) and surface active organic material (18, 19) at the selected vertical profiles along the salt wedge (figure II).

The property of surface activity, as measured by the polarographic technique, is quite generally distributed throughout the range of dissolved organic matter found in fresh, saline and estuarine waters. Systematic comparison of D.O.C. and surfactant activity values of estuarine samples (HUNTER and LISS, 1982) (20) has shown a linear relationship between the two quantities that can be expressed as surfactant activity (equivalent to Triton-X-100) mg/l = 1.3 D.O.C. mg/l, which means that about 70 per cent of dissolved organic matter is highly surface active. (This ratio depends on the molecular structure of the organic matter and can differ slightly from one river to another).

This method, which requires no pretreatment, such as filtration, which alters the dissolved and particulate matter distribution, gives the possibility to characterize the type of organic material, and to distinguish between "dissolved" organic matter and surface active aggregates (21-22). The interfacial instability of a positively charged interface dropping mercury electrode/sea-water, allows a direct characterization of single events of coalescence and transformation of aggregates of fluid surfactants into adsorbed monolayers at the interface (relaxation times 10 - 500 ms, aggregation numbers > 109, N > 1071-1)

Results

If surfactant activity is measured along the vertical profiles of stations distributed from the upper to the lower part of the estuary (Stations 3, 4, 5, 6, 7) in samples taken by a standard 20 l Niskin bottle, with the delay of > 5 hours after the sampling, the plot of surfactant activity vs. salinity (figure III) indicates more or less conservative behaviour of surface active material in the mixing process. This is in good agreement with the reported behaviour for several British and one Australian estuary (20, 23).

However, the surfactant activity value for the bottom sample, showing the highest salinity (1 m above the bottom) at Station 6 is significantly above the straight line, and the electrochemical response indicates the presence of larger (> 0.4 um) surface active aggregates : they coalesce and reorganize into the adsorbed layer at the interface - the relaxation time of this process being 0.1 s. After 3 days storage of the sample the aggregates disappeared. When the surface layer of the sediment at the same locality was extracted with distilled water, (pH = 8.2) a high content of surface active material could be measured, but with no indication of surface active aggregates. Thus, the aggregation of dissolved organic matter seems to be an irreversible process with at least one, final slow step (time scale 1-3 days).

The surface active material - salinity plot is significantly different when the depth profile at Station 6 (figure III) is sampled along the halocline with a higher resolution (= 10 cm) and the samples analysed with a minimum delay (< 5 h) show the following behaviour :

1 - No measurable aggregates are present in the surface sample (S = 2,2 ‰). Mineral particles (clays, quartz, calcite) are dispersed or trapped in nets formed by abundant diatoms (S.E.M. observations) ;

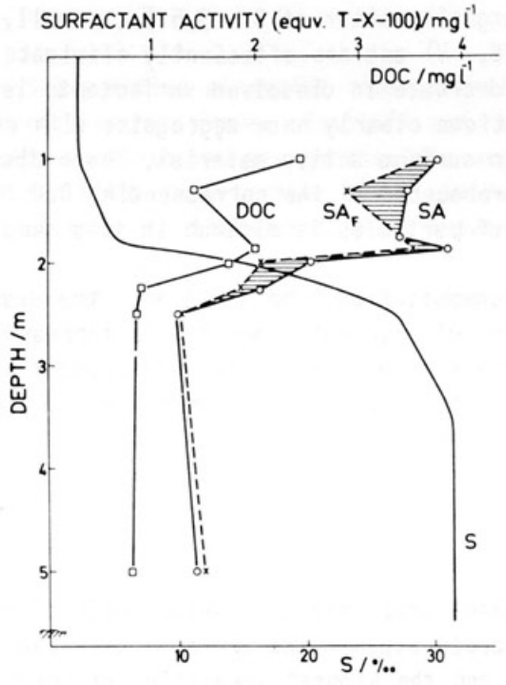


Fig. IV - D.O.C. and surfactant activity in the samples taken along the vertical profile at station 6, 20 May. Surfactant activity was measured directly (o) and in filtered samples (x).

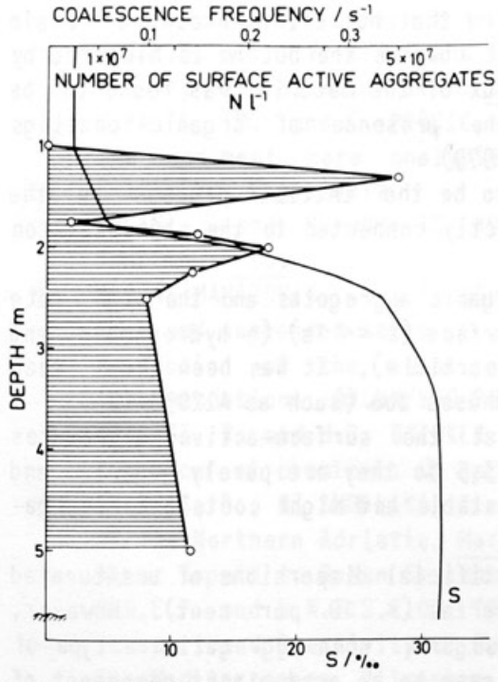


Fig.V -Distribution of surface active aggregates along depth at Station 6. The coalescence frequency was determined from the current-times curves (osciloscopic recordings) and calibrated against model dispersions (methyl oleate) by Coulter counting (23).

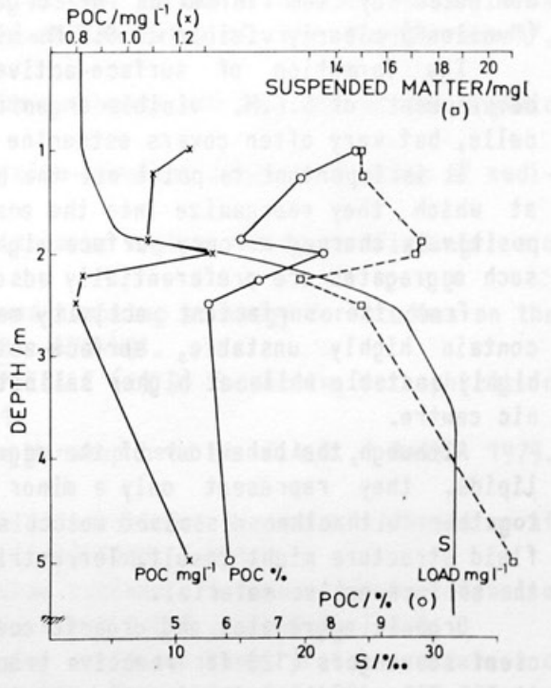


Fig. VI - Particulate organic carbon (P.O.C.) distribution (in mg/l and %) and sediment load (mg/l) along depth profile at Station 6.

2 - Intense aggregation of organic matter at S = 3,5 ‰, small, fluid and highly surface active aggregates are present (figures IV, V) and are efficiently eliminated by filtration through Whatman GF/F glass fibre filters. The decrease in dissolved surfactants is correlated with a lower D.O.C. value (figure IV). S.E.M. observations clearly show aggregates with organic coatings.

3 - A significant increase in surface active material, both dissolved and dispersed is observed at salinity 8 ‰ which is also pronounced in the corresponding D.O.C. values (figure IV). On the other hand, the organic content of particles is minimum in this sample (figure VI) where little clay aggregates are observed.

4 - When the salinity increases from 8 ‰ to 15 ‰, the dissolved surfactants and D.O.C. decrease strongly, while the number of aggregates and P.O.C. increase.

5 - Relatively larger and rather stable aggregates are present in the bottom sample (depth 5 m, 40 cm above the bottom). Mineral content is higher than in superficial layer but organic phase (diatoms, some coccoliths) is still present.

Discussion

We think that in situ surfactant production by phytoplankton (18) at the saline interface is not the predominant process which could explain the increase observed in the dissolved and dispersed organic material at salinity 8 ‰ and the highest population of organic aggregates at S = 3.5 ‰. It is more logical to explain these experimental facts as a result of in situ aggregation of riverine dissolved organic matter and to the effect of surface tension gradient at the saline interface that hinders vertical transport of the material, both dissolved and dispersed.

MONACO (1975) (24), ALOISI et al., (1975) (16) have documented the important physico-chemical and dynamic role of the mixing zone in the Aude estuary - indicating that halocline retains a certain quantity of material in suspension, and that the vertical transport towards the bottom is hindered by the saline interface (inertia of the salt wedge). The vertical flux of the material was found to be dominated by the formation of organo-mineral aggregates and the presence of organic coatings ("voiles") clearly visible in S.E.M. micrographs (ALOISI et al., 1979)

The formation of surface-active organic aggregates seems to be the initial process in the development of S.E.M. visible organic material which is not directly connected to the phytoplankton cells, but very often covers estuarine mineral particles.

It is important to point out the high adsorbability of the organic aggregates and the high rate at which they reorganize into the adsorbed layer at charged interface ($t << 1s$) (a hydrophobic and positively charged mercury surface might be considered as a model particle). It has been shown that such aggregates are preferentially adsorbed by mineral particles phases too (such as Al_2O_3) (25).

From the surfactant activity measurements it is evident that the surface-active aggregates contain highly unstable, surface-active material. At salinity 3,5 ‰ they are purely organic and highly unstable while at higher salinities they are larger, more stable and might contain an inorganic centre.

Although the behaviour of the aggregates is reminiscent of artificial dispersions of unsaturated lipids, they represent only a minor fraction of the organic material (< 10 per cent). However, together with other dissolved molecules such as polypeptides and sugars, upon aggregation a type of fluid structure might result. Terrestrial humic material does not seem to be predominant component of the surface-active material.

Organic aggregates and organic coated particles produced during estuarine mixing are most efficient scavengers (12) for reactive trace elements (such as toxic metals and actinides) (14, 26-28) ; their fate in the estuarine and coastal zone should be determined by the particulate residence time. As in Mediterranean estuarine and coastal zones, phosphorus usually limits the standing crop (1, 29, 30) as well as the production of dissolved and particulate aquatic organic matter, the input of phosphorus into the estuary appears to be a major factor controlling the production of biogenic particles and removal of reactive trace elements (31).

Conclusion

Organic matter is a less conservative parameter than generally accepted : during estuarine mixing it undergoes many transformations in phase, structure and reactivity to solutes and surfaces.

Since the relationship between dissolved and particulate organic matter and trace metals has been proved by recent field studies often to determine their residence times, it is not meaningful to investigate trace metals is estuaries without studying their interaction with organic matter (dissolved as well as flocculent and organic coated particles).

When geochemical interests centre on the interfacial effects of naturally occurring organic matter in the aqueous phase, a complete budget of organic carbon must be established, and the measurement of adsorbable organic matter is probably one of the more sensitive parameters, because we can expect that its participation in such processes is propositionally more important. The methodology of sampling and analysis presented should be used and improved in future characterization of organic matter in estuaries.

References

1. MED-POL - PHASE II Monitoring Programme of the Krka Estuary (Adriatic Sea),, Centre for Marine Research - Zagreb, Institute "Rudjer Boskovic", Yugoslav National Monitoring Programme, MED POL - PHASE II; Annual Report 1983.
2. COSOVIC, B., V. ZUTIC and G. CAUWET (1983) Characterization of dissolved organic matter in the estuary of a Karstic river. Rapp. Comm. int. Mer. Medit. 28 : 7-8.
3. JURACIC, M. and E. PROHIC, (1984) Sedimentation rate assessment in the Krka River estuary from the sediment core analysis. VIII International Symposium "Chemistry of the Mediterranean", Primosten, Yugoslavia.
4. BISCAN, J. and M. JURACIC (1984) Surface properties of the sediments and the role of the organic coating, Ibid.
5. MUSANI-MARAZOVIC, LJ., Z. KONRAD and C. SKRLEC (1984) Physico-chemical characterization of sediment and suspended matter from an estuary, Ibid.
6. Protection of the human environment in the Yugoslav Adriatic Region. Project Findings and Recommendations. DP.UN/YUG-72-004/1.
7. WOLLAST, R. and J.C. DUINKER (1982) General methodology and sampling strategy for studies on the behaviour of chemicals in estuaries, Thalassia Jugosl. 18 : 471-491.
8. DEGOBBIS, D., N. SMODLAKA, I. POJED, A. SKRIVANIC and R. PRECALI (1979) Increasing eutrophication of the Northern Adriatic. Mar. Pollut. Bull. 10 : 298-301.
9. River Inputs to Ocean Systems. Proceedings of the review Worshop held at F.A.O., Rome, 1979. U.N.E.P. and U.N.E.S.C.O., 1981.
10. Transport and Reactivity of Pollutants in the Estuary. 7th International Symposium : Chemistry of the Mediterranean, Primosten, 1982. Thalassia Jugosl. 18 (1/4) 1982, 1-361.
11. MARTEN, J.M. and V.V. GORDEEV (1982) River inputs to ocean systems : A reassessment. Estuarine processes : An application to the Tagus estuary, Portugal, U.N.E.S.C.O., U.N.E.P. I.O.C.
12. BALESTRI, L. P. BREWER and J.W. MURRAY (1981) Scavenging residence times of trace metals and surface chemistry of sinking particles in deep oceans, Geochim. Cosmochim. Acta 28 : 101-121.
13. SHOLKOVITZ, E.R. (1976) Flocculation of dissolved organic and inorganic matter during the mixing of river water and sea-water. Geochim. Cosmochim. Acta 40 : 831-845.
14. HUNT, C.D. (1983) Incorporation and deposition of Mn and other trace metals by flocculent organic matter in a controlled marine ecosystem. Limmol. Oceanogr. 28 : 302-308.

15. ALOISI, J.C., C. MILLOT, A. MONACO et H. PAUC (1979) Dynamique des suspensions et mécanismes sédimentogénétiques sur le plateau continental du Golfe du Lion. C.R. Acad. Sc. Paris, 289 : 879-882.
16. ALOISI, J.C., A. MONACO et H. PAUC (1975) Mécanismes de la formation des prodeltas dans le Golfe du Lion. Exemple de l'embouchure de l'Aude (Languedoc) Bull. Inst. Geol. Bassin Aquitaine, 18 3-12.
17. CAUWET, G. Automatic determination of dissolved organic carbon in sea-water in the sub-ppm range. Mar. Chem., 14/4, 297-306.
18. ZUTIC, V., B. COSOVIC, E. MARCENKO, N. BIHARI and F. KRSINIC (1981) Surfactant production by marine phytoplankton, Mar. Chem. 10 : 505-520.
19. COSOVIC, B., V. ZUTIC, V; VOJVODIC and T. NOVAKOVIC (1983) Determination of surface active substances and anionic detergents in seawater and sea surface microlayer in the Mediterranean. Proceedings of the 6th Workshop on Pollution of the Mediterranean, I.C.S.E.M., U.N.E.P., I.O.C. Monaco.
20. HUNTER, K.A. and P.S. LISS (1981) Polarographic measurement of surface-active material in natural waters. Water Res. 15 : 203-215.
21. PLESE, T. and V. ZUTIC On irregular pattern of polarographic maxima in surfactant dispersions. J. Electroanal. Chem., in press.
22. ZUTIC, V., T. PLESE, J. TOMACIC and T. LEGOVIC Electrochemical characterization of fluid vesicules in natural waters. Mol. Cryst. Liq. Cryst., in press.
23. HUNTER, K. (1983) On the estuarine mixing of dissolved subtances in relation to colloid stability and surface properties. Geochim. Cosmochim. Acta, 47 : 467-473.
24. MONACO, A. (1975) Les facteurs de la sédimentation marine argileuse. les phénomènes physico-chimiques à l'interface. Bull. B.R.G.M., Sect. IV, n° 3, 147-174.
25. TOMAIC, J. and V. ZUTIC (1984) On the interaction between aquatic organic matter and hydrous alumina. An electrochemical study. VIII Internatioanl Symposium "Chemistry of the Mediterranean", Primosten, Yugoslavia.
26. CAUWET, G. and D. FAGUET (1982) The role of organic matter in transport processes of metals in estuarine environments. Thalassia Jugosl. 18 : 379-392.
27. BRULAND, K.W. (1984) Residence times of trace elements within the surface waters of the Californian current. VIII International Symposium "Chemistry of the Mediterranean". Primosten, Yugoslavia.
28. FISHER, N.S., Concentration of metals in marine phytoplankton : Implication for toxicity, vertical transport, and oceanic residence times. Ibid.
29. POJED, I. and S. KVEDER, (1977) Investigation of nutrient limitation of phytoplankton production in the Northern Adriatic. Thalassia Jugosl. 13 : 13-24.
30. LEGOVIC, T. and B. SEKULIC Eutrophication trends in Rijeka Bay (North Adriatic). Modelling chlorophyl a distribution. In preparation.
31. SIGG, L. M. STURM, J. DAVIS and W. STUMM (1982) Metal transfer mechanisms in lakes. Thalassia Jugosl. 18 : 293-3311.

MODEL STUDIES OF PHYSICO-CHEMICAL INTERACTION OF CADMIUM WITH ORGANIC COATINGS AT INTERFACES*

by

Bozena COSOVIC and Zlata KOZARAC
Center for Marine Research Croatia, (Yugoslavia)
Rugjer Boskovic Institute, Zagreb

Abstract

Surface-active substances, both natural and pollutants, adsorb and accumulate at natural interfaces, modify the structure of interboundary layers and effect processes of mass and energy transfer between different phases. Adsorption processes have important effects on sedimentation and mineralogy, bubble flotation of particles and enrichment of organic and inorganic material in the sea surface microlayer. The distribution of chemical elements in natural waters is controlled to a great extent by scavenging, or adsorption on to solid surfaces.

Investigations of interfacial phenomena can be carried out on real interfaces but often it is more practical and convenient to use a model interface. Investigations of the physico-chemical interaction between surface-active substances and cadmium at the electrode/water interface showed that : a) most naturally occurring substances have little effect on the mass and charge transfer process at the electrode surface, b) synthetic compounds, like commercial detergents, slow down the kinetics of the processes at interfaces, c) some organic coatings at the surface, as for example unsaturated fatty acids, may interact with metal ions resulting in an enrichment of metal ions in the organic layer at the interfaces.

*
* *

Introduction

The distribution of chemical elements in natural waters is controlled to a great extent by scavenging, or adsorption on to solid surfaces. Fine-grained particles make up the majority of sediments entering the marine environment. These particles carry the majority of toxic metals and organic substances transported by rivers and effluents and discharged into estuaries and the ocean. Coagulation,sedimentation, adsorption, and other processes are usually controlled by physical chemistry of the solid/liquid interface (STUMM and MORGAN, 1981). Surface active substances, both natural

---

* This communication is relevant to the work carried out in the framework of the MED POL - PHASE II project "Study of the Physico-Chemical Interactions of Natural and Artificial Surface Active Substances with Toxic Substances in the Sea".

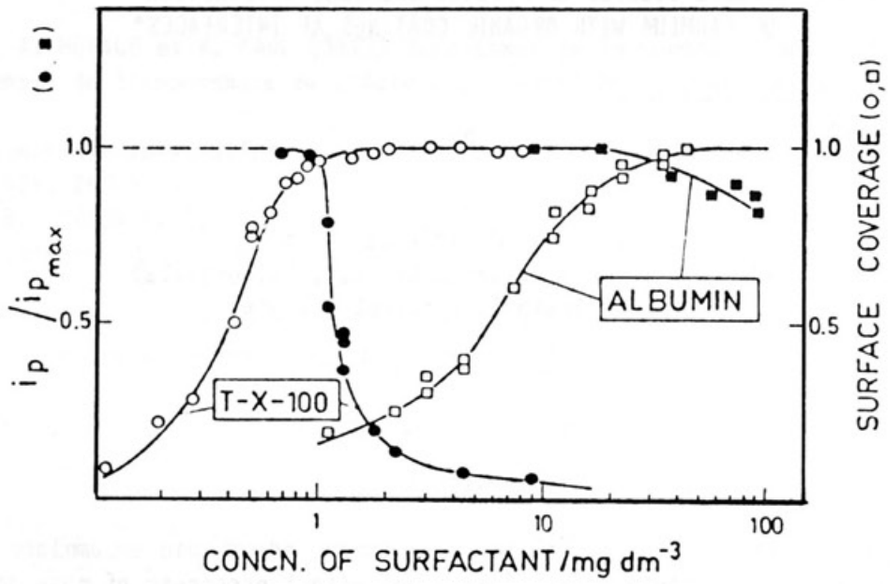


Fig. I

Adsorption isotherms of Triton-X-100 (○) and albumin (□) in sea-water, and the dependence of the peak height of the differential pulse polarogram of cadmium (10^{-4} mol dm^{-3}) on the concentration of Triton-X-100 (●) and albumin (■). Accumulation period 5 minutes by the diffusion process.

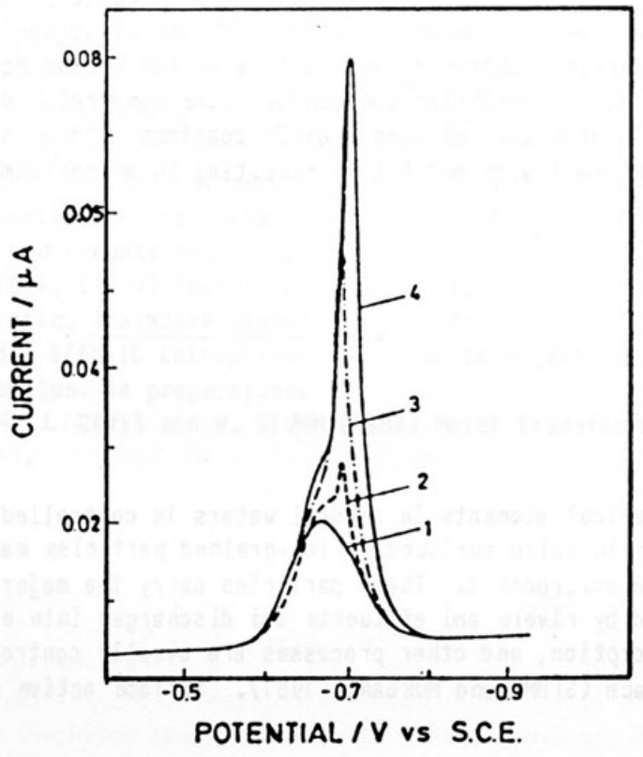


Fig. II

Differential pulse polarograms of 2×10^{-5} mol dm^{-3} Cd (II) in the presence of 36.3 mg dm^{-3} linoleic acid in 0.55 mol dm^{-3} NaCl and 3×10^{-2} mol dm^{-3} $NaHCO_3$. Adsorption time : (1) 0 ; (2) 2 ; (3) 4 ; (4) 8 minutes. Adsorption potential -0.4 V vs. SCE ; pH = 8.7.

and pollutants, adsob and accumulate at natural interfaces, modify the structure of interboundary layers and effect processes of mass and energy transfer between different phases. Natural organic surfactants have an effect on the properties of surfaces of natural particles in sea-water through the formation of films, as proposed by LOEB and NEIHOF, 1975, and HUNTER and LISS, 1979. Coagulation experiments with natural samples and with the same samples with the organic coatings removed showed that the natural coated samples coagulated significantly slower than the samples with the coatings removed (GIBBS, 1983). It was also found that significant fractions of different heavy metals were associated with surface-active and colloidal organic matter isolated from the water column of a controlled experimental ecosystem (WALLACE, 1982). Qualitative and quantitative information on the nature of these associations is still lacking.

Recently, there has been an ever-increasing interest in trying to learn more about the content and composition of organic substances in natural waters and their interactions with other macro and micro constituents, both in the bulk water and at interfaces. Investigations of interfacial phenomena can be carried out on real interfaces but often it is more practical and convenient to use a model interface. The study of adsorption phenomena and mass and charge transfer processes at charged electrodes covered, partly or completely, with the adsorbed layer of different surface-active substances, which are representative of the composition of natural and polluted waters, opens up new possibilities for simulation of interfacial phenomena and processes at natural boundaries.

Oxido-reduction processes of cadmium have been studied by differential pulse voltammetry using the hanging mercury drop electrode method in the presence of organic film which was formed in solutions of different concentrations of selected biogenic and artificial surface-active substances.

Methodology

The measurements of adsorption isotherms of surface-active substances were carried out by capacity current measurements using either the Kalousek commutator technique or a.c. polarography, as described in our previous papers (KOZARAC et al.,1976 ; COSOVIC and VOJVODIC, 1982).

Differential pulse voltammetric measurements were performed with a PAR model 174A Polarographic Analyzer connected to a Hewlett-Packard 7.045 A X-Y recorder. The organic film at the mercury electrode was formed at the adsorption potential which was more positive than the potential of the metal ion reduction.

The kinetic parameters of the electrode reaction of cadmium at the modified electrode surface were estimated from the shape and the height of the corresponding differential pulse voltammograms using theoretical curves obtained by digital simulation of the electrode process (KOZARAC et al., 1982).

Results and discussion

The adsorbed layer of organic molecules generally presents a barrier to the transport of ions and electrones at the interface. In the electrochemical measurement, adsorbed surface-active substances influence oxido-reduction processes of ion and molecules present in solution, mainly by reducing the rate of the electrode reaction (LIPKOWSKI and GALUS, 1975). In differential pulse polarography the decrease of the electrode reaction rate is usually followed by a lowering and broadening of the polarographic wave (KOZARAC et al., 1982).

Cadmium was chosen because of its very convenient electrochemical characteristics which result from its well-defined reversible and two-electron polarographic wave which appears at potentials near to the electrocapillary maximum of the mercury electrode, i.e. at potentials of maximum adsorption. Besides, cadmium is a very toxic metal and one of the potential pollutants in the aquatic

environment. Investigations were carried out in a wide concentration range of different model surfactants (detergents, proteins, fatty acids, humic substances, etc.) and using different time periods of adsorption. Special attention was paid to adsorption studies at very low concentrations of surfactants that are relevant for the composition of natural and polluted waters. The investigations with model substances showed that the synthetic compounds, such as, for example, a non-ionic tenside Triton-X-100, which is presented in figure I, inhibit the electrode reduction of cadmium at the mercury electrode at very low concentrations of surfactant.

Kinetic parameters of the electrode reduction of cadmium in the absence and in the presence of Triton-X-100 are given in table 1. On the other hand, model substances representative of biopolymers, such as albumin in figure I (the same valid for geopolymers, humic and fulvic acids) create adsorbed films which are very porous for cadmium ions, so that one can observe only a small decrease in the peak height of the differential pulse voltammogram of cadmium in the presence of albumin.

One should generally expect an interaction, most likely a complexation or co-ordination, of trace metals with the adsorbed layer of organic molecules at various interfaces. Our experiments with different surface-active compounds and with cadmium demonstrated the unique behaviour of unsaturated fatty acids (oleic and linoleic) in this respect (KRZNARIC et al., 1983 ; KRZNARIC and COSOVIC, 1985). The increase in the peak height of cadmium up to ten times the value obtained in the electrolyte without fatty acid is caused by the adsorption of metal ions at the thus modified electrode surface. The accumulation of the metal increases with an increase in the adsorption time (at potentials more positive than the reduction potential of cadmium) as shown in figure II. The reduction current of Cd(II) represents, in fact, the sum of at least two superimposed reduction peak currents. When the adsorption time is zero, a diffusion-controlled type of reduction peak is obtained. With an increase in the adsorption time, a new peak appears, the sharp form of which indicates reduction of an adsorbed Cd species.

The same was also obtained for lead and zinc.

Table 1

Kinetic parameters, the tranfer coefficient α and the rate constant k_s of electrode reduction of 10^{-4} mol dm^{-3} Cd(II) on Hg electrode in the presence of T-X-100 in seawater.

Triton-X-100 ($mg\ dm^{-3}$)	$\frac{i_p}{i_{p_{max}}}$	$E_{p/2}$ (mv)	α	R_s	$k_s = R_s\sqrt{D}/t$ ($cm\ s^{-1}$)
0	1	90	0.5	10	$\geq 1.1 \times 10^{-1}$
0.97	0.77	102	0.4	2	2.2×10^{-2}
1.30	0.44	120	0.4	0.6	6.7×10^{-3}
1.80	0.20	156	0.3	0.25	2.2×10^{-3}
2.20	0.13	186	0.3	0.13	1.5×10^{-3}
4.50	0.08	240	0.2	0.09	1.0×10^{-3}
5.00	0.06	240	0.2	0.05	5.6×10^{-4}

Salinity has a very pronounced effect on the metal adsorption at the charged surface of the mercury electrode in the presence of unsaturated fatty acids, as presented in figure III in the case of cadmium and linoleic acid. The same was found for fatty acid uptake by clay (MEYERS and QUINN, 1973). The higher the salt content, the stronger is the adsorption of fatty acid and metal. These

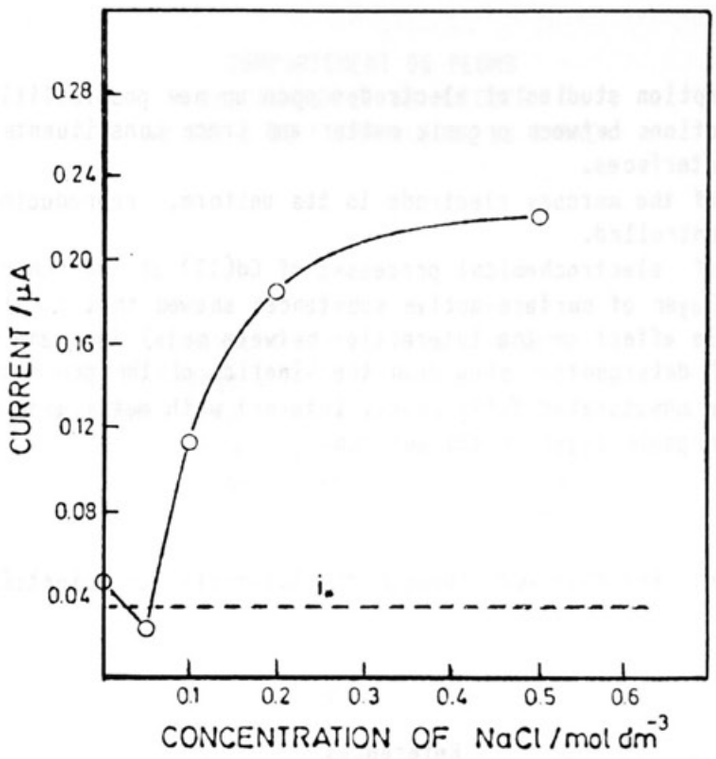


Fig III

Differential pulse reduction current of 10^{-5} mol dm^{-3} Cd(II) in 3 x 10^{-2} mol dm^{-3} $NaHCO_3$ and in the presence of 36 mg dm^{-3} linoleic acid plotted against the concentration of NaCl in solution. Adsorption time 2 min. with stirring, adsorption potential -0.4 V vs. SCE : pH = 8.7 + ou - 0.3. ix peak current of 10^{-5} mol dm^{-3} Cd(II) in the absence of linoleic acid.

phenomena are very probably closely connected with the colloidal properties of the system and might be of considerable importance for scavenging processes in natural water systems with considerable variations of salinity such as estuaries.

We have not observed the accumulation of cadmium in the adsorbed layer of humic and fulvic acids. However, one should not neglect the probability of the interaction between the adsorbed layer of humic and fluvic substances with copper ions which are known to form stronger complexes with humic material than cadmium does (SAAR and WEBER, 1982). The river water samples with a high content of surface-active humic material showed no influence on the kinetics of the electrode reduction of cadmium, the same as was obtained with the adsorbed layer of humic and fulvic acids (COSOVIC, 1983).

If the adsorbed layer of organic molecules at the surface of the mercury electrode represents an adequate model for organic coatings of the particles in natural waters, these preliminary electrochemical studies on cadmium indicate that : a) most of naturally occurring substances (biopolymers and geopolymers) may have little effect in the physico-chemical interaction between metal ions and the surface ; b) synthetic compounds, like commercial detergents, slow down to a great extent the kinetics of the processes that occur at interfaces : c) some organic coatings may specifically interact with metal ions which results in the enrichment of the metal in the organic layer at the surface.

Conclusions

Electrochemical adsorption studies at electrodes open up new possibilities in the investigation of physico-chemical interactions between organic matter and trace constituents of sea-water, both in the bulk solution and at interfaces.

The main advantage of the mercury electrode is its uniform, reproducible renewable surface, smooth and energetically controlled.

The investigations of electrochemical processes of Cd(II) at the charged mercury electrode covered with the adsorbed layer of surface-active substances showed that : a) most naturally occurring substances have little effect on the interaction between metal ions and surface ; b) synthetic compounds, like commercial detergents, slow down the kinetics of the processes at interfaces : c) some organic coatings, like unsaturated fatty acids, interact with metal ions resulting in an enrichment of metal ions in the organic layer at the surface.

Acknowledgement

The financial support for this work through the Authority for Scientific Research of the SR Croatia is acknowledged.

References

COSOVIC, B. and V.VOJVODIC (1982) The application of a.c. polarography to the determination of surface active substances in sea-water. Limm. Oceanogr. 27 :361-369.

COSOVIC, B., (1985) Aqueous surface chemistry. Adsorption characteristics of organic solutes. Electrochemical evaluation. In : Chemical Process in Lakes (Ed. Werner Stumm), J. Wiley, 55-80.

GIBBS, R.J., (1983) Effect of natural organic coatings on the coagulation of particles. Environ. Sci. Technol., 17 : 237-239.

HUNTER, K.A. and P.S. LISS, (1979) The surface charge of suspended particles in estuarine and coastal waters.Nature, (lond.) 282 : 823-825.

KOZARAC, Z., B. COSOVIC and M. BRANICA (1976) Estimation of surfactant activity of polluted sea-water by Kalousek commutator technique. J. Electroanal. Chem., 68 :75-83.

KOZARAC, Z., S. NIKOLIC, I. RUZIC and B. COSOVIC (1982) Inhibition of the electrode reaction in the presence of surfactants studied by differential pulse polarography. Cadmium (II) in sea-water in the presence of Triton-X-100. J. Electroanal. Chem, 137 : 279-292.

KRZNARIC, D. and B. COSOVIC (1983) The interaction of the Cd with linoleic acid and its influence upon the adsorbed film structure at the polarized mercury/water solution interface.J. Colloid. Interface Sci., 96 : 425-436.

KRZNARIC, D., B. COSOVIC and Z. KOZARAC (1983) The adsorption and interaction of long-chain fatty acids and heavy metals at the mercury electrode/sodium chloride solution interface. Mar.Chem. 14 : 17-29.

LIPKOWSKI, J. and Z. GALUS (1975) On the present understanding of the nature of inhibition of electrode reaction by absorbed neutral organic molecules. J. Electroanal. Chem., 61 : 11-32.

LOEB, I.G. and R.A. NEIHOF (1975) Marine conditioning films. Adv. Chem. Ser. 145 : 319-335.

MEYERS, P.A. and J.G. QUINN (1973) Factors affecting the association of fatty acids with mineral particles in sea-water. Geochim. Cosmochim. Acta, 37 : 1,745-1,759.

SAAR, R.A. and J.H. WEBER (1982) Fulvic acid : modifier of metal-ion chemistry. Environ. Sci. Technol., 16 : 510A-517A.

STUMM, E. and J.J. MORGAN (1981) Aquatic Chemistry, 2nd ed., Wiley 780 pp.

WALLACE, G.T., Jr. (1982) The association of copper, mercury and lead with surface-active organic matter in coastal sea-water. Mar. Chem. 11 : 379-394.

COMPORTEMENT DU PLOMB DU CADMIUM ET DU MERCURE AU LARGE DE L'EMBOUCHURE DU RHONE

par

Daniel SPAN*, Gilles FLATAU**
François FERNEX* et Ayed ADDED*
*Laboratoire de Géodynamique sous-marine L.A. 197 C.N.R.S.
06300 Villefranche-sur-Mer
** INSERM, Un. 40, 06300 Nice

Résumé

Autour d'un point de rejet (émissaire ou embouchure de fleuve), la répartition de diverses substances n'est pas homogène tant dans la pleine eau que dans les dépôts marins. Cela est en particulier net au voisinage de l'embouchure du grand Rhône.

En ce qui concerne le Cd, les relativement fortes concentrations sont localisées près de l'embouchure, tant dans la masse d'eau de surface (supérieur à 5 ug/l) que dans les sédiments (supérieur à 1 ug/l). Les teneurs sont relativement élevées non seulement dans la phase solide mais aussi dans les eaux interstitielles. Il y aurait donc coprécipitation dans la zone prodeltaïque. Mais une part du Cd associé aux dépôts se dissout. Il est alors susceptible de diffuser vers l'eau de mer susjacente. Les teneurs diminuent vers le large, ce qui indiquerait qu'une partie du Cd a été dissoute au cours du transit jusqu'à l'aire de dépôt. Dans la phase solide le Cd des sédiments superficiels est en bonne part extrait au moyen d'acide acétique dilué. Il se trouverait dans surtout adsorbé et associé à des carbonates, et/ou aussi lié à de la matière organique.

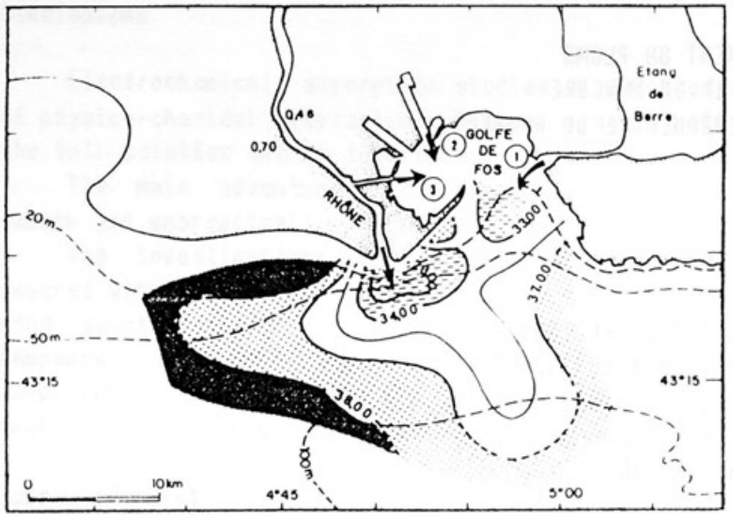
Le Pb semble surtout tranporté par des particules assez fines (silts) qui décantent progressivement en allant vers le large. Ce métal semble relativement bien fixé aux particules qui le transportent. Dans la phase solide des sédiments superficiels, le Pb se trouve en bonne part lié à des oxydes/hydroxydes (ou sous formes d'oxydes).

La répartition du Hg dans les eaux peut être expliquée par une contamination atmosphérique et industrielle. Les sédiments prodeltaïques sont peu chargés contrairement à ceux situés dans une bande orientée suivant une direction Nord-Est-Sud-Ouest.

*
* *

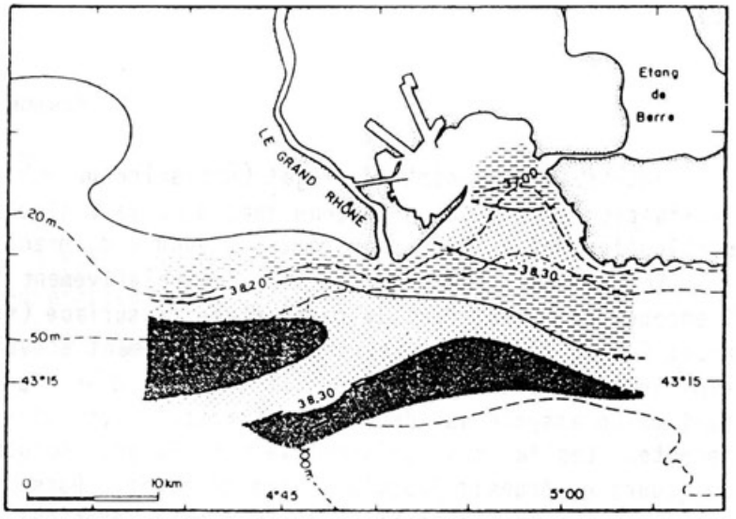
Introduction

Malgré le voisinage du complexe industriel du golfe de Fos et du port de Bouc-Lavera, l'influence du grand Rhône est largement prédominante dans un rayon de 10 Km autour de l'embouchure.

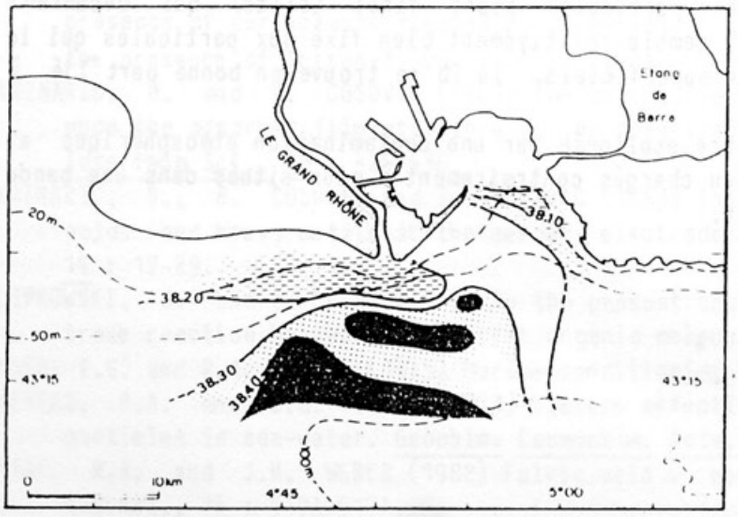


A - En surface.

(1) Eaux douces du canal de Caronte (étang de Berre).
(2) Influence rhodanienne secondaire (canal de Port-Saint-Louis).
(3) Nappe de Crau.



B - En demi-profondeur.



C - Près du fond.

Figure 1
Salinités en ‰ dans les eaux prélevées en novembre 1981

De plus, la morphologie du plateau continental devant le Delta est assez simple. En particulier, aucun accident morphologique majeur ne découpe le Plateau. Le modèle d'étude est simple. Il nous a donc paru intéressant de suivre les modalités de transit et le comportement des Pb, Cd et Hg, métaux réputés dangereux dans cet environnement ; en considérant l'eau de mer, les sédiments et les eaux interstitielles.

Cette région a déjà fait l'objet de quelques études sédimentologiques antérieures surtout dans la partie orientale qui jouxte le golfe de Fos. ALOISI et al. (1982) ont entrepris des études d'ordre sédimentologique, ARNOUX et al. (1980) ont effectué des dosages de métaux dans les sédiments. A. ADDED et al. (1980, 1981, 1984) ont établi des cartographies détaillées de répartition des métaux devant l'embouchure du grand Rhône .

Les mesures de la salinité (figure 1) montrent bien l'extension du panache en surface à partir de l'embouchure vers Sud-Est (nov. 1981). Elle mettent en évidence une remontée d'eaux profondes qui semble se produire par saccades. Ce phénomène d'up-welling semble fréquent dans cette région (ADDED, 1981). Les eaux profondes arrivant du Sud, sont déviées vers le Nord-Est à l'approche du prodelta. La circulation des eaux profondes tend évidemment à guider la répartition des métaux dans les eaux près du fond et dans les sédiments superficiels (voir en particulier la répartition du Cd).

Méthodes

Les campagnes à la mer

La principale campagne de prélèvements a eu lieu en novembre 1981 à l'aide du N.O. "Catherine-Laurence" de la station marine de Villefranche-sur-Mer (C.E.R.O.V.). Des échantillons d'eaux de mer ont été prélevés à mi-profondeur et près du fond, en une vingtaine de sites. Des sédiments superficiels ont été recueillis aux mêmes points au moyen de la benne Flucha. L'étude des eaux interstitielles a été réalisée à partir de 11 échantillons de sédiments superficiels prélevés au moyen de la benne Flucha, dans la zone prodeltaïque en septembre 1983.

Traitements des échantillons

Les eaux de mer prélevées ont été acidifiées à pH2 (mais pas filtrées). Les eaux des sédiments ont été extraites par centrifugation à 2.000 t/mn pendant 30 minutes, puis filtrées sous azote à 0,2u. Les échantillons d'eaux sont acidifiés à pH2, puis analysés.

Les sédiments ont été tamisés à 63u et séchés. La fraction fine a servi aux dosages des métaux totaux et des fractions litho-chimiques.

La méthode de fractionnement est inspirée de celle décrite par TESSIER et al.(1979), voir aussi D. SPAN (1984). Il s'agit d'extractions séquentielles au moyen d'une succession d'attaques chimiques ménagées. On obtient ainsi la part des métaux associés aux divers composés majeurs des sédiments : carbonates, oxydes, matière organique, sulfures, etc.

Techniques de dosages

Le Cadmium et le Plomb total ont été dosés par redissolution anodique et le Mercure par spectrophotométrie d'absorption atomique sans chauffage. Les eaux ont été analysées directement. Les sédiments ont subi une minéralisation en milieu nitroperchlorique (3/4 HNO_3 + 1/4 $HClO_4$). Les différentes phases litho-chimiques ont été analysées en Cadmium et en Plomb par spectrophotométrie d'absorption atomique à flamme air-acétylène, au laboratoire de Villefranche-sur-Mer.

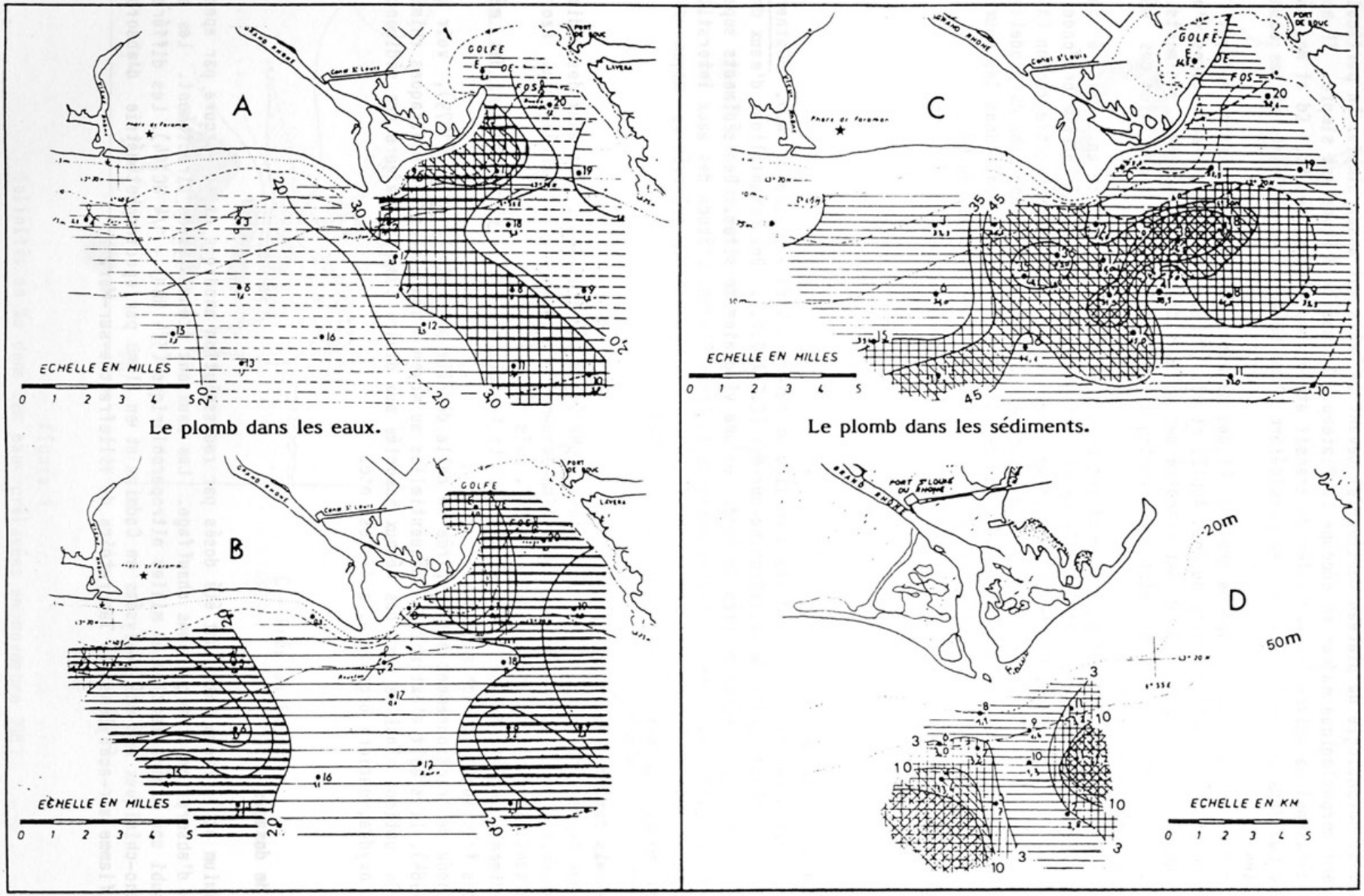


Figure 2. - Répartition du Plomb dans les eaux (µg/l) à mi-profondeur (A), près du fond (B), dans les eaux interstitielles (D) et dans les sédiments (µg/g) (C).

Résultats et discussions

Le Plomb

En novembre 1981, il est apparu que le Pb "s'écoulait" à mi-profondeur vers le S.E.,avec des teneurs supérieures à 3u/l (figure 2a). De fait, cet écoulement est parallèle à celui du panache des eaux du Rhône au même moment. Près du fond (figure 2b) seule la zone plus lointaine est chargée (sans parler du secteur le long de la flèche de la Gracieuse, à l'Est de l'embouchure). Cette direction avait déjà été mise en évidence par ADDED et al. (1980) dans l'étude des sédiments du fond. Ces derniers se trouvent, en effet, enrichis selon cette bande allongée vers le Sud-Est en Pb et en Fe. On retrouve cette image dans la cartographie du Pb associé aux oxydes (extractions à l'hydroxylamine) (figure 3).

Le Pb serait fortement associé à des particules ne sédimentant pas au voisinage immédiat de l'embouchure. D'après l'étude par fractionnement lithochimique ces particules seraient en majorité des oxydes ou plutôt des hydroxydes.

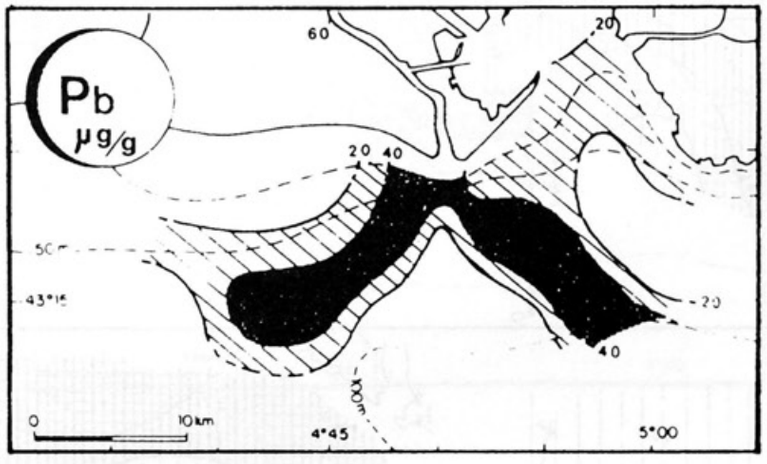


Figure 3. - Répartition en µg/g du Pb associé aux oxydes de Mn (extraction au chlorhydrate d'hydroxylamine) des sédiments. Cette disposition bilobée pourrait être due à l'action des courants remontant vers l'embouchure, scindant en 2 langues le matériel rhodanien.

La cartographie des eaux interstitielles des sédiments superficiels (figure 2d) montre une image de répartition inverse par rapport à celle de la pleine eau. Les plus faibles teneurs en Pb se retrouvent en effet dans la bande orientée vers le Sud-Est (ou Sud-Sud-Est). Cela fait penser à un axe de courant près du fond en provenance du Rhône.Etant donné l'opposition avec la carte du Pb associés aux oxydes-hydroxydes (figure 3), on peut admettre que, là le Pb coprécipite avec les oxydes ou hydroxydes de Fe et de Mn.

Le Cadmium

Les teneurs en Cd, fortes dans les eaux du Rhône au voisinage de l'embouchure, diminuent rapidement (voir figure 4a). Les eaux du large redeviennent plus chargées. Une bonne part du Cd apporté par le Rhône se dépose au voisinage de l'embouchure par phénomène de floculation au passage eau douce-eau salée.

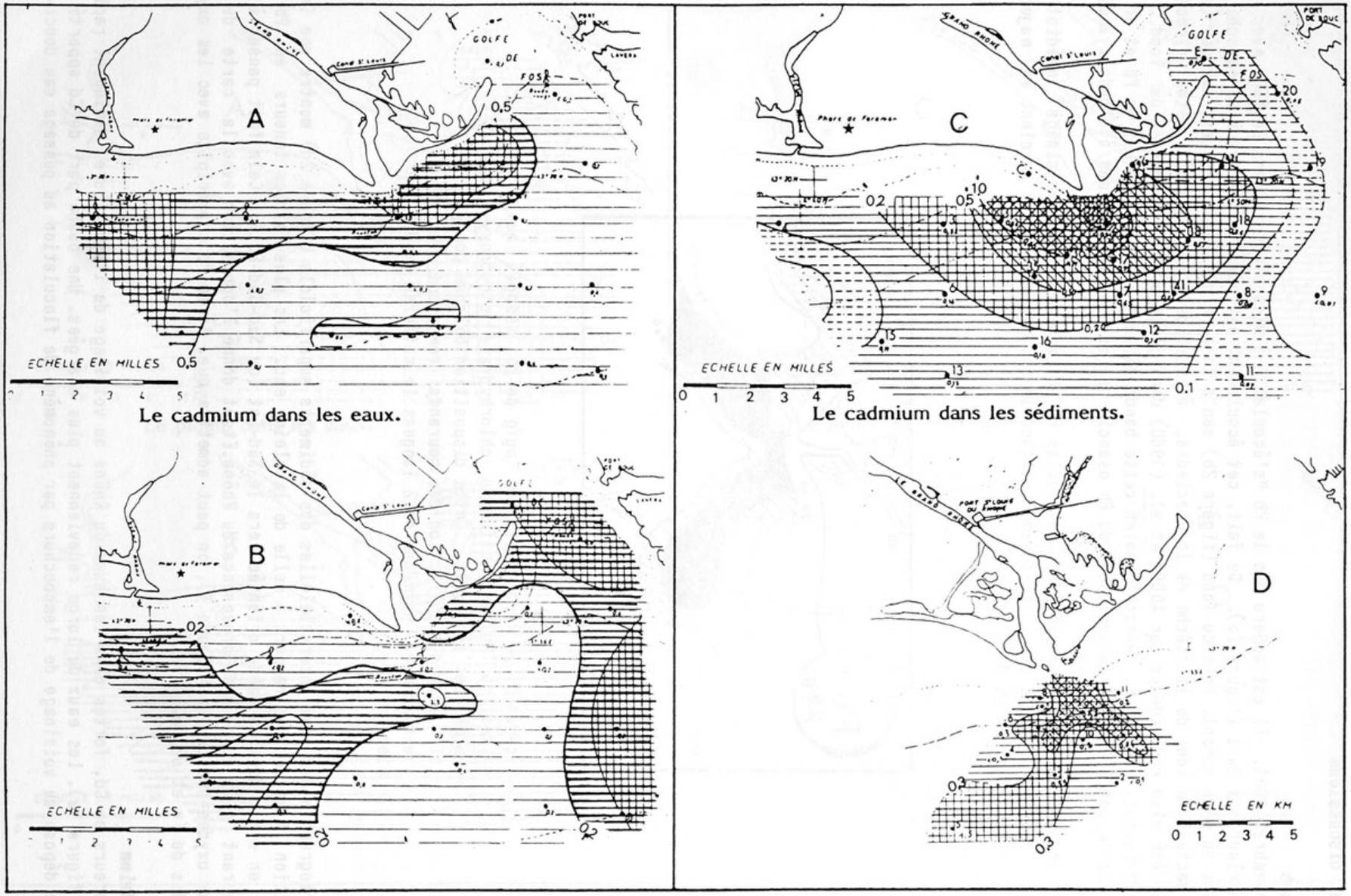


Figure 4. - Répartition du Cadmium dans les eaux (µg/l) à mi-profondeur (A), près du fond (B), dans les eaux interstitielles (D) et dans les sédiments (µg/g) (C).

Pour le reste, ce métal paraît faiblement lié aux particules déversées par le fleuve. Cela est confirmé par la relative richesse en Cd des eaux interstielles (figure 4d) et par la rapide diminution des teneurs dans les sédiments en s'éloignant de l'embouchure (figure 4c).

La seule zone où les sédiments marins sont riches en Cd est celle au voisinage de l'embouchure, là où l'accumulation de sédiments est rapide : plusieurs cm/an. L'effet de la diffusion du Cd à partir de l'eau interstitielle vers l'eau de mer sus-jacente est diminué par cette accumulation de dépôts. Le Cd de la phase solide des dépôts se trouverait essentiellement coprécipité avec les carbonates (SPAN, 1984). De fait, cette part peut être considérée comme bien piégée.

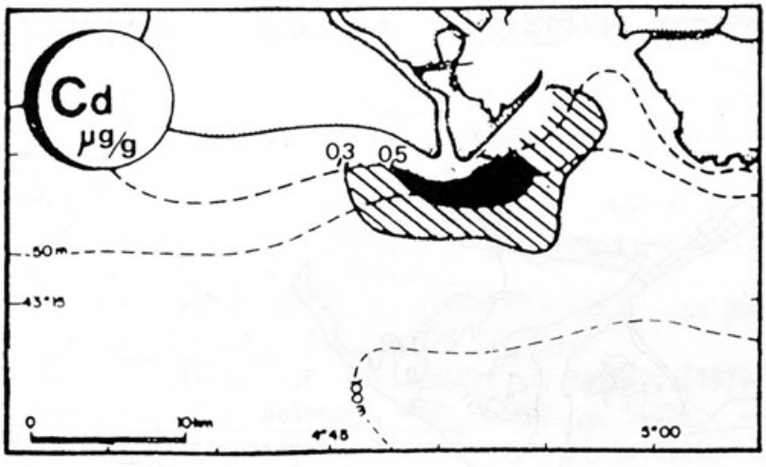


Figure 5. - Répartition en µg/g du Cd associé à la phase échangeable et carbonatée des sédiments.

Le Mercure

Bien que les eaux du Rhône en soient peu chargées les teneurs en mer sont souvent fortes. Cependant elles voisinent parfois avec des teneurs très faibles formant une répartition difficilement interprétable. Peut-être faudrait-il invoquer une contamination par l'atmosphère en provenance des zones industrielles de Lavéra, Port-Louis et Port-de-Bouc.

L'augmentation des teneurs en mercure dans les sédiments (figure 6a) avec la distance à la côte résulterait du fait que le métal serait essentiellement associé aux particules fines (sauf en présence de grandes quantités de matières organiques). Les zones à fortes teneurs au sud-est et au sud-ouest de l'embouchure ont déjà été repérées dans des missions antérieures (AUBERT et coll., 1979-1980; ADDED et coll., 1980; ADDED, 1981; ARNOUX et coll., 1980). Elles sont dues à des cheminements préférentiels et leurs positions dépendent des conditions météorologiques et courantologiques. Par ailleurs, la granulométrie joue un rôle particulièrement important dans la répartition (ADDED, 1981). Notons aussi les très fortes teneurs signalées par ARNOUX (1980) pour la région côtière de Lavéra, riche en posidonies. Il s'agit là de contaminations localisées.

Les plus fortes teneurs dans les eaux interstitielles sont situées au sud-est de l'embouchure au voisinage de zones à relativement fortes teneurs dans les couches d'eaux profondes et dans les sédiments superficiels (figure 6b).

Ainsi, encore plus nettement que pour le Cd, c'est aux mêmes endroits que l'on trouve des fortes teneurs tant dans la phase solide des sédiments que dans les eaux interstitielles.

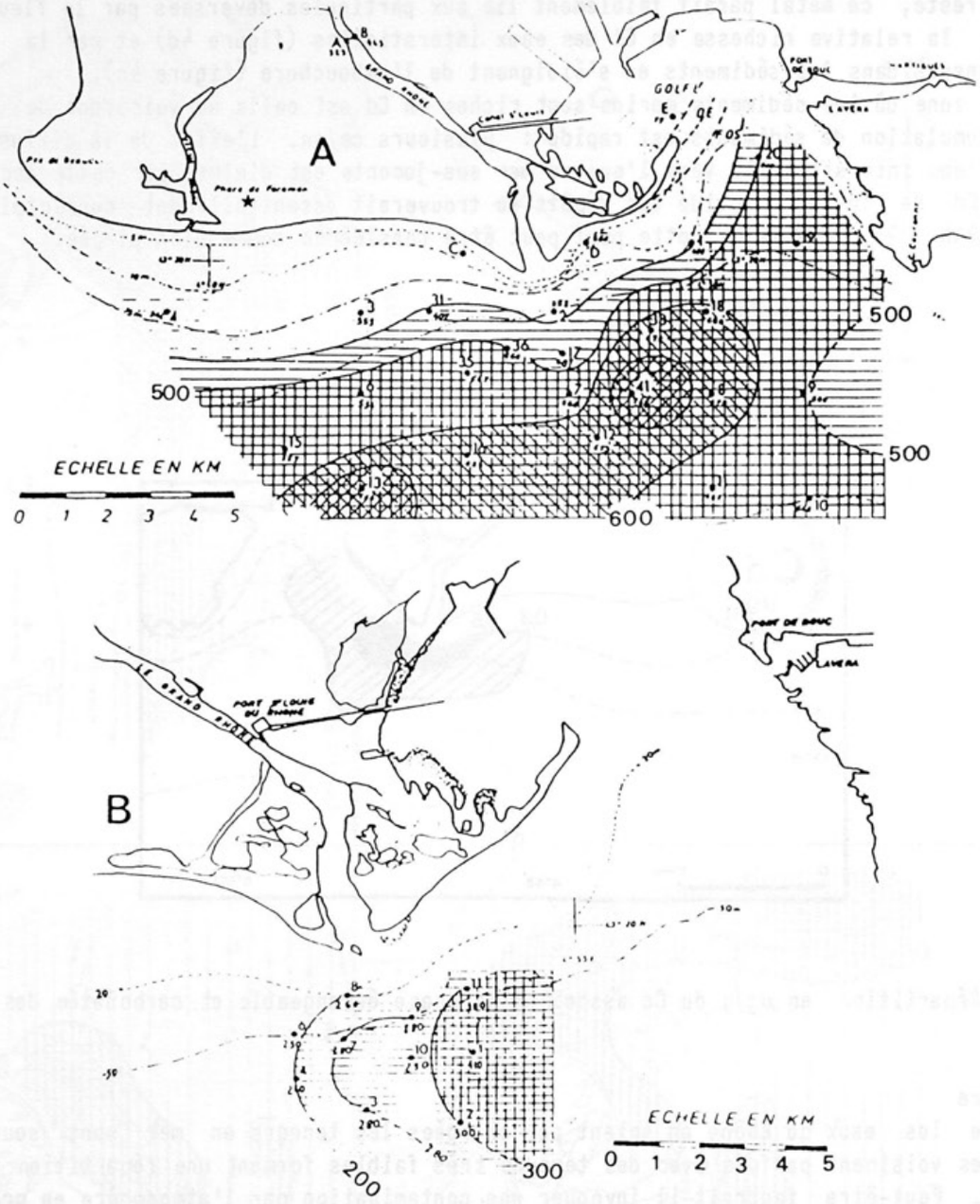


Figure 6. - Répartition du Mercure dans les sédiments (ng/g) (A) et les eaux interstitielles (ng/l) (B).

Conclusions

Certains métaux tendent à s'associer aux termes qui floculent au passage eau douce-eau salée. On ne les trouve en fortes teneurs dans les sédiments que près de l'embouchure. Tel est le cas du Cd. Les particules qui se déposent plus loin sont alors peu chargées.

D'autres métaux resteraient plutôt associés à des particules qui se déposent en grande partie plus au large, après avoir transité en pleine eau selon des voies privilégiées (surtout vers le Sud-Est ou vers le Sud-Ouest). Tel serait le cas du Pb et du Hg.

Divers métaux lourds, notamment le Pb, semblent relativement bien piégés une fois déposés dans les sédiments superficiels. En effet,les teneurs dans les eaux interstitielles ne sont pas beaucoup plus élevées que dans l'eau sus-jacente. La diffusion à partir de l'eau interstitielle vers l'eau de mer sus-jacente serait modérée.

En revanche, le Cd et le Hg dissous se présentent localement avec des teneurs relativement élevées dans les eaux des sédiments superficiels. Cela favorise une diffusion vers l'eau de mer susjacente.

Par ailleurs, il ne faut pas oublier qu'une part de la phase solide est susceptible d'être assimilée et transformée par les organismes benthiques ; par exemple les métaux lourds associés à la matière organique ou à des oxydes facilement réductibles. Ainsi, l'activité biologique benthique peut, elle aussi, conduire à un relargage d'une portion de certains métaux à partir des sédiments vers la pleine eau.

Références

A. ADDED, (1981). - Etude géochimique et sédimentologique des sédiments marins du delta du Rhône, Thèse 3e Cycle, Paris VI, 263 p.

A. ADDED, F. FERNEX, F. RAPIN, (1980). - Distribution of heavy metals in the superficial marine sediments in the vicinity of the Rhône mouths. Prog. Wat. Techn., vol. 12, n° 1, p. 89-96.

A. ADDED, J. CENCIARINI, F. FERNEX, C. SERRA, D. SPAN, (1983). - La situation des métaux dans les sédiments marins en des zones plus ou moins polluées de la Méditerranée nord-occidentale. C.I.E.S.M./P.N.U.E. (Cannes, 1982).

J.C. ALOISI, J.P. CAMBON, J. CARBONNE, G. CAUWET, C. MILLOT, A. MONACO, H. PAUC, (1982). - Origine et rôle du néphéloïde profond dans le transfert des particules au milieu marin. Application au Golfe du Lion. Océan Acta, vol. 5, n° 4, p. 481-491.

A. ARNOUX, J.L. MONOD, J. TATOSSIAN, A. BLANC, F. OPPETIT, (1980). - La pollution chimique des fonds du golfe de Fos. C.I.E.S.M./P.N.U.E., Cagliari, p. 447-458.

M. AUBERT, P. REVILLON, G. FLATAU, J.P. BREITTMAYER, J. AUBERT, (1979-1980). - Métaux lourds en Méditerranée (1er tome). Rev. Int. Océanogr. Méd., 66-67, p. 5-304.

D. SPAN, (1984). - Etude par fractionnement chimique de la distribution des métaux-traces dans les sédiments marins récents : le delta du Rhône, Thèse 3e Cycle, Paris VI, 252 p.

A. TISSIER, P.G.C. CAMPBELL, M. BISSON, (1979). - Sequential extraction procedure for the speciation of particulate trace metals, Analytical chemistry, vol. 51, n° 7, Univ. Québec, I.N.R.S.

WIND PATTERN AND EXPECTED DIRECTION OF FLOATING POLLUTANT TRANSPORT IN THE NEARSHORE ALEXANDRIA WATER

by

A.A.H. EL GINDY* and A.A. HAMED**
* Physical Oceanography, Oceanography Dept.
Faculty of Science, Alexandria University, Alexandria (Egypt)
** Hydrometeorological Office, Egyptian Navy, (Egypt)

Abstract

The petroleum industry and domestic waste water effluents are associated with the release of floating pollutants. In order to test the hypothesis that the direction of transport of floating pollutants is governed by winds, hourly records of wind velocity from a coastal meteorological station were analysed for the period from January 1976 to December 1979. Assuming that the shore is nearly straight, the monthly mean vectors were calculated and the progressive vector diagram represented. The shoreward flow dominated for most of the year, from February to November, while the alongshore flow (SW to NE) was apparent in January and December.

The onshore transport was confirmed by the driftcards experiments of GERGES (1978). Higher mean wind velocity was mostly observed in July and August when the NW wind prevailed strongly, but the mean rate of deposition of tarballs along Alexandria beaches was minimal in summer and maximal in winter (ABOUL-DAHAB and HALIM, 1980, and HALIM 1983). This contradiction could be explaited by the dominance of west winds in winter which transport the tarballs from their source, west of Alexandria. The difference in the rate of degradation of tarballs in the different seasons is also likely to affect the seasonal rate of deposition.

*
* *

Introduction

The petroleum activities located west of Alexandria and the sewage water effluents, from a number of outlets along the coast, release some floating materials at the sea surface. The flow direction of these pollutants is expected to be governed by direct wind action. The wind velocity vectors, therefore, could be taken as an indicator of their movements. In this paper the wind data are analysed to find the monthly trends of the surface drift.

Data and method of analysis

The hourly values of wind velocity at Ras-El-Tin meteorological station (figure I) in the period from January 1976 to December 1979, were considered (Records of Hydrometeorological Office).

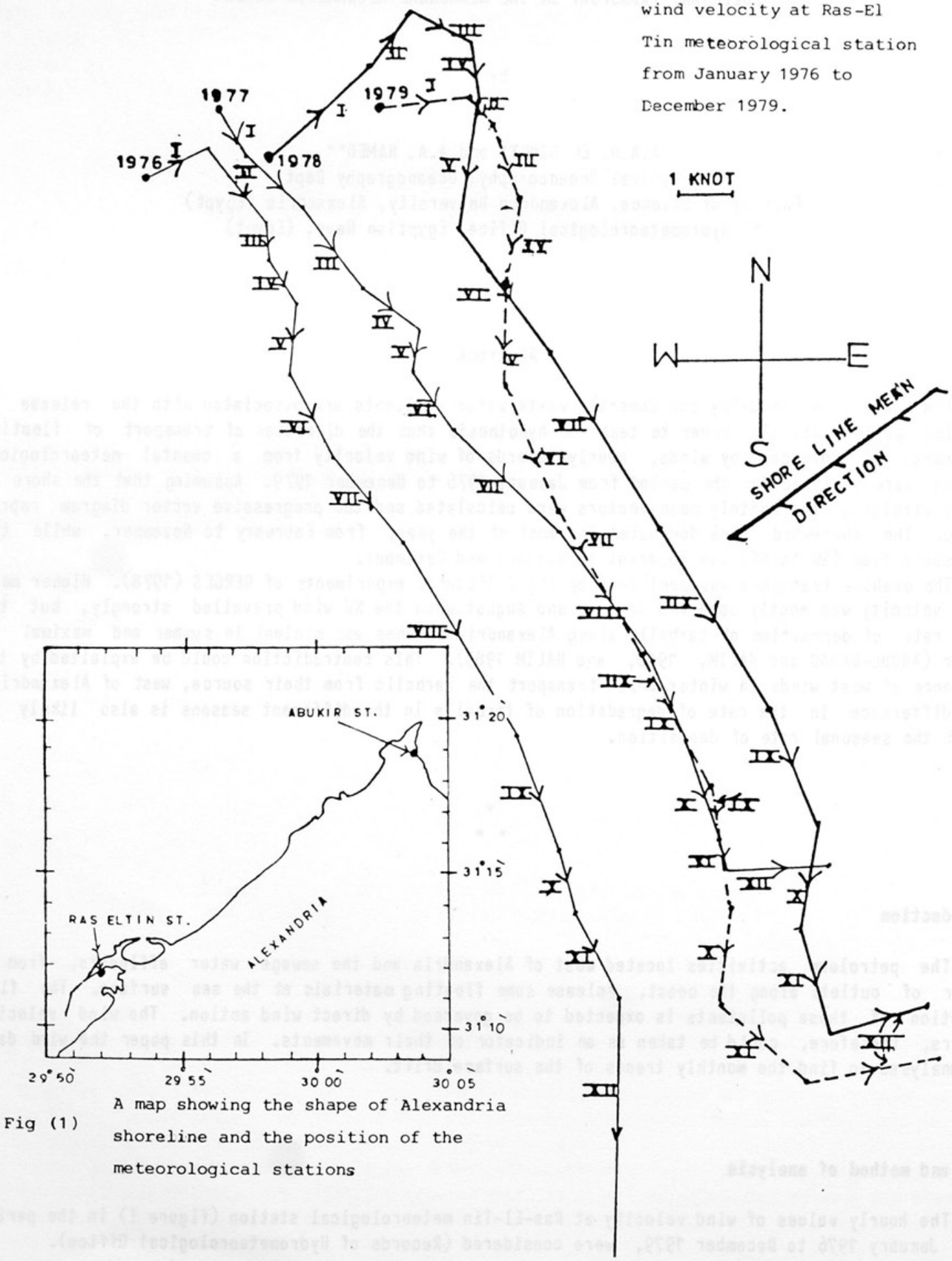


Fig (1) A map showing the shape of Alexandria shoreline and the position of the meteorological stations

Fig(2) Progressive vector diagram of monthly mean wind velocity at Ras-El Tin meteorological station from January 1976 to December 1979.

The wind velocity was analysed into alongshore and normal to shore components. The monthly mean of each component was calculated and the resultant mean velocity was estimated. The coastline is assumed to be nearly straight and oriented SW to NE.

The results of driftcards experiments in 1977 obtained by GERGES (1978) as well as the mean monthly rates of tarballs deposition along Alexandria beach, from September 1978 to June 1979, (ABOUL-DAHAD and HALIM 1980), and from September 1980 to June 1981 (HALIM, 1983) were also considered.

Results and discussion

The monthly means of wind velocity during the four years of study are represented by progressive vector diagram (figure II). It shows that the wind tends to blow towards the shore in the period from February to November, except in November 1978 when it was alongshore (SW to NE). During January and December the alongshore wind (SW to NE) was dominant except in December 1976 and January 1977 when the direction was onshore.

The monthly mean wind velocity was relatively high (6 to 9 knots) in July and August. In December 1976 and January 1978 strong mean wind velocities were also recorded. From the analysis of the wind velocity records, the highest speeds are associated with persistent wind direction while the lowest monthly mean speeds are due to direction scattering in autumn and spring : the transitional seasons.

The monthly rates of tarballs deposition along Alexandria beaches in 1978-1979 (ABOUL-DAHAB and HALIM 1980) were highest in winter and lowest in summer (figure III) i.e. it has nearly an inverse relations with the monthly mean wind speed. The seasonal periodicity in the tarballs deposition is confirmed by the observations of 1980-1981 (HALIM, 1983). The increase of tarballs deposition rate along Alexandria beaches in winter can be explained by two factors : the first being the different degradation rates of the tarballs in different seasons, the rate being higher in summer due to higher water temperature. On the other hand, the wind direction could have an important effect. In january and december the alongshore wind from SW to NE was predominant. Appreciable amounts of tarballs or other forms of petroleum products are therefore transported from the west to the east of Alexandria. The wind blowing along the coast will drive surface water against the coast and tarballs will deposit. In summer, the winds blow mostly from offshore (NW) and eastward drift of floating tarballs from their sources west of Alexandria is less frequent.

GERGES (1978) carried out driftcards experiments during 1977 in the area from the west of Alexandria to the west of Abu-kir. In this year, 1,418 cards were released during 10 experiments from which 18.55 per cent were recovered, mostly within five days after the release. The drift patterns of the cards show the dominance of the inshore flow in the nearshore zone west of Abu-kir head in March, September and November (figure IV). The flow direction agrees with that of the wind at Ras-El-Tin station. In the east of Abu-kir head, i.e. in Abu-kir Bay, the relation between wind direction and drift pattern is not clear.

Conclusion

From the above discussion it can be deduced that the mean wind velocity vector at a coastal meteorological station can be used as a tracer for the movement of the floating objects in the nearshore zone of Alexandria where the shoreline is nearly straight.

Acknowledgement

The authors are indebted to Prof. HALIM for reading the text and his constructive suggestions.

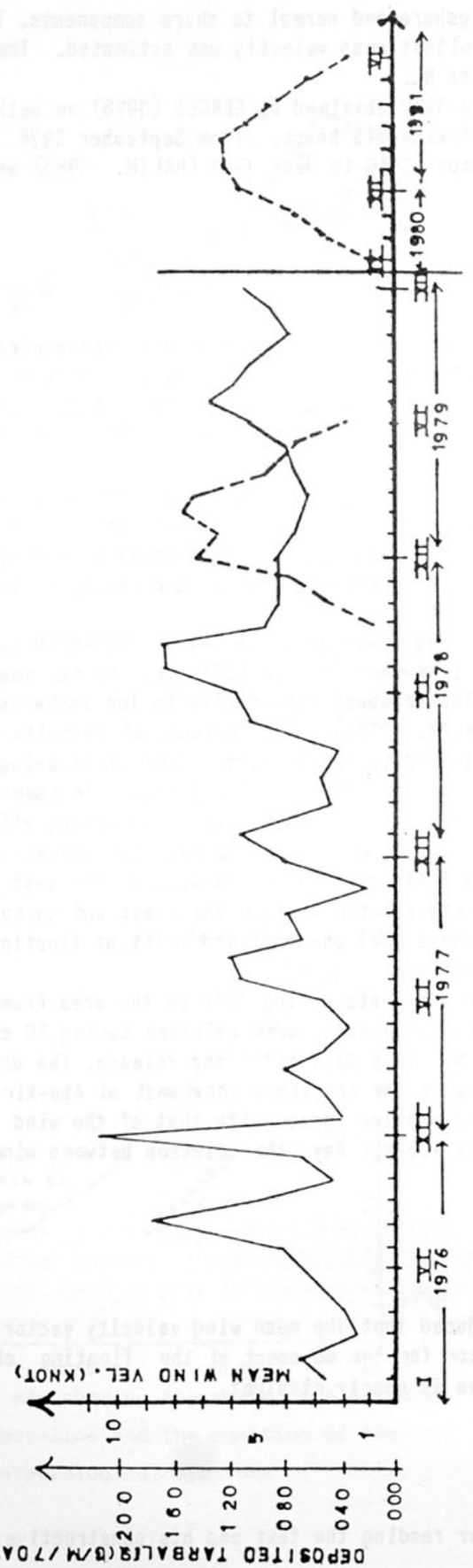


Fig.(3) Mean rates of tarballs desposition along Alexandria beaches (After Aboul-Dahb and Halim 1980, and Halim 1983)- - -and the monthly mean wind velocities at Ras-El-Tin station (——).

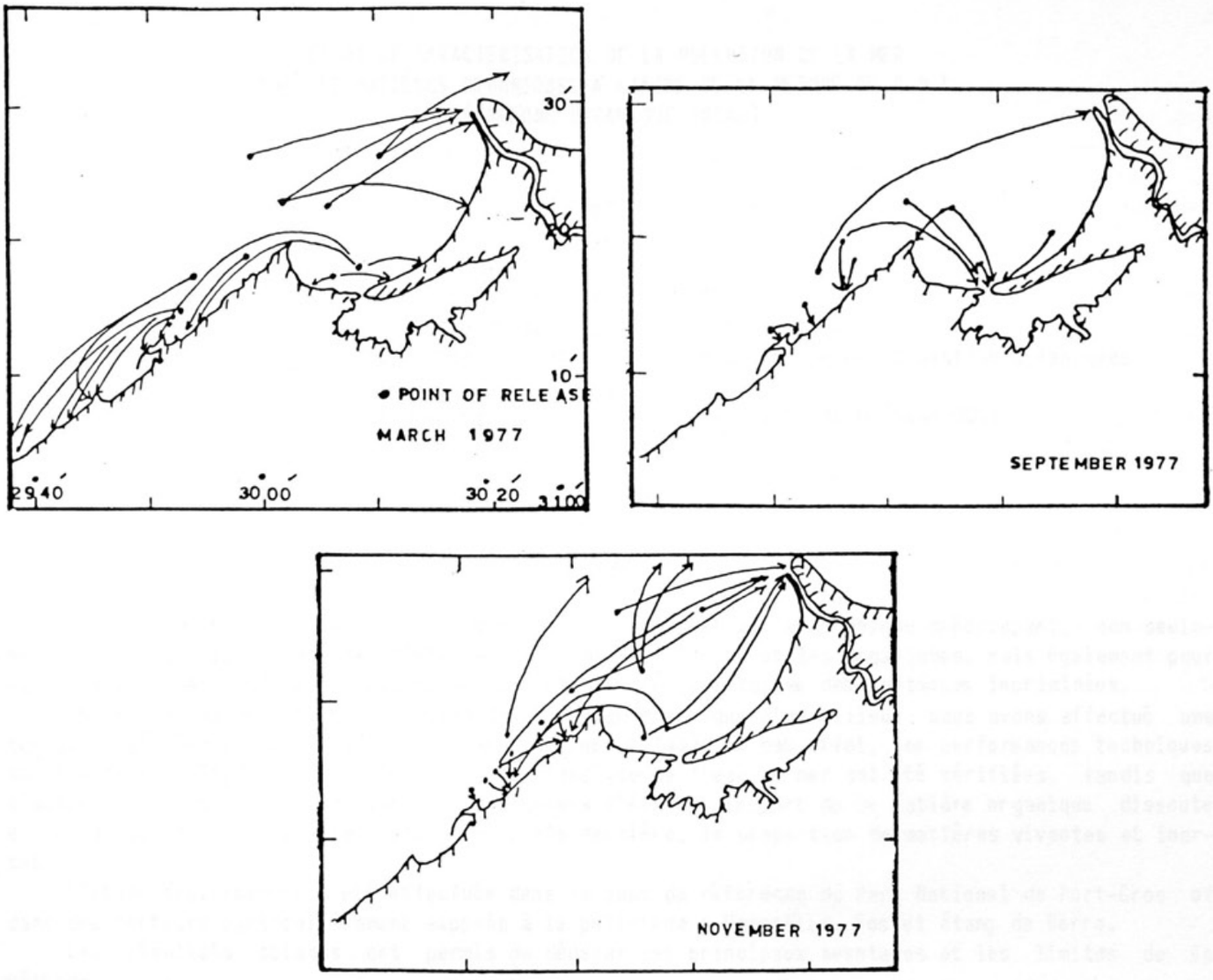


Fig. (4) Trajectories of surface drifters released in the coastal waters of Alexandria and Abukir bay

References

ABOUL-DAHAB O. and Y. HALIM (1980). - Oil pollution of the marine environment in the area of Alexandria. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. U.N.E.P. pp. 201-208.

GERGES M. (1978). - Trajectories and speed of surface currents near the Egyptian Mediterranean coast as deduced from the movement of surface drifters. IVes Journées Etud. Pollutions. Antalya C.I.E.S.M. U.N.E.P. pp. 573-587.

HALIM Y. (1983). - Mid-term technical report. Aquatic Environmental pollution project EGY/73/058. Hydrometeorological office, Egyptian Navy, wind records in the period 1976-1979.

ESSAI DE CARACTERISATION DE LA POLLUTION DE LA MER PAR LES MATIERES ORGANIQUES A L'AIDE DE LA MESURE DU C.O.T. (CARBONE ORGANIQUE TOTAL)

par

H. AUGIER et M. SESNEC
Laboratoire de Biologie végétale marine
Groupe d'Etudes et de Recherches de Biologie, Ecologie, Nuisances et Gestion Océaniques (B.E.N.G.O.)
Faculté des Sciences de Luminy, 13288 Marseille Cedex 9 (case 901)

Résumé

La contamination de la mer par les matières organiques est un problème préoccupant, non seulement à cause des perturbations qu'elle entraîne pour le milieu et les organismes, mais également pour la définition des critères d'évaluation quantitative et qualitative des subtances incriminées.

Parmi les voies possibles d'étude de la charge organique d'un milieu, nous avons effectué une tentative d'ulisation du C.O.T. (Carbone Organique Total). A cet effet, les performances techniques de l'appareil ASTRO ECOLOGY 1800 L.T.O. à l'analyse de l'eau de mer ont été vérifiées, tandis que d'autres techniques préparatives nous ont permis d'évaluer la part de la matière organique dissoute et de la matière particulaire, et, dans cette dernière, la proportion de matières vivantes et inertes.

L'étude exploratoire a été effectuée dans la zone de référence du Parc National de Port-Cros et dans des secteurs particulièrement exposés à la pollution : Marseille, Fos et étang de Berre.

Les résultats obtenus ont permis de dégager les principaux avantages et les limites de la méthode.

*
* *

Abstract

Sea pollution by organic material is a crucial problem not only with regard to the environment and the organisms, but also with regard to the definition of criteriors concerning quantitative and qualitative estimation of incriminated substances.

Among the possible ways of organic load study, we have carried out an attempt of utilizing T.O.C. (Total Organic Carbon). For that purpose, we have checked the technical performances of the ASTRO-ECOLOGY analysator, model 1800 L.T.O. adapted to the sea water. Whereas other preparative technics have enabled us to estimate the part of dissolved and suspended organic material, and in the latter, the proportion of living and inert materials.

The preliminary studies have been carried out in the reference area of the National Park of Port-Cros, and in areas particularly exposed to pollution : Marseille, Fos, étang de Berre.

The results have enabled to find out the main advantages and the limits of the method.

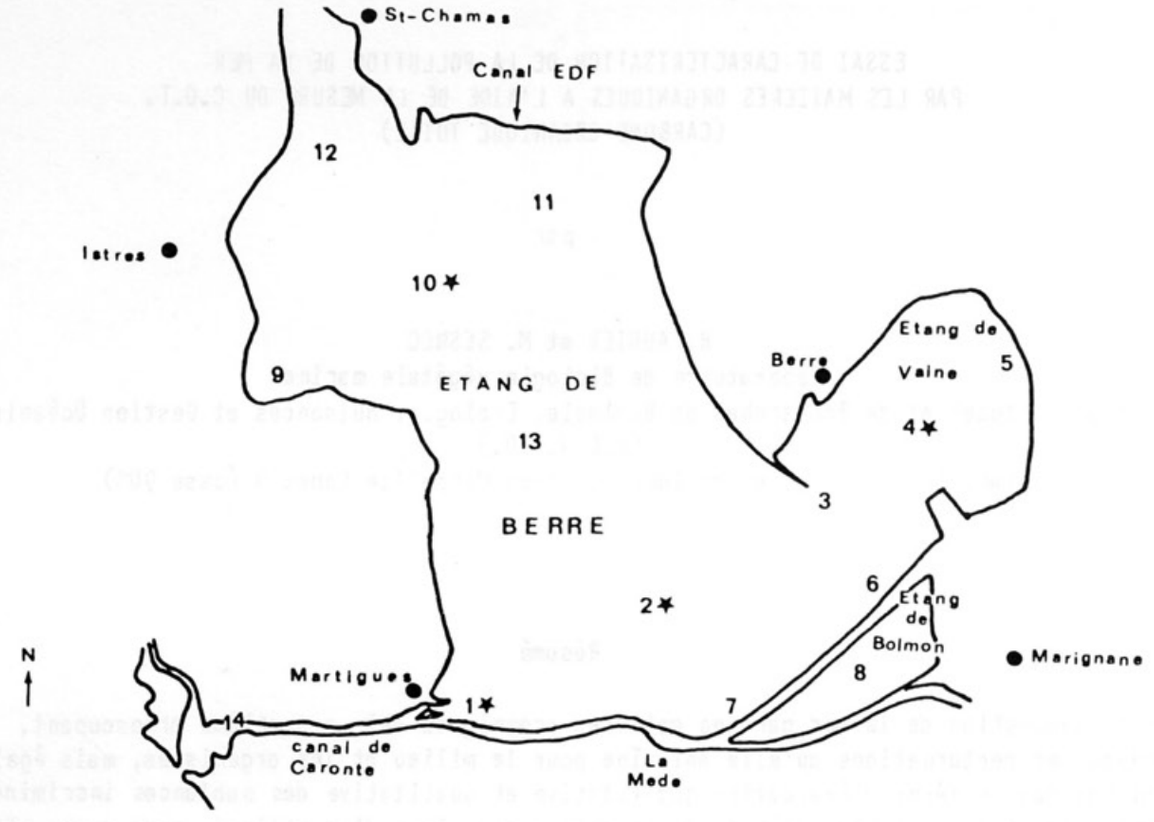


Figure 1. - Stations d'études de l'étang de Berre.

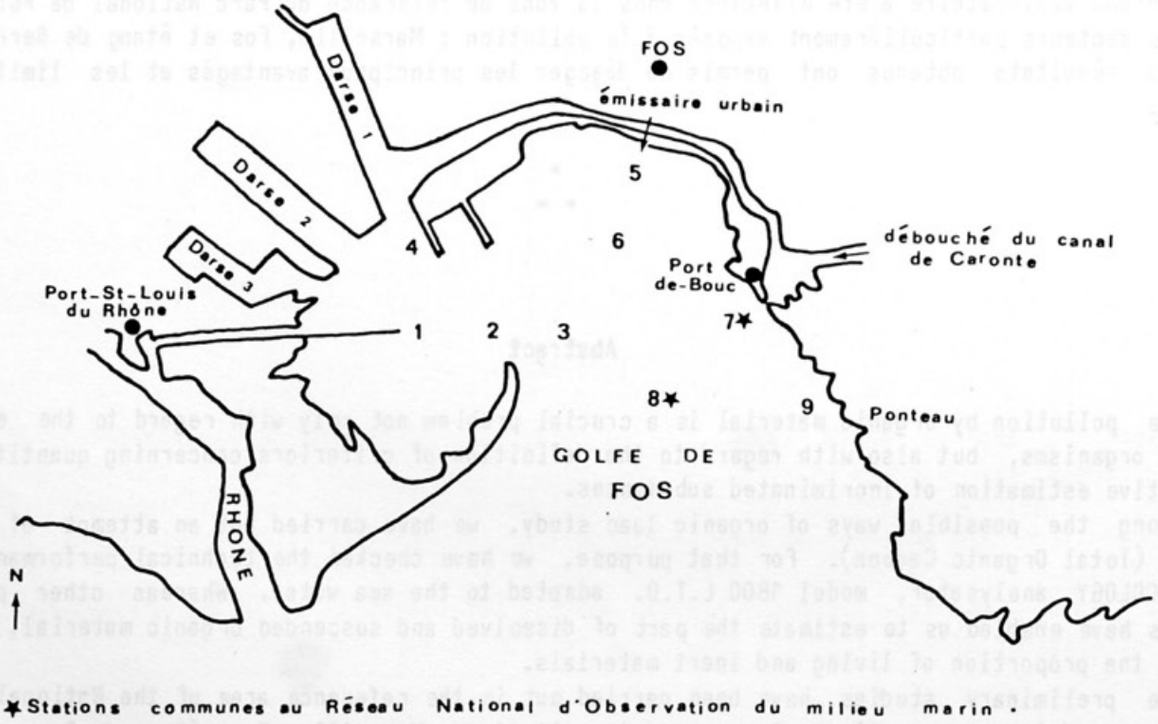


Figure 2. - Stations d'études du golfe de Fos.

Introduction

Nos connaissances sur les matières organiques contenues dans les eaux marines sont encore incomplètes et fragmentaires et ceci certainement en raison du nombre et de la variété chimique des composés organiques impliqués, de l'instabilité d'un grand nombre d'entre eux et enfin des difficultés que l'on rencontre habituellement dans leur dosage et leur identification. La complexité du problème ressort bien des formes multiples de la matière organique : d'origine naturelle ou provenant des rejets polluants sous forme vivante ou inerte, en solution vraie, en suspension colloïdale ou en suspension particulaire, sous forme d'humus, etc.

A l'heure actuelle, pour contrôler la charge en matières organiques d'une eau, à défaut d'identifier et de doser chacune des subtances prises séparément, on a la possiblité d'utiliser deux indicateurs différents : l'oxygène et le carbone.

Dans la pratique courante, on emploie préférentiellement l'oxygène ; la charge en matières organiques s'exprime alors en quantité d'oxygène nécessaire à leur oxydation, c'est-à-dire en quantité d'oxygène demandée par voir chimique (D.C.O.) ou biologique (D.B.O., D.B.O. 5). Ces mesures sont malheureusement inopérantes en mer (BEBIN et al., 1970), reste donc la voie passant par le carbone.

Sur la base de la détermination du carbone organique, de nombreuses tentatives ont été mises en oeuvre dans le passé, mais les techniques trop laborieuses et les appareils trop peu sensibles en limitaient la pratique (ERHARDT 1969, BRIGGS 1976, CHANDLER et al., 1976, WAITE 1978, ...). Une génération d'appareils plus performants ont permis d'obtenir récemment des résultats intéressants, notamment sur les eaux douces (SMALL 1980, MARTY 1981, BRASS 1982, ...) ; Il nous a, par conséquent, paru opportun d'engager une série d'essais sur les possibilités d'utilisation du C.O.T. dans la mesure de la charge organique du milieu marin et pour tester s'il peut être considéré comme un paramètre hydrologique courant d'évaluation de la qualité des eaux marines.

Méthodes

1. Sites

L'étude exploratoire a été effectuée dans la zone de référence du Parc National de Port-Cros et dans trois autre secteurs présentant des caractéristiques différentes au plan de la pollution et du degré de confinement (fig. 1 à 4).

- le secteur de Cortiou, zone ouverte placée sous l'influence directe du rejet non épuré des eaux usées urbano-industrielles de la ville de Marseille (1.500.000 EQH) ;
- le golfe de Fos, plus enclavé et caractérisé par une pollution industrialo-portuaire dominante ;
- l'étang de Berre, très confiné, à charge organique importante et à tendance eutrophique dans certains secteurs.

2. Echantillonnage

L'eau de surface est prélevée avec un seau et celle du fond et de la profondeur intermédiaire à l'aide d'une bouteille à clapets traditionnelle. Les échantillons sont transportés et stockés à une température inférieure à 10° C (OTSON et al., 1979).

3. Mesure du C.O.T.

Le C.O.T. est déterminé à l'aide de l'analyseur ASTRO ECOLOGY 1800 L.T.O. et selon les indications de BONNEFILLE et al., (1982). Les performances, la sensibilité de l'appareil et les différents paramètres de variation des dosages ont été contrôlés, notamment l'effet de la température, l'interférence des chlorures et le rendement de l'oxydation.

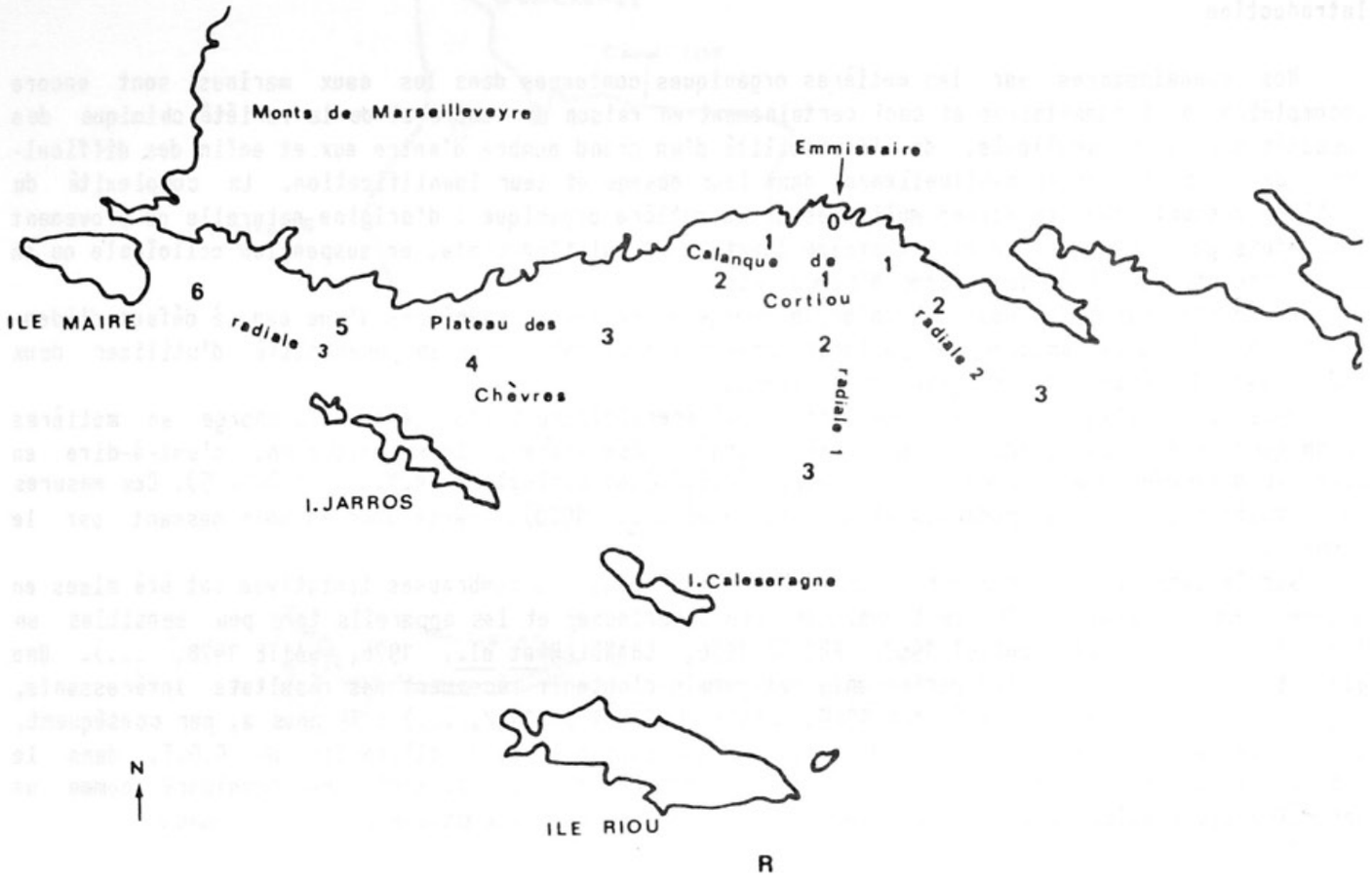


Figure 3. - Stations d'études de l'émissaire de Cortiou.

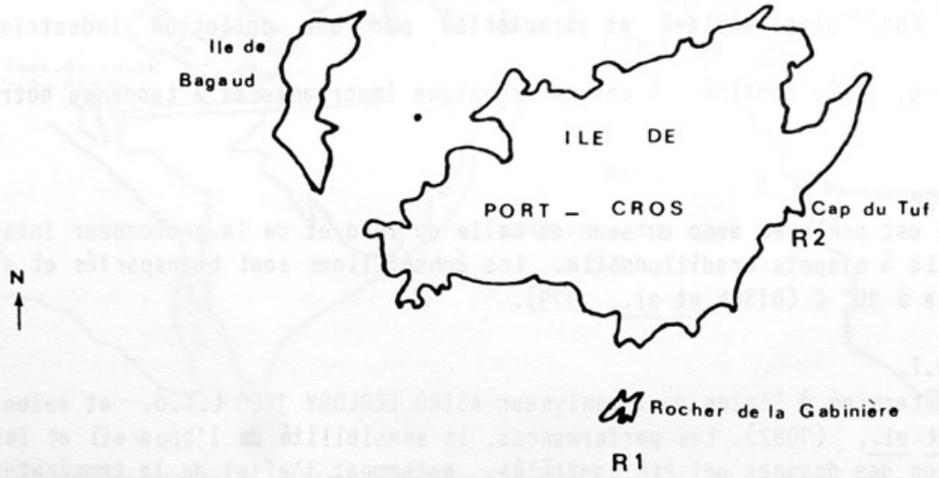


Figure 4. - Stations de Référence (R).

Echantillon	Volume plasmique total (mmg)	Carbone vivant (ppm)
Berre (13/12)		
1 - Surface	1.166	4.5
4 - Surface	0.336	1.3
6 - Surface	0.274	37.7
7 - Surface	1.023	3.9
8 - Surface	0.291	0.07
10 - Surface	1.913	7.3
12 - Surface	0.596	2.3
12 - Fond	0.119	0,5
Cortiou (26/10)		
I - 2 Surface	0.094	0.04
I - 1 Demi-prof.	0.023	0.09
I - 1 Fond	0.047	0.02
O - Fond	0.036	0.14
II - 2 Fond	0.016	0.06
III - 2 Surface	0.015	0.06
III - 4 Demi-prof.	0.042	0.02
Fos (22/11)		
5 - Demi-prof.	.0,0803	0.3
3 - Surface	.0,025	0.09
3 - Surface	.0,015	0.06
1 - Fond	pas de phyto	0

TABLEAU I : Résultats des calculs du carbone de la matière vivante par la formule de SMAYDA (1965).

Stations		21/12/82		25/9/83	
		Brut	Filtré	Brut	Filtré
R_1	S	0,3	0,3	0,3	0,3
	DP	0,4	0,3	04,	0,3
	F	0,5	0,5	0,5	0,4
R_2	S	0,7	0,5	0,5	0,3
	DP	0,9	0,4	0,5	0,4
	F	1,2	0,4	0,7	0,5

TABLEAU III : Taux de C.O.T. (en ppm) dans les échantillons d'eau des stations de référence du Parc national de Port-Cros (S = Surface, DP = Demi-Profondeur, F = Fond)

Site	Minimum absolu	Maximum absolu
Berre (oct.)	1,5	5,9
Berre (déc.)	1,5	4,3
Fos	0,5	1,4
Cortiou (juil.)	0,9	3,4
Cortiou (oct.)	0,3	3,6
en moyenne	0,7	3,6

TABLEAU II : Valeurs minimales et maximales absolues du C.O.T. observées sur chaque site (en ppm.)

Stations		Brut	Filtré	Stations		Brut	Filtré
1	S	1,4	0,9	6	S	1,0	0,9
	DP	1,1	0,7		DP	0,8	0,8
	F	0,9	0,5		F	0,9	0,7
2	S	0,6	0,6	7	S	1,2	1,2
	DP	1,4	0,6		DP	0,8	0,8
	F	0,8	0,5		F	0,6	0,6
3	S	1,1	0,9	8	S	0,9	0,7
	DP	0,6	0,6		DP	0,6	0,5
	F	0,5	0,5		F	0,7	0,6
4	S	0,8	0,7	9	S	0,7	0,6
	DP	0,6	0,5		DP	0,8	0,7
	F	0,8	0,5		F	0,6	0,6
5	S	0,9	0,8				
	DP	1,0	0,8				
	F	0,7	0,5				

TABLEAU IV : Taux de C.O.T. (en ppm) dans les échantillons d'eau du golfe de Fos (S = Surface, DP = Demi-Profondeur, F = Fond).

Le C.O.T. est mesuré sur l'échantillon brut et sur le filtrat après passage sur membranes millipores de 0,45 u. Nous avons également tenté de faire la part du carbone organique des organismes vivants et du matériel inerte par la méthode de calcul du volume cellulaire d'UTERMOHL, in SMAYDA (1965).

Résultats et discussion

1. Le carbone de la matière vivante

Comme le montre le tableau I, la méthode de SMAYDA (1965) conduit à des résultats contradictoires, soit qu'ils concordent avec les valeurs du C.O.T., soit qu'ils conduisent à des surestimations considérables, dépassant même quelquefois la valeur elle-même du C.O.T.

Compte tenu des anomalies, nous n'avons pas étendu le calcul à l'ensemble des échantillons et nous conseillons, en attendant mieux, de se fier préférentiellement à la mesure du C.O.T. après filtration. Cela n'empêche pas, malgré le handicap, qu'en matière de pollution, il peut être intéressant de connaître la part représentée par le matériel particulaire. Le paramètre M.E.S.T. fournit déjà le renseignement global et le C.O.T. peut renseigner sur la part de matière organique, en particulier inerte, en valeur approchée. Rappelons, toutefois, qu'à l'heure actuelle, la technique employée fournit des valeurs où les matières dissoutent représentes souvent plus de 90 % du contenu organique de l'échantillon.

2. Le C.O.T. dans la zone de référence du Parc National de Port-Cros.

Les résultats obtenus dans le Parc National de Port-Cros (tableau III) ne sont pas tous représentatifs d'une zone de référence. Pour expliquer les valeurs élevées obtenues notamment à la station R2, on peut mettre en doute la pureté des eaux de l'île, car des anomalies de ce genre ont été déjà observées comme, par exemple, pour les détergents (AUGIER et al., 1984). On peut également incriminer l'intrusion et les remaniements de matières et de sédiments au niveau des fonds sous l'influence de forts courants, tel qu'il a pû être constaté, maintes fois, en plongée.

Les résultats les plus représentatifs d'une station de référence seraient encore ceux concernant la station R1 où les valeurs oscillent de 0,3 à 0,5 ppm.

3. Le C.O.T. dans les zones polluées

L'examen des résultats (tableaux IV à VI) montrent que les bornes de variation des valeurs du C.O.T. sont très variables suivant le site étudié, aussi pour faciliter la discussion nous avons reporté dans un tableau distinct les valeurs maximales et minimales du C.O.T., pour chaque site (tableau II).

Dans tous les cas, les valeurs minimales ou maximales du C.O.T. sont toujours plus importantes dans les échantillons bruts que dans les échantillons filtrés, ce qui est logique puisque la filtration élimine la partie organique des matières particulaires. Les taux sont en général supérieurs en surface, sauf à Cortiou en été. De même, à l'exception de Fos, le minimum absolu est toujours observé au fond sur un échantillon filtré et le maximum absolu sur un échantillon brut de surface.

Si l'on compare les résultats obtenus entre les échantillons filtrés et non filtrés, on constate que l'écart maximal est de 2,2 (Berre, profondeur intermédiaire), l'écart minimal nul (Fos, en surface) et la moyenne des écarts égale à 0,5. Il apparaît donc que la matière organique dissoute est prépondérante dans la mesure du C.O.T.

Enfin, dans l'ensemble, les valeurs obtenues ne sont pas aussi élevées que celles auxquelles on aurait pu s'attendre pour des zones polluées, notamment à Cortiou. Le facteur de dilution doit entrer pour une grande part dans l'explication de ce phénomène. Les sites peuvent être classés par ordre croissant des valeurs du C.O.T. : Port-Cros, Cortiou l'hiver, Fos, Cortiou l'été, étang de Berre.

a) Emissaire de Cortiou

Des gradients de concentration à partir du point de rejet ont pu être mis en évidence (tableau V) :

- En été, la pollution s'étend loin en profondeur comme le montre la situation au niveau des radiales 1 et 2 ; elle atteint même la zone de référence certainement par intrusion de nappes polluantes poussées par la Tramontane très forte la veille des prélèvements. La radiale 3 présente des valeurs plus importantes que les autres et marque donc une dérive vers l'ouest.

- En hiver, la charge organique s'étend encore vers le fond, mais elle diminue plus rapidement en allant vers le large ; il convient cependant de préciser que le vent qui soufflait ce jour-là vers la côte devait contribuer à freiner l'extension des eaux usées vers le large. La station de référence joue bien son rôle cettre fois, les valeurs du C.O.T. y sont aussi faibles qu'à Port-Cros (0,3 ppm).

Stations		8/7	26/10	
		Brut	Brut	Filtré
0	S	3,4	2,5	1,8
	DP	1,7	1,5	0,9
	F	1,2	1,0	0,9
1	S	1,7	3,6	2,7
	DP	1,6	1,1	1,0
	F	1,3	1,0	0,9
2	S	1,6	1,0	0,8
	DP	1,7	0,8	0,7
	F	1,3	0,5	0,7
3	S	–	0,6	0,3
	DP	–	0,9	0,4
	F	–	0,5	0,4
4	S	2,4	1,2	1,0
	DP	2,2	1,0	0,7
	F	1,9	0,7	07
5	S	2,2	0,9	0,9
	DP	2,2	0,6	0,3
	F	1,4	1,0	0,7
6	S	1,7	0,9	0,8
	DP	2,5	0,6	0,5
	F	1,9	0,6	0,4

Stations		8/7	26/10	
		Brut	Brut	Filtré
7	S	2,5	2,8	1,9
	DP	1,7	0,6	0,5
	F	1,3	0,5	0,4
8	S	1,7	1,1	0,9
	DP	1,5	0,8	0,5
	F	1,6	0,7	0,4
9	S	–	1,1	0,7
	DP	–	0,6	0,6
	F	–	0,7	0,5
10	S	–	0,7	0,6
	DP	–	0,6	0,4
	F	–	0,6	0,3
11	S	–	0,6	0,4
	DP	–	0,5	0,4
	F	–	0,6	0,4
12	S	–	0,5	0,3
	DP	–	0,5	0,4
	F	–	0,7	0,4
13	S	0,9	0,6	0,4
	DP	1,2	0,6	0,4
	F	1,0	0,6	0,3

TABLEAU V :

Taux de C.O.T. (en ppm) dans les échantillons d'eau de Cortiou
(S = Surface, DP = Demi-Profondeur, F = Fond)

Stations		18/10		13/12	
		Brut	Filtré	Brut	Filtré
1	S	2,4	2,3	2,2	2,0
	DP	2,0	1,6	–	–
	F	1,8	1,5	2,0	2,0
2	S	2,5	2,5	2,1	1,9
	DP	2,5	2,3	–	–
	F	2,4	2,2	2,2	1,7
3	S	–	–	2,3	1,8
	DP	–	–	–	–
	F	–	–	2,2	1,7
4	S	5,7	4,0	2,8	2,3
	DP	5,9	3,7	–	–
	F	5,8	3,8	2,4	2,3
5	S	–	–	2,9	2,2
	DP	–	–	–	–
	F	–	–	–	–
6	S	–	–	4,3	3,0
	DP	–	–	–	–
	F	–	–	–	–
7	S	–	–	2,8	2,5
	DP	–	–	–	–
	F	–	–	–	–

Stations		18/10		13/12	
		Brut	Filtré	Brut	Filtré
8	S	–	–	9,7	9,2
	DP	–	–	–	–
	F	–	–	–	–
9	S	2,3	2,2	2,1	1,9
	DP	–	–	–	–
	F	–	–	–	–
10	S	3,0	2,7	2,5	2,1
	DP	2,8	2,5	–	–
	F	2,6	2,5	2,4	1,8
11	S	–	–	2,8	2,4
	DP	–	–	–	–
	F	–	–	2,5	1,9
12	S	2,5	2,0	2,1	1,8
	DP	–	–	–	–
	F	–	–	2,0	1,5
13	S	–	–	3,1	2,6
	DP	–	–	–	–
	F	–	–	2,9	2,4
14	S	2,4	2,3	–	–
	DP	2,0	1,6	–	–
	F	1,8	1,5	–	–

TABLEAU VI :

Taux de C.O.T. (en ppm) dans les échantillons d'eau de l'étang de Berre
(S = Surface, DP = Demi-Profondeur, F = Fond)

b) Golfe de Fos

Sur les radiales, les gradients sont difficiles à mettre en évidence (tableau IV), ce qui traduit une certaine uniformisation de la charge organique dans le golfe. Un gradient paraît cependant exister de 1 vers 3 à la profondeur intermédiaire et au fond et un autre, moins marqué, de 5 vers 3 à la profondeur intermédiaire. En surface, la situation est assez complexe, mais les valeurs maximales ont été trouvées cependant dans les parties les plus confinées, à proximité des rejets industriels de Lavera, au débouché du canal de Port-Saint-Louis (influence du Rhône) et à la sortie du canal de Caronte (influence de l'étang de Berre).

Les valeurs du C.O.T. ne sont, en général pas aussi élevées que celles qu'on attendait d'un milieu recevant d'aussi nombreux rejets d'eaux usées, mais cette situation peut être facilement expliquée. On sait, en effet, que depuis quelques années la situation du golfe de Fos s'est nettement améliorée (réduction de 90 % de la pollution en terme de $D.B.O._5$, D.C.O. et M.E.S.T.) grâce aux moyens de lutte et de prévention mis en oeuvre. On sait aussi que les eaux sont très diluées par un apport considérable d'eau douce provenant principalement du Rhône et de la dérivation de la Durance dans l'étang de Berre.

c) Etang de Berre

Les valeurs du C.O.T. dans l'étang de Berre sont très élevées (tableau VI), elles sont tout à fait significatives de la situation de l'étang et confirment certains faits bien connus : l'influence des rejets domestiques et des industries riveraines à vocation surtout pétrolière et celle aussi des eaux sortant de la centrale hydroélectrique de Saint-Chamas. Le rôle du confinement est également bien mis en évidence dans l'étang de Vaïne et dans l'étang de Bolmon qui ne sont pas situés dans la zone centrale de drainage.

Si l'on excepte les cas particuliers des étangs de Vaïne et de Bolmon, il existe aussi, comme dans le golfe de Fos, une certaine uniformisation de la charge en matières organiques de l'étang, notamment dans son axe principal partant de Saint-Chamas. De même les différences enregistrées entre la surface et la demi-profondeur et la demi-profondeur et le fond ne sont pas très significatives et sont le reflet d'une absence de stratification de la charge organique ; ce constat nous a incités à supprimer les prélévements intermédiaires lors de la deuxième campagne. Il convient cependant de noter qu'un gradient s'établit souvent sur l'axe allant de l'étang de Vaïne au canal de Caronte, moins soumis au drainage des eaux de la Durance.

Si on compare maintenant la campagne d'octobre à celle de décembre, on s'aperçoit que les valeurs obtenues la première fois sont plus élevées, surtout pour les concentrations maximales. Le phénomène résulte certainement d'une charge plus importante en composés organiques, mais certainement aussi de la présence d'une plus grande quantité de plancton en automne qu'en hiver, tel qu'on peut le voir dans l'examen des populations planctoniques. Dans cette hypothèse, une partie au moins du matériel vivant est oxydé et entre dans la valeur du C.O.T. ... ce qui est confirmé par l'écart observé entre les échantillons bruts et filtrés.

Conclusion

L'étude entreprise a permis de vérifier les performances de l'appareillage utilisé : bonne reproductibilité des mesures, linéarité du signal facilitant la lecture directe des résultats, automatisation pour passage en continu des échantillons. La limite de sensibilité, bien qu'intéressante (0,2 à 0,3 ppm) gagnerait encore à être améliorée.

Sur le plan de la mesure, la valeur du C.O.T. ne prend pas toujours en compte la totalité des matières en suspension. Il est donc possible de ne travailler que sur le filtrat pour des mesures de routine, la fraction dissoute représentant environ 90 % du C.O.T. mesuré.

Au plan opérationnel, les résultats obtenus montrent tout l'intérêt de la mesure du C.O.T. comme critère de pollution générale, susceptible de se modifier au contact des contaminants et de donner

l'alerte (CARSIN, 1976) ; d'autant plus qu'elle introduit une évaluation quantitative du taux de matières organiques et peut compléter ainsi les paramètres classiques, en général inopérants en mer, comme la D.B.O. et la D.C.O. Plusieurs auteurs considèrent d'ailleurs que ces mesures sont indissociables dans le contrôle de la pollution des eaux douces et ont mis au point des correlations entre ces divers paramètres (BORIES 1976, WAITE 1978, CONSTABLE et Mc BEAN 1979, BONNEFILLE et al;, 1982).

Nos résultats soulignent d'autre part, l'intérêt de prendre en compte la mesure du C.O.T. dans l'étude des milieux à tendance eutrophique, notamment dans les zones plus ou moins confinées. La mesure du C.O.T. est également un élément très précieux pour des études spécifiques dans lesquelles la matière organique a un rôle important à jouer ; nous développerons prochainement des résultats intéressants à ce sujet grâce à la prise compte du C.O.T. dans la recherche des conditions hydrologiques conditionnant l'apparition des eaux rouges.

Enfin, dans les milieux ouverts sur la pleine mer, comme à Cortiou, les valeurs du C.O.T. sont apparues peu élevées en regard de la masse polluante : des causes hydrologiques (forte dilution, courants...) météorologiques et topographiques peuvent être évoquées, mais on peut également incriminer la méthodologie. L'utilisation de la mesure du C.O.T. pour les milieux ouverts est donc subordonnée à la mise en oeuvre de recherches complémentaires.

Remerciements

Il nous est agréable de remercier M.M. EVRARD, APLINCOURT, BONNEFILLE, TABONNET, PEYRE et SALIBA de l'Agence de Bassin Rhône-Méditerranée-Corse, Mme BERTRANDY et M.M.PATRONE et PERFETTI des cellules anti pollution de Marseille et de Martigues, M.M. MANCHE, ROBERT, GERARDIN et PERROCHEAU du Parc National de Port-Cros, Mme BECKER du laboratoire d'Hydrobiologie de Marseille-Luminy.

Références

M. AUBERT, H. AUGIER, P. VON UNRUH, M. LAFAURIE, C. TAFANI, A. MATHIEU, M. COLLET-PLANAS, R. CLEMENT (1984). - Etude de la prévention des risques liés à l'apparition des eaux rouges. Rev. Intern. Océan. Médic. 5-25.

H. AUGIER, G. MONNIER-BESOMBES, M. SIGOILLOT (1984). - Influence des détergents sur Posidonia oceanica, Ile de Porquerolles 13-15 octobre 1983. G.I.S. Posidonies Edit. (sous presse).

J. BEBIN, J. BOULENGER, J. OBSZEUSMI (1970). - La demande chimique en oxygène et la demande biochimique en oxygène. Degremont, Edit. Cahier d'analyse no 1 (2e Edit.).

M. BONNEFILLE, DUCAS R., PEYRE D., TABONET A., LONGVERT G. (1982). - Carbone Total et Carbone Organique Total. Oxydation par ultra-violet ; Analyseur ASTRO ECOLOGY - Modèle 1800 L.T.O. Rapport de l'Agence de Bassin Rhône-Méditerranée-Corse : 113 p.

A. BORIES, (1977). - Le Carbone Organique Total. Méthodes modernes de mesure et intérêt de la détermination dans les eaux résiduaires. Ann. Technol. Agvic., Fr. 25 (3) : 273-289.

H.J. BRASS, (1982). - Procedures for analyzing organic contaminants in drinking water. Am. Water works Assoc., USA, 74 (2) : 107-112.

R. BRIGGS, (1976). - Instrumental methods of monitoring organic pollution. J. Inst. Wat. Pollut. Cont., 1 : 47-56.

J.L. CARSIN, (1976). - Intérêt de l'indice de pollution en Océanographie appliquée. Indices de pollution générale. Indices de pollution par hydrocarbures. EPSHOM-E-OC., Fr.,124 : 32 p.

R.L. CHANDLER, J.C. O'SHAUGHNESSY, F.C. BLANC (1976). - Pollution monitoring with total organic carbon analysis. J. Wat. Pollut. Control Feder., USA, 42 (12) : 2771-2803.

T.W. CONSTABLE, E.R. Mc BEAN (1979). - BOD/TOC correlations and their application to water quality evaluation. Wat. Air Soil Polut. NLD. 11 (3) : 363-375.

M. ERHARDT (1969). - A new method for the automatic measurement of dissolved organic carbon in sea water. Deep-Sea Researd, GB, 16 : 393-397.

J. MARTY (1981). - Oxydation photochimique de matières organiques. Application à la mesure du COT - Ministère Environ. Edit. : 23 p.

R. OTSON, D.T. WILLIAMS, P.D. BOTHWELL, R.S. Mc CULLOUGH, R.A. TATE (1979). - Effects of sampling, shipping, and storage on Total Organic Carbon levels in water samples. Bull. Env. Contamin. Toxicol. USA, 23 (3) : 34-318.

J.W. SMALL (1980). - All about TOC analyzers. Pollut. Eng., USA, 9 : 63-65.

T.J. SMAYDA (1965). - A quantitative analysis of the phytoplankton of the Gulf of Panama. II. On the relationship of the between C14 assimilation and the diatom standing crop. Bull. Intern. Amer. Trop. Tunna Comm., 9 (7) : 467-531.

T.D. WAITE (1978). - An evaluation of non specific measurements on organic pollutants in waters and wastewaters. Research project., Aust., 74 (66) : 162 p.

BIOMETHYLATION OF TIN IN THE MARINE ENVIRONMENT

by

S YEMENICIOGLU, C. SAYDAM and I. SALIHOGLU
Middle East Technical University
Institute of Marine Sciences
P.K. 28 Erdemli-Içel (Turkey)

Abstract

The concentrations of tin and methyltin in sea-water, sediments and organisms from the north-eastern Mediterranean were determined throughout a year. For the analysis a hydride generation technique was employed. The hydrides produced were detected by atomic absorption spectrophotometer. The sea-water samples collected from Iskenderun Bay throughout a year were found to contain 9.2-236 ng/l of Sn (IV) 12-16 ng/l of CH3SN+3, 2.6-26 ng/l of (CH3)2Sn+2 and no trimethyl tin. On the other hand, tin components in sea-water samples taken from the Goksu River Delta and Tasucu Bay showed seasonal variations both in concentrations and chemical speciation, in other words besides mono and dimethyl tin, trimethyl tin was also observed. The highest total tin concentrations in sea-water were measured in samples collected close to an iron and steel complex. Acid extractable tin contents of sediments were also analysed and samples taken from Iskenderun Bay were found to contain between 0.19-2.3 ug/g (dry weight) acid extractable tin and 0.1-4.5 ng/g (dry weight) methyltin. Samples taken from the Goksu River Delta and Tasucu Bay showed acid extractable tin in the range 0.17-1.53 ug/g (dry weight) and methyltin in the range 0.1-99 ng/g (dry weight). Seasonal and local variations in methyl tin compounds are explained by biomethylation behaviour of inorganic tin.

*
* *

Introduction

Tin and its alloys have been economically important for men since the beginning of the bronze age. Today inorganic tin and organotin compounds have several industrial and agricultural uses, many of which are dispersive (ZUCKERMAN et al., 1978). Such uses imply an important anthropogenic input of tin into the environment. Inorganic tin compounds are relatively non-toxic, but organotin compounds used industrially are toxic to most aquatic and terrestrial organisms (HALLAS and COONEY, 1981 ; ZURCKERMAN et al., 1978). The degree of toxicity of organotin compounds depends on the nature of the organic group and degree of substitution (THAYER, 1974 ; PRICE, 1977). WOOD and GOLDBERG (1977) placed tin in the very toxic group due to the powerful toxic effect of organotin compounds relative to its inorganic form. Some laboratory studies demonstrated that Sn (IV) and trimethyltin can be biomethylated in the marine environment by micro-organisms (GUARD et al., 1981 ; HALLAS et al., 1982). RIDLEY et al., (1977) had proposed a vitamin B12 dependent mechanism for biomethylation of the toxic substances. The biomethylation of toxic substances in the environment is of vital importance

from the standpoint of public health (TOLBA, 1980). While the laboratory studies on biomethylation of tin are well documented, relatively little work has been done on their natural distribution and seasonal variations in the marine environment. Based upon the work done by HODGE et al., (1979) a hydride generation technique was employed for the speciation of tin compounds in the marine environment.

Experimental

Apparatus : A Varian-techtron Model AA-6 Atomic Absorption Spectrophotometer (AAS- equipped with a varian tin hollow cathode lamp was used for the analysis of the samples and output was routed to varian Model A-25 strip chart recorder set on 2 mV to read full scale for 0.2 absorbance unit. The water trap (12 x 1.5 Cm. i.d.), hydride trap (14 x 0.6 Cm. i.d.) packed with 3 % OV-1 on chromosorb W AW-DMCS 60/80 (mesh size), quartz-tube burner (8 x 0.8 Cm. i.d.) and the reaction vessel (250 ml capacity) are illustrated in figure I. The AAS parameters were adjusted as follows : wavelength 286.3 nm, slit width : 0.5 nm, lamp current : 3.0 mA and the gas flow rates were : air : 150 ml/min, hydrogen : 250 ml/min, and helium : 80 ml/min. Under these conditions the detection limits are 0.2 ng for inorganic tin and methyltins. Precisions is _ 6 per cent. Background correction was used throughout the analysis.

Table 1: Inorganic tin levels (ng/l) in discharged water samples of some of industrial activities established on the shore of Iskenderun Bay, (Nomethyltins were detected in these samples)

Source	Sampling date	sni
Isdemir Iron and stell complex		
Discharge: 1	September, 1982	15
Discharge: 2	September, 1982	16
Discharge: 3	September, 1982	1115
Toros Fertilizer plant	September, 1982	-
Sarıseki fertilizer and phosphoric acid complex	September, 1982	240

(-) : Not detected
Sni : Inorganic Sn (IV)

Reagents : All solutions were prepared with distilled deionized water (ddw). HCl (Analar), HNO3 (Merck, GR), HClO4 (Aristar), NaOH (Baker Analysed Reagent) were used as received. 4 % $NaBH_4$ (MCB Reagents) solution was prepared and purified as follows : 4 g of $NaBH_4$ was dissolved in 25 ml of sea-water, then 0.5 ml of 2 N NaOH was added and stirred for about 15 min. Finally the solution was gravity-filtered through Watman N° 2 filter paper and diluted to 100 ml. TRIS-HCL buffer was obtained from Ventron (Danvers,Mass.).

Standards : All methyltins and $SnCl_4$ standards were obtained from Ventron (Danvers, Mass.). and prepared in ddw as described by HODGE et al., (1979).

Sampling and sample preservation : Sea-water samples were collected from the surface, by means of a polyethylene bucket from a rubber boat upwind of the ship and transferred into 2 L capacity acid-cleaned bottles. Then the samples were brought to the laboratory within a few hours and analysed, or 2 ml of concentrated HCl was added and the samples were preserved in refrigerator until the time for analysis. To avoid hydrowire contamination a nylon hydrowire was used for sampling.

Sediment samples were collected with a Vaan-Vien type grab sampler which collects the top 10 cm and placed into acid-cleaned polyethylene bags and sealed. The samples were then kept in a deep freezer at-30 °C until analysis.

The organism samples (limpets) were collected by hand. After washing with ddw they were placed into acid-cleaned polyethylene bags and sealed. The samples were then stored in a deep-freezer until the time for analysis.

Sea-water analysis : For inorganic Sn (IV) analysis 100 ml of sea-water sample was put into the reaction vessel. If less then 100 ml of sample is used the final volume is brought to 100 ml with ddw. 1 ml of 20 % TRIS-HCl buffer was added and purged with helium carrier gas for 1 min during which time the hydride trap was of liquid nitrogen (when 1 ml of concentrated HCl had been added per litre of sea-water sample for preservation the samples were analysed without buffer addition). Then the procedure given by HODGE et al., (1979) was followed. For methyltin determination 250 ml of sea-water was used. The stripping time was extended to 7 min. 2ml of buffer and 2 ml of 4 % $NaBH_4$ were used and the procedure given above was followed. For qualitative and quantitative determination the standard addition method was employed.

Sediment analysis

Acid extractable tin : 1 g of dried and ground sediment sample was leached with 6 N cold HCl as described by SEIDEL et al., (1980) and TUGRUL (1982).

Methyltin : 1-5 g of the dried and ground sediment sample, depending on the concentration of methyltin content, was shaken ultrasonically in 40 ml of ddw for half an hour . The sample was then transferred into the reaction vessel and the volume was brought up to 100 ml with ddw 1 ml of buffer was added and reacted with 1 ml of 4 % $NaBH_4$ following the procedure given for water analysis. The standard addition method was used for the identification of peaks and quantitative analysis.

Organism analysis

Total tin : 4-5 g of wet tissue (soft parts) of limpet samples were digested in concentrated HNO_3 as deseribed by TUGRUL, (1982). 0.5-1.0 ml of the solution was transferred into reaction vessel, diluted to 100 ml with ddw. The same procedure given for the analysis of water samples was followed. The standard addition method was used for each sample.

Methyltins : 0.5-2 g dried soft parts of limpets were homogenized in a pyrex homogenizer cup by addition of 20 ml of 0.04 N HCl. The solution was then tranferred into the reaction vessel and diluted to 100 ml with ddw. The analysis was carried out by the same procedure used for water analysis.

In order to prevent foaming in the reaction vessel, the helium bubbler was shortened and the reaction vessel was degassed above the solution. The reaction vessel was then filled up to below the helium entrance, and stirred with a magnetic stirrer during the collection of the hydrides. Standards were run under the same conditions.

Table 2 Inorganic Sn(IV) and Methyltin levels in sea-water samples (ng/l)

Location and station (a)	S.Date	Sn_i	Me-Sn	Me_2-Sn	Me_3-Sn
Göksu River Estuary (2)	Nov.1982	35	ND	ND	ND
Taşucu Bay (3)	Nov.1982	28	ND	ND	ND
station 10 (1)	Nov.1982	10.4	ND	ND	ND
station 9-A (2)	Nov.1982	46	ND	ND	ND
station 7 (1)	Nov.1982	8	ND	ND	ND
Göksu River Estuary (2)	Janu.1983	63.5	ND	ND	ND
Taşucu Bay (3)	Janu.1983	178	ND	ND	ND
station 7 (1)	Janu.1983	178	ND	ND	ND
Göksu River Estuary (3)	March.1983	15	ND	2.2	ND
station 14 (2)	March.1983	34	ND	2.2	ND
station 7 (2)	March.1983	25	ND	2.2	ND
Taşucu Bay (6)	March.1983	36	ND	ND	ND
station 9-A (1)	March.1983	42	ND	ND	ND
Göksu River Estuary (2)	April.1983	24	ND	ND	ND
Taşucu Bay (2)	April.1983	4.5	ND	ND	ND
station 14 (1)	April.1983	5.5	ND	ND	ND
Göksu River Estuary (3)	July.1983	23	7.5	43	2.6
Taşucu Bay (2)	July.1983	24	5.2	27	2.6
station 7 (1)	July.1983	12	9.9	13	2.0
Lamasharbor inside (2)	Aug.1982	1.7	ND	ND	ND
" " (2)	Sep.1982	33	ND	ND	ND
" outside (1)	Dec.1982	11.7	ND	ND	ND
" inside (2)	Dec.1982	12	ND	ND	ND
" " (4)	Janu.1983	11.8	ND	ND	ND
" outside (4)	Janu.1983	16	ND	ND	ND
" River Estuary (5)	Janu.1983	11.7	3.4	8.7	3.4
" Harbor inside (2)	Feb.1983	12.6	ND	ND	ND
" " outside (1)	Feb.1983	12.5	ND	ND	ND
" " inside (2)	March.1983	10.3	ND	ND	ND
" " outside (2)	March.1983	10.0	ND	ND	ND
" " inside (2)	July.1983	129	9.9	1.3	2.0
Lamas Harbor outside (1)	July.1983	250	11	2.5	9.7
Lamas River (2)	Aug.1982	3.6	ND	ND	ND
" " (2)	Sep.1982	5.5	ND	ND	ND

Table 2 Inorganic Sn(IV) and Methyltin levels in sea-water samples (ng/l)

Location and station (a)		S.Date	Sn_i	Me-Sn	Me_2-Sn	Me_3-Sn
Lamas River	(1)	Nov.1982	6.5	ND	ND	ND
" "	(2)	Janu.1983	1.1	ND	ND	ND
" "	(1)	March.1983	7.8	ND	ND	ND
" "	(1)	May.1983	3.6	ND	ND	ND
" "	(2)	July.1983	2.5	ND	ND	ND
" "	(2)	Aug.1983	5.0	ND	ND	ND
İskenderun Bay						
Ceyhan river estuary		July.1982	23.9	15.8	12.8	-
Infront of ısdemir		July.1982	203.5	13.3	14.8	-
station S.5		July.1982	19	-	8.5	-
Iskenderun Bay						
Station 22	(1)	Aug.1982	722	15	17	ND
Infront of Isdemir	(3)	Aug.1982	4635	16	13	ND
Station S-6	(1)	Aug.1982	125	ND	ND	ND
Ceyhan River Estuary	(2)	Aug.1982	123	12	21	ND
Station 22	(1)	Sep.1982	219	ND	26	ND
Infront of Isdemir	(2)	Sep.1982	236	ND	20	ND
Station S-5	(1)	Sep.1982	98	ND	ND	ND
Ceyhan River Estuary	(2)	Sep.1982	52	ND	ND	ND
Station 5	(1)	Sep.1982	181	ND	ND	ND
Station (22)	(2)	Nov.1982	111	ND	5.2	ND
Infront of Isdemir	(4)	Nov.1982	27	ND	2.6	ND
Infront of Botaş	(3)	Nov.1982	53	ND	4.3	ND
Ceyhan River Estuary	(2)	Nov.1982	56	ND	ND	ND
Station 5	(1)	Nov.1982	11.3	ND	ND	ND
Station 11	(1)	Nov.1982	32	ND	5.3	ND
Station 22	(2)	Apr.1983	9.2	ND	ND	ND
Infront of Isdemir	(2)	Apr.1983	80	ND	ND	ND

ND: Not detected

(a); Number of samples analyzed from the same station area

Sni: Inorganic Sn (IV)

Me-Sn: Monomethyltin

Me_2-Sn: Dimethyl tin

Me_3-Sn: Trimethyl tin.

Table 3 Acid extractable tin (µg/g dry weight) and methyltin (ng/g) dry weight) in sediment samples.

Location and station (a)	S.Date	Depth(m)	A.E.Sn_i	Me-Sn	Me_2-Sn	Me_3-Sn
ISKENDERUN BAY						
Ceyhan River Estuary (3)	July.1982	2 0	0.78	0.5	2.8	0.6
station 3 (1)	July.1982	107	0.66	2.7	2.7	2.7
station 18 (1)	July.1982	50	1.80	0.8	1.4	0.3
Ceyhan River Estuary (2)	Aug.1982	20	0.62	0.1	0.3	0.1
station 8 (1)	Aug.1982	90	0.54	0.6	4.5	0.3
station 11 (1)	Aug.1982	50	2.30	0.3	1.5	0.2
station 22 (1)	Aug.1982	50	0.86	0.2	0.4	0.1
Ceyhan River Estuary (2)	Sep.1982	20	0.38	0.2	0.7	0.1
Infront of Isdemir (2)	Sep.1982	60	1.50	0.3	1.6	0.1
station S-6 (1)	Sep.1982	30	0.85	0.1	0.2	0.1
Ceyhan River Estuary (3)	Nov.1982	20	0.19	Trace	0.2	0.1
station 11 (1)	Nov.1982	50	0.36	Trace	0.2	0.1
station S-6 (1)	Nov.1982	30	0.48	Trace	0.1	0.1
Infront of Isdemir (2)	Nov.1982	60	0.56	Trace	0.1	0.1
TAŞUCU						
Taşucu Bay (2)	Apr. 1980*	35	0.94	0.2	1.0	4.3
Station 7 (1)	Apr. 1980*	50	0.71	0.1	1.4	9.9
Göksu River Estuary (6)	Apr. 1980*	40	0.90	0.3	0.8	2.9
Taşucu Bay (3)	Aug.1982	40	0.80	0.3	0.3	0.6
Taşucu Bay (3)	March.1983	25	1.53	Trace	0.3	ND
Göksu River Estuary (3)	March.1983	40	1.14	0.5	0.9	ND
station 12 (1)	March.1983	60	0.17	ND	1.6	ND
Lamas River Estuary (2)	Apr.1980	40	1.06	0.2	1.3	3.1
" " " (2)	Janu.1983	70	1.27	ND	0.9	ND
" Harbor outside (3)	Janu.1983	50	1.51	ND	0.4	0.2

* Samples are collected in April, 1980 dried and kept until analysistime.

ND : Not detected

(a) : Number of samples analyzed from the station area.

A.E.Sn_i : 6 N HCl extractable tin.

Table 4 Avarage total tin and methyltin concentrations in limpet (ng/g dry weight).

Location and station (a)	S.Date	Length(cm)	ΣSn_i	Me-Sn	Me_2-Sn	Me_3-Sn
LAMAS HARBOR						
Station 3 (8)	June.1982	3.5	210	4.2	4.6	9.5
Station 1 (4)	June.1982	3.5	160	1.7	6.4	0.9
Station 1 (10)	July.1982	3.5	59	2.3	5.4	0.6
Station 3 (16)	July.1982	2.9	121	3.1	3.2	8.6
Station 3 (7)	Aug.1982	3.9	41	1.1	0.4	2.6
Station 1 (5)	Aug.1982	4.4	19	-	1.0	-
Station 1 (7)	Sep.1982	3.8	6.5	0.7	1.9	-
Station 3 (15)	Sep.1982	3.6	20	1.3	2.1	0.7
Station 3 (6)	Oct.1982	3.5	50	1.1	2.2	0.5
Station 1 (14)	Oct.1982	4.0	59	0.7	1.8	0.5
Station 1 (10)	Janu.1983	3.0	24	-	0.8	-
Station 3 (10)	Janu.1983	3.4	43	0.9	0.8	0.6
Station 3 (4)	March.1983	3.5	71	2.8	3,1	7.6
Station 1 (7)	March.1983	3.4	37	1.4	5.5	1.1
Taşucu (13)	Janu.1983	2.9	4.5	13.9	3.5	-
Yumurtalık(9)	Nov.1983	2.8	48.9	0.3	-	-

(a): Numbers in paranthesis indicate the number of individual samples in composite samples.

-: Not detected.

Results and discussions

Water : The results obtained from the water analysis are given in table 2. The inorganic tin concentration in Lamas River waters ranges between 1.1-7.8 ng/l with an average of 4.5 ngl. No methyltins were detected in the Lamas River waters. The behaviour of tin in the Lamas River estuary was studied. The inorganic tin content of this estuary ranges between 1.7-33 ng/l with an average of 13 ng/l (excluding the high values obtained in July, 1983). Methyltins were detected in the samples obtained in January and July, 1983. in the samples obtained in January, 1983 the dominant methylated tin species was dimethyltin, while in July, 1983 monomethyl and trimethyltin concentrations exceeded the dimethyltin concentrations. The inorganic tin concentration of this estuary was also high in July, 1983.

The average inorganic tin concentration in Tasucu bay and the Goksu River estuary was 39.9 ng/l, ranging between 4.5 and 178 ng/l. In March, 1983 only dimethyltin was detected while, in July 1983, besides dimethyltin mono-and trimethyltin were also detected in comparable amounts.

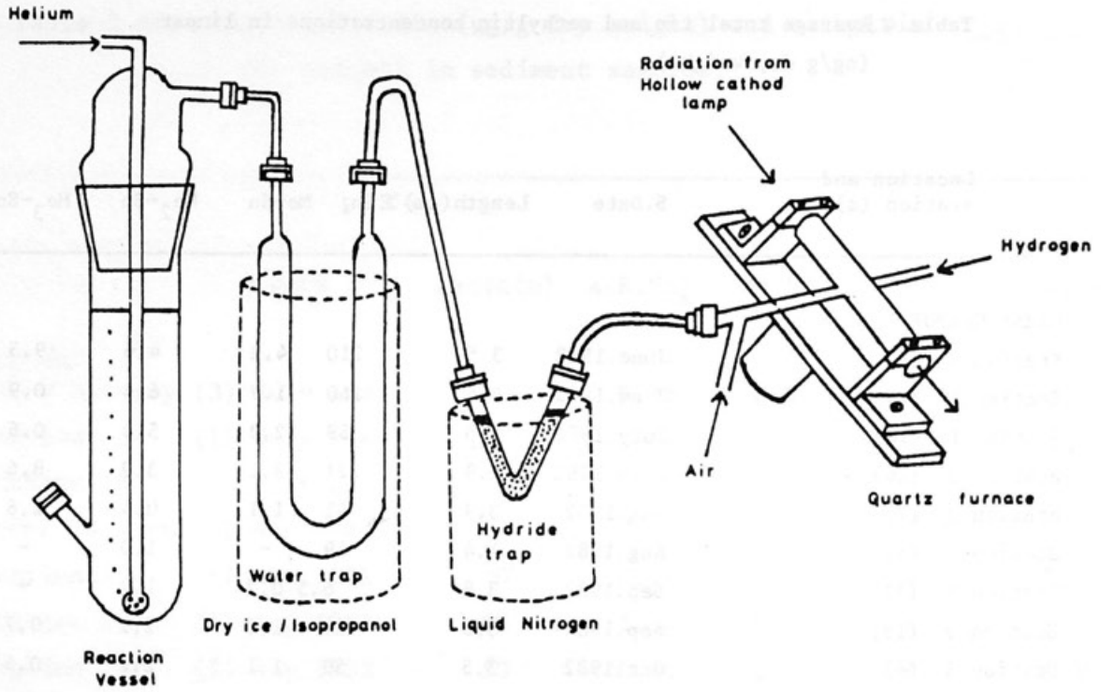


Figure 1. - Apparatus Setup.

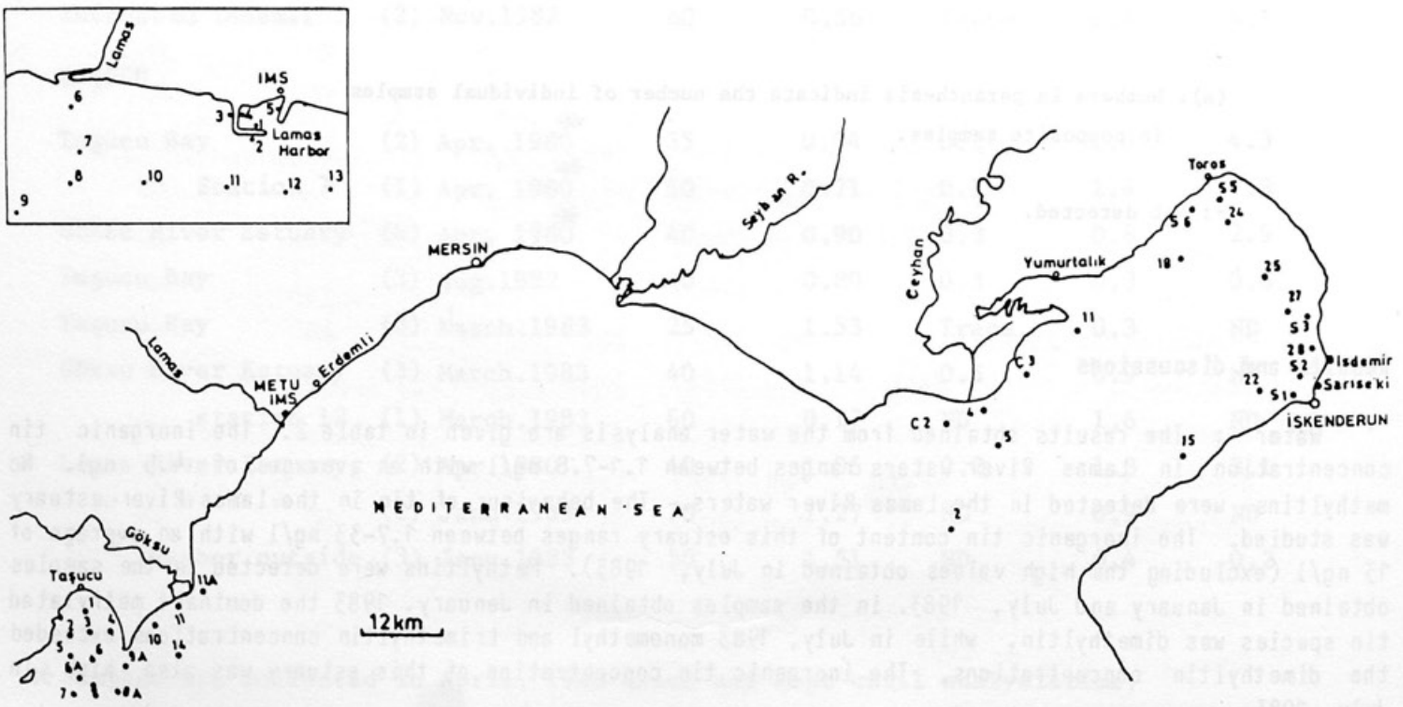


Figure 2.-Sampling Stations.

Inorganic tin concentration in Iskenderun Bay waters was very high with an average of 398 ng/l. The methyltins were detected in all sampling periods with the exception of April, 1983, when methylated tin species fell below the detection limit of the method.

The occasional appearance of methyltins above the detection limit of the method employed i.e. at ng levels, most probably originates from the seasonal changes in populations of micro-organisms able to methylate inorganic tin and to degrade methyltins. In addition. changes in physical parameters such as salinity, temperature and light intensity could be important. On the other hand, the results obtained from the analysis of Iskenderun Bay waters indicate that, if there is a continuous anthropogenic tin flux into a productive coastal water, so that the inorganic tin concentration exceeds its natural level, tin is steadily biomethylated. The industrial activities established on the shores of Iskenderun Bay discharge their waste into the sea without any pretreatment. The tin levels in discharged waters from some of these industrial activities are given in table 1. Extensive boating and shipping activities also exist in the waters of Iskenderun Bay. The high inorganic tin concentration in Iskenderun bay water may arise from these industrial and shipping activities.

Sediment : Results obtained from the sediment analysis for water extractable methyltins and cold 6 N HCl extractable inorganic Sn (IV) are shown in table 3. In order to correlate the inorganic Sn (IV) that is susceptible to biomethylation and the methyltins, the sediments were digested by cold 6 N HCl as described by SEIDEL et al., (1980), and TUGRUL (1982). As can be seen from table 3; there are no significant changes in the acid extractable tin concentrations of the sediments. No direct relationships were found between inorganic Sn(IV) and methyltin content. The methyltin concentrations in sediment samples were found to show regional and seasonal differences. The Iskenderun sediments were found to contain mono-di-and tri-methyltin, but the predominant biomethylation product in these sediments is dimethyltin. In sediment samples obtained from Tasucu Bay, Goksu River Estuary and Lamas River Estuary the major biomethylation product was also found to be dimethyltin, except those samples obtained in April, 1980. The predominant methylation product in April 1980 samples were found to be trimethyltin. These samples were collected in April, 1980. dried and kept until the time for analysis. This difference may most probably be due to the two years' storage ; nevertheless, the distribution pattern of methyltins in sediments was found to be more or less the same as the distribution pattern of methyltins in overlying water.

Organisms : The analytical results obtained from the analysis of limpets are summarized in table 4, and the sampling points are shown in figure II. Limpet soft parts, collected from the outside area (Station 3) of Lamas harbor contain relatively high amounts of methyltins in spring and summer. As can be seen from table 4 the trimethyltin is higher than the mono-and dimethyltin. In winter and autumn a considerable decrease in trimethyltin content was observed in the samples of this station. This pattern suggests that Me_3Sn is produced by biomethylation reactions. Then it is converted into inorganic forms depending on the changes in some physical parameter, such as salinity. temperature, light intensity, or type and amount of nutrient uptake. This station is affected by the cold Lamas River waters. The seasonal changes of total methyltin and inorganic tin in the samples collected from station 1 (inside Lamas harbour) are found to be same, dimethyltin being the main methylated form of tin. Samples obtained from Tasucu and Yumurtalik were found to contain monomethyltin as the main methylated form of tin and no trimethyltin was detected in these samples.

Conclusion

The present study provides evidence that inorganic tin can be biomethylated by the microorganisms in the marine environment, and can be mobilized in the ecosystem. Because of the increased

toxicities of methylated tin compounds relative to inorganic tin. the studies on environmental samples must be extended to longer periods, and besides the marine samples, atmospheric and more biological samples must be included in order to learn more about their biogeochemical cycle.

Acknowledgment

This work was partially supported by TUBITAK (Turkish Scientific and Technical Research Council).

References

GUARD H.E., COBET A.B., COLEMAN W.M., (1981), "Methylation of Trimethyltin Compounds by Estuarine Sediments", Science, 213. p. 770.

HALLAS L.E., COONEY J.J., (1981), "Effects of Stannic Chloride and Organotin Compounds on Estuarine Microorganisms", Developments in Industrial Microbiology, 22, p. 529.

HALLAS L.E., MEANS J.C., COONEY J.J., (1982), "Methylation of Tin by Estuarine Microorganisms", Science, 215, p. 1505.

HODGE V.F., SEIDEL S.L. and GOLDBERG E.D., (1979), "Determination of Tin (IV) and Organotin Compounds in Natural Waters,Coastal Sediments and Macroalgae by AAS", Anal. Chem., 51, p. 1256.

PRICE J.W., (1977), "Tin in the World of Today", Anal. Chem., 288, p. 257.

RIDLEY W.P., DIZIKES L.J., WOOD J.M., (1977), "Biomethylation of Toxic Elements in The Environment", Science, 197, p. 329.

SEIDEL S.L., HODGE V.F., GOLDBERG E.D., (1980), "Tin as an Environmental Pollutant", Thalassia Jugoslavica, 16, p. 209.

THAYER J.S., (1974), "Organometallic Compounds and Living Organisms", J. Organomet. Chem, 76, p. 265.

TOLBA M.K., (1980), "The state of the Environment : Selected Topic", Environ. Intern: 4, p. 129.

TUGRUL S., (1982), "Natural Distribution of Alkyltin Compounds in the Marine Environment", A Ph.D. thesis in Marine Sciences, METU.

WOOD J.M. and GOLDBERG E.D., (1977), "Impact of Metals in the Biosphere, in Global Chemical Cycles and Their Altrations by Man", W.Stumm, Ed., p. 137, Berlin: Dahlem Konferenzen.

ZUCKERMAN J.J., REISDORF R.R., ELLIS, III, H.V., and WILKERSON R.R., (1978), "Organotins in Biology and the Environment", Am. Chem. Soc. Symp., 82 p. 388.

RESULTATS PRELIMINAIRES D'UNE RECHERCHE SUR LA PRESENCE DE MERCURE ET DE SELENIUM DANS LES MUSCLES BLANCS ET ROUGES DES PELAMIDES (Sarda sarda) DE LA MER LIGURIENNE

par

R. CAPELLI, V. CONTARDI, B. COSMA, V. MINGANTI et G. ZANICCHI
Institut de Chimie Générale, Université de Gênes (Italie)

Résumé

Cette communication fournit les résultats préliminaires d'une étude sur la présence de Mercure (total et organique) et de Sélénium dans les muscles blancs et rouges des Pélamides (Sarda sarda) pêchés dans la mer Ligure.

Les premières données obtenues montrent l'absence de rapports entre la teneur en mercure et la teneur en Sélénium relevées dans ces tissus, alors qu'elles mettent en évidence des différences significatives entre la concentration du Sélénium présent dans les muscles rouges et celle des muscles blancs.

Un comportement analogue avait déjà été constaté avec le Cuivre et en plus faible proportion avec le Zinc et le Manganèse.

L'importance que le mode de prélèvement et d'homogénéisation de l'échantillon présente pour la représentativité de celle-ci est donc mise en évidence.

Abstract

This paper presents the preliminary results obtained during a study of mercury (total and organic) and selenium distribution in red and white muscle of Atlantic Bonitos (Sarda sarda) from the Ligurian sea.

Data reported show the lack of correlation between mercury and selenium concentrations, whereas they show a meaningful difference between red and white muscle tissue content.

Similar behaviour has been observed for copper, and in a lesser way, for Zinc and manganese.

The sample preparation methodology and its homogenization are stressed.

*
* *

Introduction

A l'occasion des VIes Journées d'Etudes sur les Pollutions Marines qui se sont déroulées à Cannes en 1982 nous avons présenté une communication concernant les résultats préliminaires d'une étude sur la teneur en métaux dans les tissus et les organes des Pélamides (Sarda sarda) pêchées dans le golfe de Gênes (CAPELLI et al., 1982).

Les données préliminaires obtenues laissaient apparaître des différences de concentration en fonction du métal et de l'organe considéré. En outre, il était évident, en ce qui concerne le mercure, qu'il existait une corrélation entre sa concentration dans les différents organes (branchies, foie, tissu musculaire, etc.) et la dimension du poisson.

Dans ce même travail nous avons mis en relief une différence de concentration des éléments tels que Cu, Mn, et Zn dans les fibres musculaires rouges et blanches présentes dans la Pélamide.

Durant ces deux dernières années nous avons poursuivi la recherche d'éléments en trace dans les différents organes et dans les tissus des Pélamides de la mer Ligurienne. Nous avons également étendu cette étude au Sélénium. Nous avons considéré en particulier les tissus musculaires rouges et blancs afin d'obtenir des renseignements sur la présence d'éléments en trace dans ces mêmes tissus (assez rares dans la littérature) et de contrôler, du point de vue analytique, l'influence que peuvent avoir les méthodologies d'échantillonnage, de préparation et d'homogénéisation de l'échantillon sur les données de l'analyse.

Dans cette étude figurent les résultats obtenus jusqu'à présent concernant la teneur en Hg (total et organique), Cu, Mn, Se et Zn des tissus musculaires blancs, mixtes et rouges. Ils sont mis en relation avec la représentativité de l'échantillon.

Echantillonnage

Les Pélamides ont été pêchées à Camogli (20 km à l'est de Gênes) où il est possible de pêcher ce poisson presque toute l'année.

Immédiatement après leur capture, les Pélamides étaient lavées avec de l'eau de mer propre et placées en chambre frigorifique à 0° C.

Au laboratoire, les poissons étaient mesurés (longueur standard), pesés et surgelés à - 25° C jusqu'au moment de l'analyse.

Avant qu'elles ne soient complètement dégelées les Pélamides étaient sélectionnées selon les règles du manuel de la F.A.O. (BERNHARD, 1976), en cherchant surtout à réduire autant que possible les risques de contamination.

De plus, différents organes (branchies, rate, foie, reins, coeur) étaient échantillonnés, deux tranches d'1 cm d'épaisseur prélevées dans la région post-cranienne et, en même temps, deux échantillons de tissu musculaire rouge et blanc. De cette façon, nous disposions d'un échantillon conforme aux normes suggérées par la F.A.O.-U.N.E.P. et de deux autres échantillons pouvant fournir des indications sur la teneur en éléments en trace dans ces tissus.

Méthodologie analytique

La détermination du Cu, du Mn et du Zn était exécutée par spectrophotométrie d'absorption atomique avec flamme air-acétylène en utilisant les solutions obtenues en minéralisant, sous reflux, 4-5 g de chair par l'acide nitrique à 90 % dans des ballons de pyrex (CAPELLI et al., 1976).

La même solution a été utilisée pour la détermination du Hg (total) par spectrophotométrie d'absorption atomique selon la méthodologie des vapeurs froides (CAPELLI et al., 1976), et celle du Se suivant la méthodologie analytique que nous avons mise au point (CAPELLI et al., 1983) et qui sera brièvement résumée ci-dessous.

Les solutions qui provenaient de la minéralisation effectuée par l'acide nitrique ont été portées à un volume de 50 ml. Sont alors prélevés 10 ml de cette dernière solution, portés à un volume de 50 ou 100 ml (ce volume dépend de la quantité de Se présent dans l'échantillon), ceci après avoir ajouté de l'acide chlorhydrique à 37 % en quantité suffisante pour obtenir une solution finale de 5 N.

5 ml de cette dernière solution sont placés dans l'appareil de réduction avec 5 ml d'acide chlorhydrique 5 N ou avec 5 ml de solution standard de Se dans l'acide chlorhydrique 5 N car la détermination a été faite en employant la méthode des additions tarées.

Après avoir enlevé entièrement l'air au moyen d'un courant d'azote, le Se est réduit en hydrure en utilisant 2 ml d'une solution à 2,5 % (p/v) de bore hydrure de sodium en 2 % (p/v) d'hydroxyde de sodium.

L'hydrure de Se est transporté par le gaz (3 l/mn) dans une cellule en quartz rechauffée (800-1.000° C) qui se trouve sur le rayon optique d'un spectrophotomètre d'absorption atomique.

La source est constituée par une lampe EDL (i = 196.0nm; slit 2.0 nm) et il a été fait usage du correcteur de fond.

Au même moment et avec les mêmes modalités sont effectuées les analyses de deux ou trois "blancs" pour contrôler la pureté des réactifs et la présence d'éventuelles contaminations: Le "blanc". dans le cas de Se. n'a jamais constitué une difficulté.

Le mercure organique (méthylmercure) a été déterminé par spectrophotométrie d'absorption atomique, suivant la méthodologie que nous avons mise au point (CAPELLI et al., 1979). Celle-ci consiste en l'extraction par le toluène du Hg organique (méthylmercure) d'un échantillon préalablement homogénéisé et acidifié avec de l'acide bromhydrique à 47 %. Le Hg organique est ensuite extrait du toluène au moyen d'une solution de L-cystéine (WESTOO, 1974) et réduit par une solution de chlorure d'étain (II) + chlorure de cadmium en milieu basique (MAGOS, 1971). Le Hg est enfin déterminé en employant la spectrophotométrie d'absorption atomique à vapeurs froides.

Dans le même temps ont été effectuées des déterminations sur deux "blancs" dans le but de contrôler la pureté des réactifs et la présence d'éventuelles contaminations. Dans chacun des cas, il a été fait usage d'un correcteur de fond (lampe au deutérium) et l'on a employé la méthode des additions tarées.

Toutes ces méthodologies, excepté celle du méthylmercure, ont été mises au point en employant des échantillons de l'U.S. National Bureau of Standards et contrôlées à l'occasion de différents essais d'intercalibrage effectués par la F.A.O.-U.N.E.P.-I.A.E.A. (Tableau 1).

Tableau 1

Résultats du contrôle analytique. Les concentrations sont exprimées en ug/g poids sec.
A) nos résultats. Le nombre de nos déterminations est mentionné entre parenthèses
B) valeurs certifiées résultats d'un essai d'intercalibrage.

Echantillon		Hg	Zn	Cu	Mn	Se
NBS SRM 1577a	A)		126±5(3)	162±7(3)	10.2±0.8(3)	
bovine liver	B)		123±8	158±7	9.9±0.8	
NBS SRM 1566	A)	0.054±0.014(6)				2.2±0.3(6)
oyster tissue	B)	0.057±0.015				2.1±0.5
IAEA MA-A-2 fish	A)	0.51±0.05(5)	33±1(5)	4.2±0.5(3)		
flesh homogenate	B)	0.49±0.02	33±1	4.5±0.3		

Résultats et discussion

Les résultats obtenus jusqu'à présent figurent dans le tableau 2. Sont également indiquées les concentrations (en µg/g poids frais) relatives au Hg (total et organique), au Cu, au Mn, au Se, et au Zn que nous avons trouvées dans le tissu musculaire rouge, blanc et mixte de 10 Pélamides pêchées en mer Ligurienne.

En outre, nous avons reporté le poids en grammes et la longueur standard de chaque poisson, de façon à fournir des renseignements sur leur taille: aucune distinction n'a été faite en ce qui concerne leur sexe.

Tableau 2

Concentrations des éléments en traces (µg/g poids frais) dans le tissu musculaire blanc (B), mixte (M) et rouge (R) des Pélamides (Sarda sarda) échantillonnées à Camogli (golfe de Gênes).

Poids g	Long. Stand. cm	Tissu musc.	Hg-t	Hg-o	Hg-o%	Cu	Mn	Se	Zn
3400	68	B	1.75	1.68	96	0.5	0.2	0.28	3.2
		M	1.70	1.52	89	1.7	0.3	0.43	5.
		R	1.55	1.35	87	4.4	0.5	1.2	7.8
3300	60	B	2.50	2.25	90	0.5	*	0.41	4.3
		M	2.80	2.01	72	0.6	*	0.83	5.0
		R	2.52	1.55	62	4.3	0.4	1.7	7.0
3280	56	B	1.21	1.15	95	0.5	0.2	0.45	4.7
		M	1.19	1.10	92	1.1	0.1	1.0	5.4
		R	1.00	0.82	82	4.1	0.4	2.4	7.7
3190	57	B	1.65	1.57	95	0.6	0.3	0.37	3.4
		M	1.60	1.55	97	0.6	*	0.38	6.1
		R	1.48	1.40	95	4.5	0.3	1.7	7.9
2600	50	B	0.56	0.56	100	0.6	*	0.28	4.3
		M	0.70	0.56	80	0.4	*	0.51	4.1
		R	0.64	0.61	95	5.3	0.6	1.6	7.0
2530	50	B	1.25	1.20	96	0.7	0.2	0.23	7.2
		M	1.30	1.10	85	0.6	0.2	0.53	3.4
		R	1.48	1.25	84	4.9	0.5	1.2	8.9
2450	55	B	1.42	1.18	83	0.6	0.2	0.39	5.4
		M	1.38	1.05	76	1.7	0.4	0.68	6.1
		R	1.15	0.76	66	4.4	0.6	2.4	8.3
2080	53	B	0.61	0.47	77	0.8	0.3	0.24	5.9
		M	0.61	0.44	72	1.1	0.5	0.34	7.9
		R	0.61	0.46	75	4.6	0.6	1.6	11.0
1653	48	B	0.48	0.25	52	0.4	0.1	0.19	3.7
		M	0.48	0.28	58	1.0	0.3	0.41	5.3
		R	0.46	0.13	28	4.1	0.5	1.9	7.6
794	37	B	0.32	0.18	56	0.6	0.2	0.23	3.9
		M	0.35	0.12	34	0.8	0.2	0.31	4.8
		R	0.43	0.17	40	3.1	0.4	1.0	6.2

* valeur inférieure à 0.1 ug/g poids frais.

Les données confirment, ce qui a déjà fait l'objet d'une communication précédente (CAPELLI et al., 1982), la corrélation entre le Hg (total) et la taille du poisson. En outre, elles sont suffisantes pour dire qu'il y a également une relation entre le Mercure organique et la taille. De même, aucune corrélation n'a été observée pour les autres éléments en trace examinés.

Les données obtenues montrent l'absence de rapports entre la teneur en Hg et la teneur en Se relevées dans ce tissu.

Pour le Hg on peut constater qu'il n'y a pas de différences significatives entre le tissu musculaire rouge, blanc et mixte.

En ce qui concerne le Cu, Mn, Se et Zn on peut observer au contraire une différence de distribution avec des teneurs plus élevées dans le tissu rouge. Les différences sont plus particulièrement élevées pour le Cu et pour le Se.

On doit tenir compte de cette situation que d'autres auteurs ont déjà rencontrée pour d'autres organismes marins (VIVIANI et al., 1979 ; MALCOM LOVE, 1980). chaque fois qu'il est nécessaire d'obtenir une donnée moyenne relative à un poisson dans lequel existent des tissus musculaires rouges et blancs.

Considérons que ces tissus sont distribués d'une façon non homogène dans tout le corps du poisson : on peut facilement comprendre que l'analyse effectuée sur une tranche de poisson dans le cas du Hg peut fournir des données suffisamment correctes, alors que dans le cas du Cu, du Zn et du Se, elle peut fournir de fausses valeurs ou, tout au moins, des valeurs ne répondant pas à la concentration moyenne de l'organisme tout entier.

Les différences de concentration, rencontrées pour le Cu, Zn et Se mettent aussi en évidence la nécessité d'une homogénéisation de l'échantillon qui doit être d'autant plus poussée que la quantité de chair utilisée pour l'analyse est réduite.

Le problème de la représentativité de l'échantillon est un des premiers problèmes qui doit être affronté et résolu. En effet, toute analyse, exécutée d'une façon correcte sera inutile si elle est effectuée sur un échantillon qui n'est pas représentatif ou conforme au but de la recherche.

Références

M. BERNHARD (1976). - F.A.O. Fish. Tech. Pap. (158) : 132 p. Manuel de la recherche sur l'environnement aquatique. Troisième partie. Echantillonnage et analyse du matériel biologique (Directives destinées au projet commun coordonné F.A.O.(C.G.P.M.)/P.N.U.E. sur la pollution en Méditerranée). F.A.O. manuals.

R. CAPELLI, V. CONTARDI et G. ZANICCHI (1976). - Enquête explorative sur la teneur en métaux lourds (Cd, Co, Cu, Cr, Mn, Ni, Pb) dans des échantillons des moules (Mytilus galloprovincialis). IIIes Journées Etud. Pollutions, Split, C.I.E.S.M. : 83-88.

R. CAPELLI, C. FEZIA, A. FRANCHI et G. ZANICCHI (1979). - Extraction of methylmercury from fish and its determination by atomic-absorption spectrometry. Analyst 104 : 1197-1200.

R. CAPELLI, V. CONTARDI, B.COSMA, V. MINGANTI et G. ZANICCHI (1982). - Résultats préliminaires d'une recherche sur la teneur en métaux dans les tissus et les organes de Pélamides (Sarda sarda échantillonnées dans le golfe de Gênes. VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M. : 315-318.

R. CAPELLI, B. COSMA, V. MINGANTI et G. ZANICCHI (1983). - Messa a punto di due apparecchiature per la determinazione del selenio e del mercurio in matrici complesse mediante spettrofotometria di assorbimento atomico. 5° Comgresso della Associazione Italiana di Oceanologia e Limnologia;, Stresa 19-22 Maggio 1982 : 229-238.

R. MALCOM LOVE (1980). - The chemical biology of fishes. Volume 2 : advances 1968-1977. Ed. : Academic Press 1980.

L. MAGOS (1971). - Selective atomic absobption determination of inorganic mercury and methyl-mercury in undigested biological material. Analyst 96 : 847-912.

R. VIVIANI, G. CRISETIA, E. CARPENE, P. CORTESI, G. SERRANZANETTI, O. CATTANI, L. MANCINI et R. POLETTI (1979). - Residui di inquinanti chimici in organismi dell'alto e medio Adriatico Atti Conv. Scientifico Naz. C.N.R., 2 : 845-854.

G. WESTOO (1974). - Methodology for mercury and methyl-mercury for food contaminant monitoring. Rep WHO-FAO/FCM/ 74-17.

SEEKING BIOLOGICAL INDICATORS FOR HEAVY METAL (Pb,Cd,Cu AND Zn) POLLUTION IN WARM MEDITERRANEAN WATERS

by

Boris S. KRUMGALZ and Zhenia FLEISHER
Chemical Oceanography and Marine Pollution Dept.
Israel National Oceanographic Institute
Tel-Shikmona, Haifa 31080 (Israel)

Abstract

It is well known that heavy metals may be accumulated by aquatic biota to levels far above those found in the surrounding environment. Therefore, some benthic organisms can be used as biological indicators of the degree of local heavy metal pollution . The greatest advantage in the use of biological indicators is that the biological availability of the pollutants is measured directly. The most frequently used species is Mytilus edulis. However, these mussels can exist only in waters of salinities from 5 to 35 ‰ and up to 26 °C. This means that in the warm and saline waters of the southern Mediterranean Sea, high temperatures (during summer months) up to 30 °C and high salinities up to 39 ‰ are lethal for this mussel. In earlier studies of our laboratory,two molluscs, Arcularia gibbosula and Arcularia circumcincta, were discovered to be suitable as indicators of mercury pollution, since they accumulate mercury to a great extent in their tissues when residing in the polluted area. In the present study, the levels of Pb, Cd, Zn and Cu in surficial sediments and in various specimens of benthic fauna collected in Haifa Bay (Israel) have been studied. Among the benthic fauna the following specimens have been examined: Crustacea: Diogenes pugilator: Mollusca Bivalvia: Rudicordium tuberculatum, Dosinia lupinus, Chamelea gallina, Mactra corallina corallina, Mactra corallina stultorum, Donax venustus: Mollusca Gastropoda: Rhinoclavis kochi, Neverita josephinia, Murex trunculus, Murex brandaris, Sphaeronassa mutabilis, Arcularia gibbosula, Arcularia circumcincta, Hinia reticulata. The concentrations of cadmium,lead, zinc and copper in the sediments of Haifa Bay are not high compared to other industrial areas of the Mediterranean. The concentrations of the heavy metals under consideration were generally higher in gastropod molluscs than in bivalves. This difference can probably be explained by the different feeding habits of these molluscs.

*
* *

Introduction

The use of benthic organisms as biological indicators for heavy metal pollution permits us to measure directly the biological availability of the pollutants and provides results of heavy metal accumulation integrated over a certain period of time. However, there are some disadvantages in the use of indicator organisms. The biggest of the disadvantages is the possible interference of extraneous parameters with the uptake of pollutants by biota. The parameters that may interfere include

differences in species, age, weight, sex, sexual cycle, diet, water temperature, etc. It should also be taken into account that different species respond to different portions of the total pollution load in the ecosystem.

The most frequently used mussel, Mytilus edulis, as an indicator organism for heavy metal pollution studies cannot survive in the warm and saline waters of the Mediterranean Sea. KRUMGALZ and HORNUNG (1982) found that the molluscs Arcularia gibbosula and Arcularia circumcincta were suitable as indicator organisms for mercury pollution. In order to find benthic organisms which can serve as biological indicators for Pb, Cd, Cu and Zn pollution of the marine environment, the levels of these elements in surficial sediments and in various specimens of benthic fauna collected in Haifa Bay (Israel) have been studied. Haifa Bay is known to be relatively polluted by some heavy metals owing to industrial discharge from a considerable number of chemical plants and other industries into the Bay. Another source of heavy metal pollution is waste waters from agricultural run-off and domestic sewage.

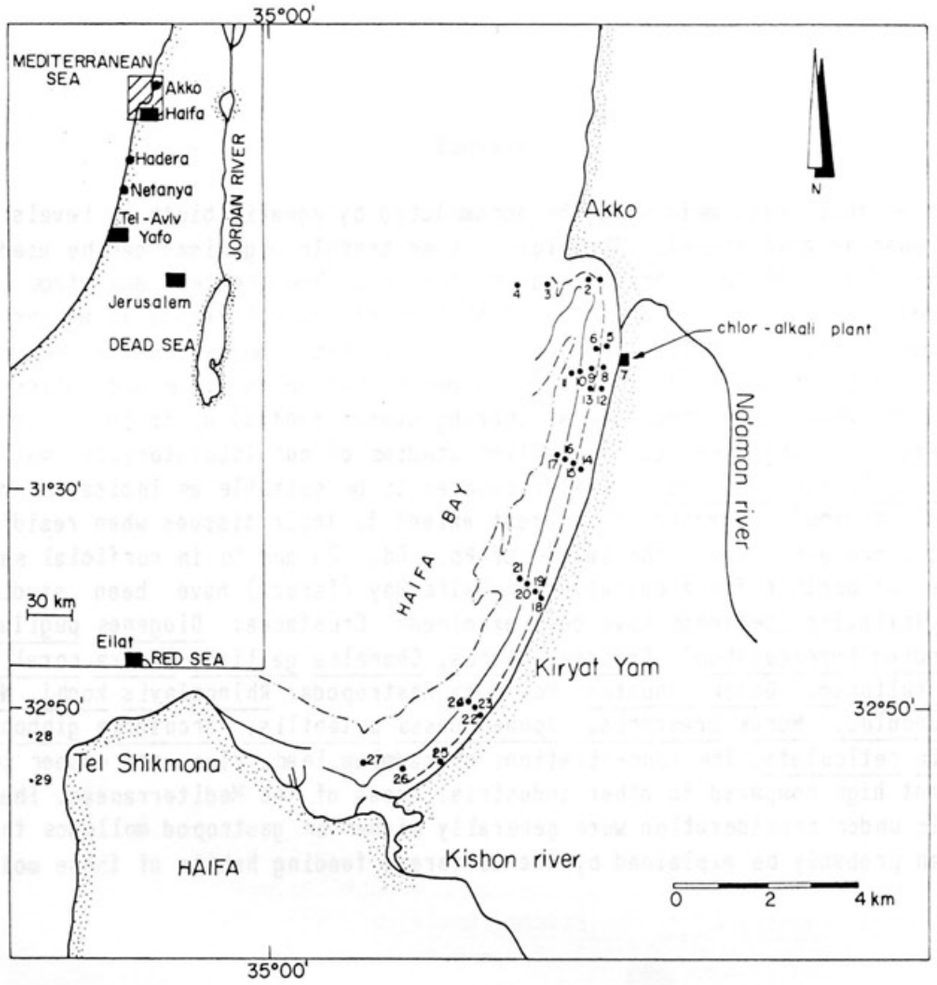


Figure I. - Map of the study area showing locations of the sampling stations.
Depth contours : ---------- 5 m: _____ 10 m: _._._._._. 15 m.

Experimental part

Sampling station locations are shown in figure I. Surficial sediment (up to 10 cm) samples along with benthic fauna were collected by diving. Sediment samples were freeze-dried on a Leybold-Heraeus

GT2 lyophilizer at 0.1 torr pressure for 48 hours, sieved, and only the fractions smaller than 250 µm were taken for analysis for heavy metal content. This fraction was found to be predominant in all samples. The benthic organisms were lyophilized in the same manner then homogenized to a fine powder with an agate mortar. The digestion of the dry samples of sediments and benthic organisms was conducted in teflon-lined, high-pressure decomposition vessels, with concentrated nitric acid (65 %) for 3 hours at 140 °C. The Pb, Cd, Cu and Zn concentrations in the samples examined were determined using an atomic absorption spectrophotometer (IL 951). The accuracy of all analytical procedures was checked by analysing samples of reference standardized research materials. These samples were analysed for heavy metal concentrations in a similar manner to those of sediments and benthic fauna. The comparison between recommended values and those obtained by us is presented in table 1.

Table 1. Mean and standard deviation of replicate analyses of trace metals in reference standards (values expressed as µg/g d. weight)

Reference material	Cd	Pb	Cu	Zn
MESS-1, reported value	0.59±0.10	34.0±6.1	25.1±3.8	191±17
present study	0.4±0.1	34.4±1.6	25.1±1.3	178±1.8
BCSS-1, reported value	0.25±0.04	22.7±3.4	18.5±2.7	119±12
present study	B.D.L.*	23.5±1.9	15.9±0.3	117.4±5.4
SRM 1571 Orchard leaves (NBS)				
reported value	0.11±0.02	45±3	12±1	25±3
present study	B.D.L.*	46.1±2.0	11.0±0.4	25.7±1.0

* B.D.L. - below detection limit of an atomic absorption spectrophotometer used (the detection limit for Cd equal to 0.4 µg/g).

Results and discussion

The concentrations of Pb, Cd, Cu and Zn in the surficial layer of marine sediments sampled in May 1982 at Haifa Bay are summarized in table 2. the data obtained indicate that the average pollution level of the Haifa Bay sediments by the heavy metals studied is relatively low compared to other industrial areas in the Mediterranean (table 3). However the area along the Kishon estuary is considerably polluted by Pb, Cu and Zn. This is direct evidence that the main influx of these metals into Haifa Bay is the Kishon River, into which most of the industries situated in the area discharge their waste waters. As a result of Kishon Port building construction (figure I), The Kishon River stream does not enter the sea at the shoreline but deeper in the sea. For this reason, no clear radial distribution of the metals copper, zinc and lead from the source has developed in Haifa Bay. The picture is different from that observed for mercury distribution in Haifa Bay (KRUMGALZ and HORNUNG, 1982). In the latter case, mercury distribution is of a pronounced radial character, since

Table 2. Concentration of trace metals (μg/g d.weight) in surface sediments of Haifa Bay collected in May 1982

Stn	Depth, m	Pb	Cu	Zn	Cd
1	3	18.1 ± 0.9	2.6 ± 0.5	14.6 ± 0.9	2.5 ± 0.1
2	6	12.1 ± 1.2	3.6 ± 0.5	36.8 ± 2.9	1.4 ± 0.6
3	9	11.9 ± 1.0	5.9 ± 0.9	39.3 ± 3.1	1.4 ± 0.3
4	12	14.4 ± 1.3	1.5 ± 0.9	40.1 ± 3.4	1.9 ± 0.7
5	3	16.6 ± 1.4	4.0 ± 1.1	40.1 ± 2.6	0.7 ± 0.2
6	6	19.9 ± 1.7	1.9 ± 0.7	16.5 ± 0.3	1.9 ± 0.1
8	3	24.1 ± 2.3	4.0 ± 0.7	18.2 ± 2.1	1.9 ± 0.5
9	6	20.1 ± 2.0	1.9 ± 0.6	33.3 ± 2.3	1.8 ± 0.5
10	9	19.6 ± 1.0	4.1 ± 1.0	24.2 ± 2.3	2.1 ± 0.3
11	12	11.4 ± 0.9	1.5 ± 0.7	30.5 ± 2.8	1.9 ± 0.6
12	3	16.0 ± 1.6	3.9 ± 0.2	24.4 ± 2.1	2.0 ± 0.4
13	6	8.7 ± 0.7	2.8 ± 0.3	19.7 ± 1.3	1.9 ± 0.3
14	3	13.1 ± 1.7	3.7 ± 0.4	20.2 ± 1.7	4.5 ± 0.9
15	6	16.8 ± 2.0	3.0 ± 0.3	21.0 ± 2.1	2.5 ± 0.7
16	9	20.9 ± 2.1	8.7 ± 2.0	38.0 ± 3.9	1.8 ± 0.1
17	12	14.7 ± 1.9	6.9 ± 1.3	46.6 ± 3.8	1.0 ± 0.3
18	3	14.7 ± 1.9	2.8 ± 0.5	24.4 ± 2.2	1.0 ± 0.1
19	6	20.2 ± 2.0	4.8 ± 1.1	20.3 ± 1.0	0.9 ± 0.1
20	9	14.9 ± 1.5	1.9 ± 0.5	23.6 ± 0.3	0.9 ± 0.1
21	12	12.9 ± 0.6	1.3 ± 0.6	30.8 ± 2.8	2.1 ± 0.3
22	3	25.6 ± 3.3	28.3 ± 3.2	18.1 ± 1.6	4.8 ± 0.2
23	6	12.7 ± 1.1	18.3 ± 3.1	35.0 ± 3.2	0.9 ± 0.3
24	9	13.1 ± 0.9	6.1 ± 0.6	17.9 ± 2.5	2.1 ± 0.5
25	3	11.9 ± 1.1	4.0 ± 0.5	48.9 ± 1.9	1.4 ± 0.3
26	6	30.8 ± 3.9	36.1± 3.9	81.3 ± 3.5	1.6 ± 0.4
27	9	42.8 ± 4.1	35.6± 3.9	97.4 ± 4.3	2.0 ± 0.1
28	3	13.9 ± 1.3	2.0 ± 0.1	14.0 ± 0.4	B.D.L.*
29	9	12.8 ± 1.9	1.9 ± 0.2	14.6 ± 0.3	0.3 ± 0.1

* B.D.L. - below detection limit of an atomic absorption spectrophotometer used (the detection limit for Cd was equal to 0.4 μg/g).

solutions containing mercury flowing from chemical facilities enter the sea from the factory outlet situated on the beach.

The Kishon River flowing through the Zevulun Valley carries a substantial amount of alluvial particles. The quantities increase substantially in the winter season as a result of soil erosion caused by heavy winter rains. The water stream reaching the sea carries mostly a hardly-precipitating, fine fraction of alluvial particles (A. Golik, personal communication). This fraction consists mainly of clay. Clay mineral montmorillonite prevails in the soil of northern Israel (HAGIN and RAVINA, 1976) and consequently this mineral prevails in the alluvium carried out by the Kishon water stream. Montmorillonite has the highest cation exchange capacity among clay minerals, about 100 meq/100g (BABCOCK, 1963).

Ions of heavy metals enter the Kishon River with industrial effluents. Part of them undergoes ion exchange adsorption and is probably carried out to sea and finally precipitates there, bonded to the montmorillonite particles. Althrough the content of the clay fraction in Haifa Bay sediments is low (NIR, 1973), due to the high cation exchange capacity. it may contain considerable amounts of metals.

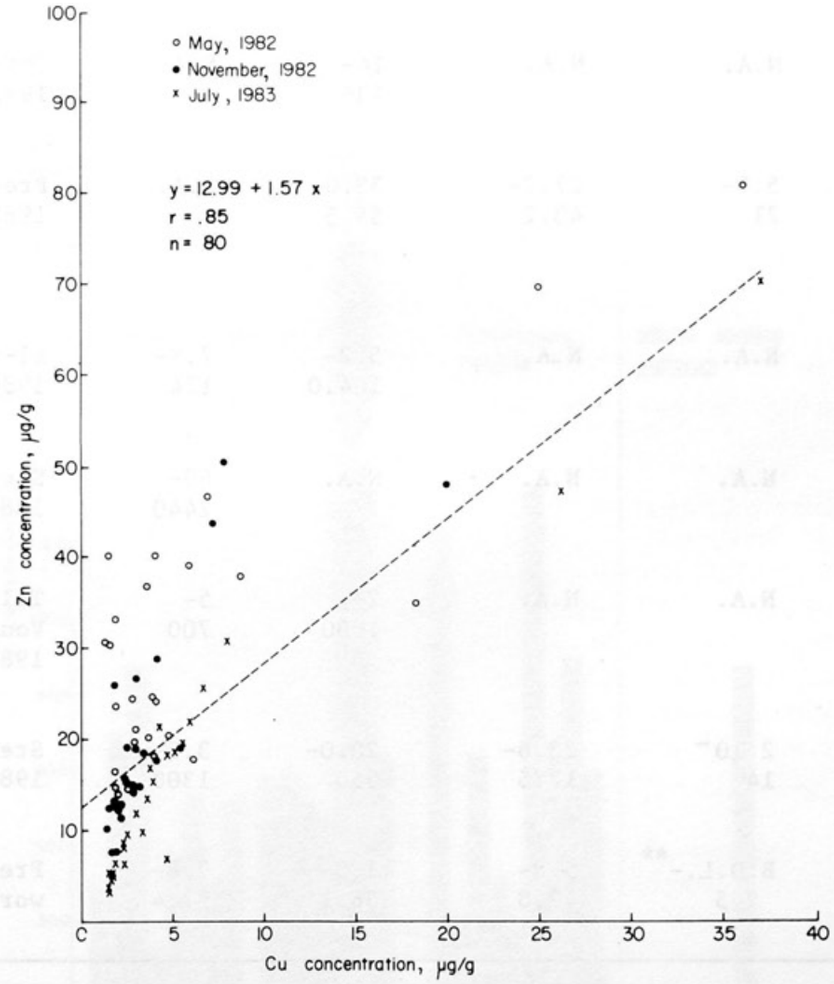


Figure II. - Relationship between copper and zinc in surface sediments in Haifa Bay.

The assumption presented above explains the distribution of copper, zinc and lead with depth found in the present research. In general, the concentration of these metals in the sediments in-

Table 3. Metal concentration in sediments[#] (µg/g d.weight) from various Mediterranean regions

Location	Cd	Pb	Cu	Zn	Reference
Tel Aviv coast	N.A.*	10-27	6-60	20-80	Amiel & Navrot, 1978
Gulf of Venice	0.5-5.6	5.3-49.9	2.8-44.2	48-446	Angela et al., 1980
Ligurian Sea	N.A.	N.A.	14-135	N.A.	Cosma et al., 1979
Ligurian & Tyrrhenian coasts	5.5-21	27.7-43.2	33.0-55.3	N.A.	Breder et al., 1980
Alexandria Harbour	N.A.	N.A.	5.2-104.0	7.8-174	El-Sayed et al., 1980
Gulf of Elefsis	N.A.	N.A.	N.A.	60-2440	Scoulos, 1981
Saronicos Gulf & Elefsis Gulf	N.A.	N.A.	2-1000	5-700	Taliadouri-Voutsinou, 1981
Adriatic Sea	2.10^{-} 14	23.6-1275	20.0-550	3.3-1300	Stegnar et al., 1980
Haifa Bay	B.D.L.-** 2.5	5.8-42.8	1.3-36.1	7.8-97.4	Present work

*N.A. - not analyzed

**B.D.L. - below detection limit equal to 0.4 µg Cd/g.

#It should be emphasized that the different extraction techniques were used by various authors.

creased with the depth of the water column up to 9 m and then decreased. This distribution seems to correspond to the distribution of the fine fraction in the sediments of Haifa Bay (HORNUNG et al., 1982). This picture of metal distribution with depth was more pronounced in the winter months after the relatively quiet summer period and was less pronounced in the summer months after the period of winter storms, when heavy waves raise small clay particles even at 12 m depth.

The distribution of copper and zinc by the same pathway (and also the similarity in their chemical behaviour) is illustrated in figure II, which shows a correlation between zinc and copper concentrations in Haifa Bay sediments.

It is well known that trace metals may be accumulated by aquatic biota to levels far above those found in the surrounding marine environment. The following specimens have been examined for their heavy metal content: Crustacea: Diogenes pugilator: Mollusca Bivalvia: Rudicordium tuberculatum, Dosinia lupinus, Chamelea gallina, Mactra corallina corallina, Mactra corallina stultorum, Donax venustus; Mollusca Gastropoda: Rhinoclavis kochi, Neverita josephinia, Murex trunculus, Murex brandaris, Sphaeronassa mutabilis, Arcularia gibbosula, Arcularia circumcincta and Hinia reticulata.

In this brief article we will present only part of our findings.

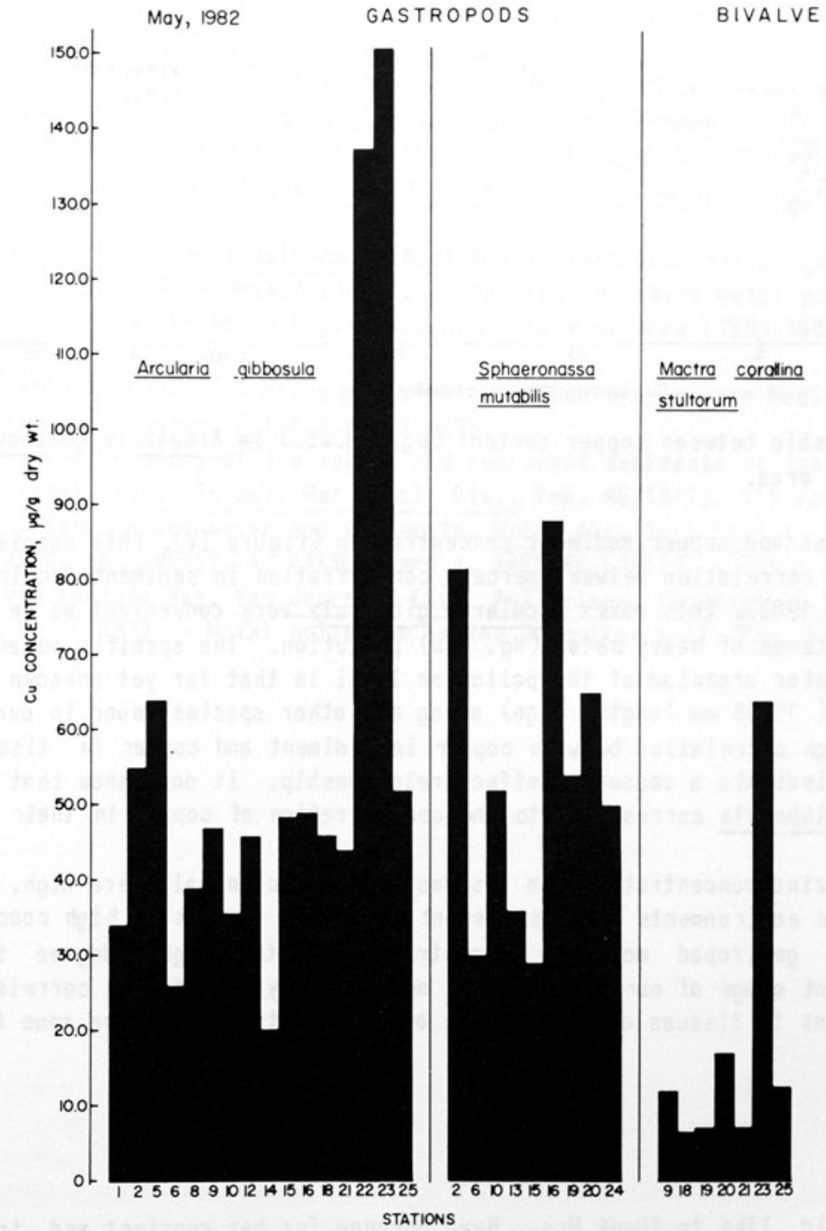


Figure III. - Copper content (µg/g d.wt.) in molluscs along the sampling sites in May 1982.

The concentrations of copper in different species generally follow the copper concentration profile obtained from sediment analysis. It is more or less uniform along Haifa Bay. Part of the results concerning copper content in some molluscs is presented in figure III. As can be seen from this figure, the concentrations of copper in molluscs were higher in gastropods than in bivalves. This difference can probably be explained by the different eating habits of these molluscs. The bivalves are filter feeders and respond to dissolved and particulate metals, while the gastropods found are carnivores feeding on detritus or live molluscs. Thus their food is much more concentrated in copper. Only for Arcularia gibbosula was a significant positive correlation found between copper

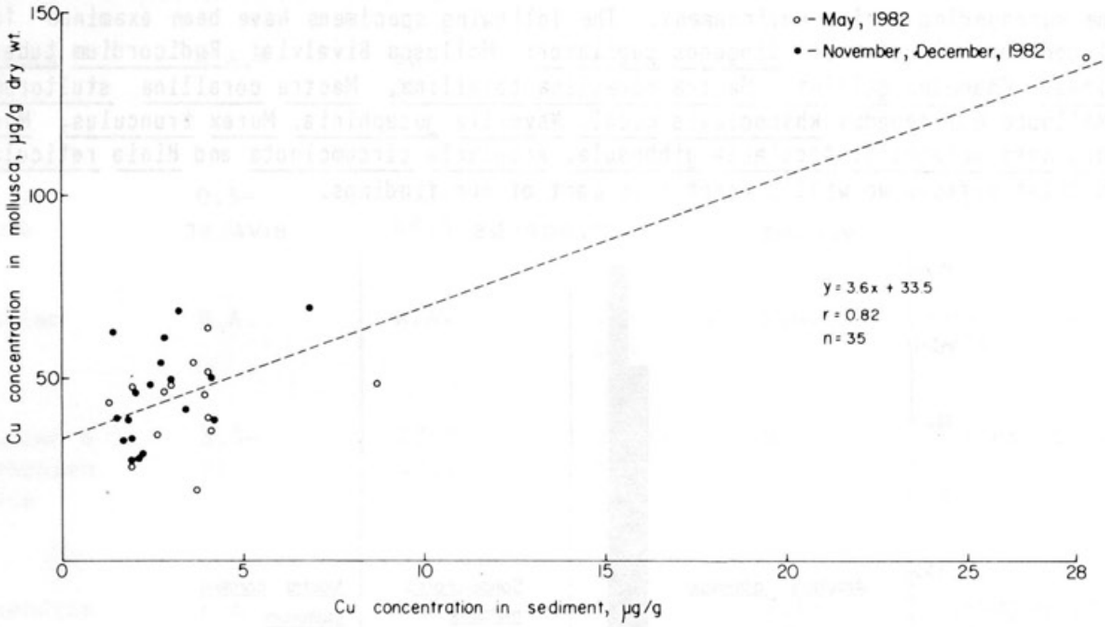


Figure IV. - Relationship between copper content (µg/g d.wt.) in Arcularia gibbosula and the surface sediments of the study area.

concentration in tissues and copper sediment concentration (figure IV). This species has already been found to show a high correlation between mercury concentration in sediment and in mollusc tissues (KRUMGALZ and HORNUNG, 1982). This makes Arcularia gibbosula very convenient as an indicator organism for a monitoring programme of heavy metal (Hg, Cu) pollution. The specific advantage of Arcularia gibbosula as an indicator organism of the pollution level is that for yet unknown reasons it has the least size variations (15-18 mm length range) among all other species found in our surveys. Although the existence of a high correlation between copper in sediment and copper in tissue concentrations does not necessarily indicate a cause-and-effect relationship, it does show that the copper concentration in Arcularia gibbosula corresponds to the concentration of copper in their dwelling environment.

On the average, zinc concentrations in tissues of benthic animals were high, which reflects the fact that in unpolluted environments zinc is present in living tissues in high concentrations. As in the case of copper, gastropod molluscs concentrated zinc to a higher degree than did bivalves. However, at the present stage of our study we did not find any significant correlation between zinc and lead concentrations in tissues of the benthic organisms studied and the same heavy metal content in sediment.

Acknowledgements

The authors would like to thank Mrs. Hava Hornung for her constant and invaluable help at different stages of our work, and divers from the Physical Oceanography Department for sample

collecting. This work was supported by the Ministry of the Interior, Environmental Protection Service, Israel.

References

AMIEL A.J. and J. NAVROT (1978). - Nearshore sediment pollution in Israel by trace metals derived from sewage effluent. Mar. Pollut. Bull. 9: 10-14.

ANGELA G., R. DONAZOLLO, O. HIEKE MERLIN, L. MENEGASSO VITTURI, A.A. ORIO, B. PAVONI, G. PERIN and S. RABITTI (1980). - Heavy metal contents in bottom sediments from the Gulf of Venice and comparisons of their nature. Ves Journees Etud. Pollutions, Cagliari, C.I.E.S.M. 399-406.

BABCOCK K.L. (1963). - Theory of the chemical properties of soil colloidal systems at equilibrium. Hilgardia 34(11): 417-542.

BREDER R., H.W. NURNBERG and M. STOEPPLER (1980). - Toxic trace metal levels in water and sediments from the estuaries of the southern Ligurian and northern Tyrrhenian coasts: a comparative study. Ves Journées Etud. Pollutions Cagliari, C.I.E.S.M., 285-292.

COSMA B., M. DRAGO, M. PICCAZZO, G. SCARPONI and S. TUCCI (1979). - Heavy metals in Ligurian Sea sediments Distribution of Cr, Cu, Ni and Mn in superficial sediments. Mar. Chem. 8: 125-142.

EL-SAYED M.Kh., M.A. EL-SAYED and A.A. MOUSSA (1980). - Anthropogenic material in sediment from the eastern harbour of Alexandria, Egypt. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. 215-222.

HAGIN I. and I. RAVINA (1976). - Soil Science. Mahlol Press, Technion, Haifa. (Hebrew).

HORNUNG H., Z. FLEISHER and B.S. KRUMGALZ (1982). - The study of heavy metal pollution in sediments, benthic fauna and fishes in Haifa Bay, including the Akko area (1981/1982). Israel Oceanogr. Limnol. Res., Haifa, Final Report.

KRUMGALZ B.S. and H. HORNUNG (1982). - Mercury pollution indicators in warm Mediterranean water. VIes Journées Etud. Pollut., Cannes, C.I.E.S.M. 271-276.

NIR Y. (1973). - Geological history of the recent and subrecent sediments of the Israel Mediterranean shelf and slope Geol. Surv. Israel, Mar Geol Div., Rep. MG/10/73, 135 pp.

SCOULOS M.J. (1981). - Zinc in sea-water and sediments. Water Air, Soil Pollut 16: 187-207.

STEGNAR P., I. VUKADIN, B. SMODIS, A. VAKSELJ and A. PROSENC (1980). - Trace elements in sediments and organisms from Kastela Bay. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. 595-600.

TALIADOURI-VOUTSINOU F. (1981). - Metal pollution is the Saronikos Gulf. Mar. Pollut. Bull. 12: 163-168.

SURVEY OF METAL POLLUTION IN GREEK SEDIMENTS

by

Fanny VOUTSINOU-TALIADOURI
Institute of Oceanographic and Fisheries Research
GR-166 04 Hellinikon (Greece)

Abstract

The Patraikos Gulf and the adjoining lagoon of Messolongi, the Pagassitikos Gulf, the Bay of Kavala and various parts of the East Aegean Sea were investigated for the purpose of detecting metal pollution in sediments. For that purpose, the following determinations were carried out: granulometric composition, organic carbon, calcium carbonate, iron, manganese, zinc, copper, lead, chromium, nickel and cobalt. All the above areas were found unsullied, except in the Kavala Bay under the oil-platforms and near a fertilizer factory. Unaffected sediments exhibited metal levels that depended on the area.

*
* *

Introduction

In 1974, a systematic quest for metal pollution in Greek marine sediments was initiated. The elements looked for were iron, manganese, zinc, copper, lead, chromium, nickel and cobalt. The grain size, as well as the organic carbon and the calcium carbonate contents were also determined, since they provide most needed complementary information. Results obtained in the Saronikos and Thermaikos gulfs, around which most of the country's population and industrial and port activities concentrate, have already been reported (VOUTSINOU-TALIADOURI, 1981; VOUTSINOU-TALIADOURI, and SATSMADJIS, 1983). The present work concerns other regions poorly studied in that respect. These are (see map): the Patraikos Gulf and its northern extension, the Messolongi Lagoon, in the east Ionian Sea; the Pagassitikos Gulf, in the west Aegean Sea; the Bay of Kavala, of interest because of its oil-platforms, in the north Aegean Sea different localities, including some near Lesbos Island and its Bay of Gera, in the east Aegean Sea.

Methodology

Sampling took place in '80-81 in the Patraikos Gulf and East Aegean Sea and in '83 in the other areas. The number of stations were for each area as follows: Patraikos 21, Messolongi 16, Pagassitikos 16, Kavala 14, Lesbos 10 and Aegean 10.

The sediment samples were gathered with either a 0.1 m2 van Veen grab or a 0.045 m2 Ponar one, dropped more than once to compensate for its smaller volume. They were stored in deep-freeze, to avoid lump formation and the bacterial decomposition of the organic matter.



Figure 1
Sampling locations

Before processing, they were left at room temperature for several hours and thoroughly mixed. One portion was set aside for the granulometric analysis. Another one was dried in a ventilated oven at 105° C, then crushed in a mortar to pass a 0.45 mm nylon sieve.

The particle size composition was estimated according to BUCHANAN's (1971) technique as adapted and modified by SATSMADJIS and VOUTSINOU-TALIADOURI, (in press). Part of the sample, weighing 10 to 100 g wet, is treated with hydrogen peroxide to destroy interfering organic matter. Sand is isolated on a 62 µm sieve in the presence of water containing hexametaphosphate, to facilitate the separation. The remainder is transferred to a glass cylinder and its volume brought to 1 litre. Immediately following violent shaking, 20 ml are pipetted at a depth of 20 cm, to determine silt (4 to 62 µm) + clay + dissolved salts. After 2 h 3 min, with the temperature kept constant at 20° C, another 20 ml are withdrawn at a depth of 10 cm, to measure clay + dissolved salts. One or two days later, 20 ml are again sucked in from the clear surface layer, to evaluate the dissolved salts.

The organic carbon was determined by oxidizing it with a hot mixture of potassium dichromate solution and concentrated sulphuric acid. The excess reagent was titrated with a standard solution of ammonium ferrous sulphate. Diphenylamine was used as indicator (GAUDETTE et al., 1974).

For the estimation of the metals, 5 g of the dried sediment were shaken in a plastic bottle with 75 ml of 2 N HCl for 16 hours at room temperature. The insoluble matter was retained on a glass fiber filter. The difference between the weight of the sample and that of the residue was deemed to correspond to calcium carbonate. The volume of the filtrate was brought up to 100 ml. The concentrations of the elements were measured on a Perkin-Elmer 305 B atomic absorption spectrophotometer, equipped with a deuterium background corrector.

Results and discussion

Tables I to VI provide a summary of the results obtained. They indicate, for each parameter and area, the mean of the concentration, its range, standard deviation coefficient of variation. They show too the ratio of the highest figure to the mean and of the mean to the average of all the areas. The correlation coefficients of iron. calcium carbonate. sand. organic carbon and clay, with the other parameters are also reported.

The sediments in the Patraikos Gulf generally present enhanced levels of metals, especially manganese, copper and cobalt. This does not reflect pollution, only presumably their great degree of fineness, since the highest values occur in the central part of the gulf, away from the likely sources of contamination. such as the industrial area of Patras and the mouths of the rivers. VARNAVAS and FERENTINOS (1983) have also investigated the level of concentrations of sediments in the Patraikos Gulf. Whenever the sampling stations were the same the results their obtain did not differ significantly from those reported here. From table I, we can see that sand, clay and iron consistently exhibit a correlationship with metals, but not calcium carbonate and organic carbon.

The Messolongi Lagoon, though the north-west extension of the Gulf of Patras, differs greatly from it, owing to its depth not exceeding 3 metres. The metal levels, which vary greatly, tend to rise as the iron and clay contents go up and those of sand, organic carbon and calcium carbonate decrease. However the mean correlation coefficient of sand. clay and organic carbon with the metals are small.

The Pagassitikos Gulf resembles the Messolongi Lagoon in that its sediments present extreme texture contrasts, with attending wide changes in metal contents, in spite of any significant anthropogenic disturbance. The concentration of most metals, especially of chromium, nickel and manganese, increases in the deeper central part of the embayment, but also in the eastern section, pointing to natural enrichment there. Sand, iron and organic carbon display high correlation coefficients with the metals, but not clay and calcium carbonate.

The sediments of the Bay of Kavala contain amounts of metals very variable. We notice on table IV that the ratio mean/general mean of lead, zinc and copper is respectively 11.6, 3.0 and 2.4. This results from the oil-platforms raising, at stations near them, the level of lead up to 50 times and that of zinc up to 10 times. A fertilizer factory on the shore causes an equally heavy contamination of the sea floor by these changing the sign of the correlation coefficients between iron or sand and copper, lead, zinc and nickel, so that contrarilly to what happens elsewhere no constituent shows a high correlation coefficient with the metals.

Next to the coast of the south east corner of Lesbos Island, including the rather shallow Bay of Gera the sediments are poor in copper, iron, manganese, zinc and cobalt. The absence of any significant anthropogenic impact explains the close relationship between the clay, iron and sand contents with those of the metals, excluding nickel the level of which presents major disparities. The values reported in this work are similar to those found by SCOULLOS and DASSENAKIS (1983) outside the area affected by a large tannery.

In other parts of the East Aegean Sea, away from the land and at much greater depths (75 to 730 m), the sediment, much finer, holds more metals, especially iron, manganese, copper and nickel, though considerably less lead and chromium. Clay, organic carbon, iron and calcium carbonate have high correlation coefficients with the metals.

Table VII enables us to make a comparison between the various areas taking as a basis the concentration of nickel in the sediments, since elevated levels of this element are rarely of anthropogenic origin.

The ratio $\underline{q}$, of the concentration of a metal to that of nickel shows very clearly that the Bay of Kavala is polluted by lead, zinc and copper, since the $\underline{q}$ figures of the other areas are considerably lower. It can also be seen that the $\underline{q}$ figures in the Bay of Kavala are also high for chromium, iron and cobalt.

TABLE I: Concentrations and correlatio coefficients of the various parameters in the Patrailos Gulf.

	mean	max.	min.	stand. deviat.	coef. var.	max./mean	mean/gen.mean	Correlation coefficient Fe	$CaCO_3$	sand	org.C	clay
Sand (%)	4.9	45.7	0	11	216	9.3	0.15	-0.758	-0.102	-	-0.308	-0.907
Silt (%)	43.2	61.0	24.9	10	23	1.4	0.95	0.361	0.827	0.254	0.228	-0.356
Clay (%)	51.9	65.8	28.8	13	25	1.3	2.37	0.728	0.158	-0.907	0.560	-
$CaCO_3$(%)	33.8	36.6	30.7	1.7	5	1.1	0.85	0.739	-	-0.102	0.628	0.158
org.C(%)	0.67	0.87	0.43	0.1	18	1.3	0.92*	0.613	0.628	-0.308	-	0.560
Fe (‰)	28.3	32.2	16.5	4	13	1.1	1.43	-	0.739	-0.738	0.613	0.728
Mn (ppm)	1419	2610	750	58	40	1.8	1.82	0.598	-0.092	-0.967	0.101	0.860
Zn (ppm)	72	88	43	12	17	1.2	1.41**	0.955	0.512	-0.887	0.542	0.877
Cu (ppm)	35	43	16	8	22	1.2	1.75**	0.441	-0.270	-0.891	-0.009	0.821
Pb (ppm)	16	20	10	3	18	1.3	0.80**	0.693	0.992	-0.053	0.584	0.073
Cr (ppm)	100	119	55	16	15	1.2	0.73	0.991	0.668	-0.790	0.515	0.744
Ni (ppm)	110	132	60	18	16	1.2	1.22	0.543	-0.157	-0.929	0.108	0.880
Co (ppm)	19	23	11	3	16	1.2	1.58	0.443	-0.275	-0.907	0.031	0.825
mean only for the metals								0.666	0.265	-0.770	0.313	0.726

* excluding Messolongi Lagoon

** excluding Kavala Bay

*

* *

TABLE II: Concentrations and correlation coefficients of the various parameters in the Messolongi Lagoon.

	mean	max	min.	stand. deviat.	coef. var.	max./mean	mean/gen.mean	Correlation coefficient Fe	$CaCO_3$	sand	org.C	clay
Sand (%)	35.5	76.8	2.0	25	70	2.2	1.12	-0.570	0.280	-	0.846	-0.912
Silt (%)	46.2	67.3	17.2	17	37	1.5	1.00	0.582	-0.295	-0.947	-0.730	0.734
Clay (%)	18.2	41.6	5.0	11	58	2.3	0.85	0.467	-0.218	-0.912	-0.860	-
$CaCO_3$(%)	46.0	63.4	32.4	10	21	1.38	1.15	-0.931	-	0.280	0.078	-0.218
org.C(%)	3.2	5.88	0.91	1.7	52	1.84	4.38*	-0.411	0.078	0.846	-	-0.860
Fe (‰)	18.1	28.5	6.80	6	31	1.57	0.91	-	-0.931	-0.570	-0.411	0.467
Mn (ppm)	772	2005	349	450	59	2.60	1.00	0.486	-0.339	-0.149	-0.526	-0.092
Zn (ppm)	56	109	18	23	40	1.93	1.10**	0.319	-0.437	0.321	-0.199	-0.230
Cu (ppm)	20	30	5	7	35	1.51	1.00**	0.783	-0.620	-0.755	-0.759	0.820
Pb (ppm)	14	23	11	4	27	1.64	0.70**	0.453	-0.348	-0.728	-0.497	0.858
Cr (ppm)	116	246	11	70	60	2.12	0.84	0.994	-0.952	-0.496	-0.329	0.391
Ni (ppm)	70	104	27	21	30	1.49	0.78	0.991	-0.919	-0.591	-0.383	0.463
Co (ppm)	9	13	2	3	35	1.46	0.75	0.946	-0.884	-0.675	-0.419	0.606
mean only for the metals								0.710	-0.679	-0.455	-0.366	0.410

* excluding Messolongi Lagoon

** excluding Kavala Bay

TABLE III: Concentrations and correlation coefficients of the various parameters in the Pagassitikos Gulf.

	mean	max.	min.	stand. deviat.	coef. var.	max./mean	mean/gen.mean	Correlation coefficient Fe	$CaCO_3$	sand	org.C	clay
Sand (%)	22.9	63.2	1.7	23	98	2.76	0.72	-0.773	-0.092	-	-0.764	-0.278
Silt (%)	70.4	95.9	34.0	23	32	1.36	1.50	0.639	-0.010	-0.957	0.854	-0.011
Clay (%)	6.7	22.9	1.3	6	92	3.42	0.30	0.551	0.345	-0.278	-0.199	-
$CaCO_3$ (%)	29.9	36.2	19.1	6	19	1.21	0.75	0.508	-	-0.092	0.318	0.345
org.C (%)	0.6	1.08	0.29	0.2	30	1.80	0.82*	0.624	0.318	-0.764	-	-0.199
Fe (‰)	23.7	29.9	14.6	4	17	1.26	1.20	-	0.508	-0.773	0.624	0.551
Mn (ppm)	897	2298	246	600	66	2.56	1.15	0.851	0.731	-0.744	0.759	0.375
Zn (ppm)	58	74	45	8	13	1.27	1.14**	0.790	0.238	-0.981	0.825	0.282
Cu (ppm)	21	32	14	5	24	1.52	1.05**	0.542	-0.216	-0.921	0.675	0.151
Pb (ppm)	26	34	20	30	72	1.31	1.30**	0.458	0.210	-0.775	0.630	0.032
Cr (ppm)	264	395	97	85	32	1.50	1.92	0.914	0.309	-0.875	0.760	0.420
Ni (ppm)	117	238	40	57	49	2.03	1.30	0.856	0.352	-0.939	0.777	0.429
Co (ppm)	15	21	9	5	33	1.40	1.25	0.956	0.528	-0.864	0.720	0.526
mean only for the metals								0.767	0.332	-0.859	0.721	0.346

* excluding Messolongi Lagoon

** excluding Kavala Bay

*

* *

TABLE IV: Concentrations and correlation coefficients of the various parameters in the Kavala Bay.

	mean	max.	min.	stand. deviat.	coef. var.	max./mean	mean/gen.mean	Correlation coefficient Fe	$CaCO_3$	sand	org.C	clay
Sand (%)	68.1	97.5	4.3	29	42	1.43	2.14	-0.976	0.345	-	-0.501	-0.982
Silt (%)	27.2	83.4	1.9	23	85	3.10	0.60	0.968	-0.335	-0.998	0.501	0.970
Clay (%)	4.7	24.8	0.7	7	145	5.30	0.21	0.981	-0.379	-0.982	0.488	-
$CaCO_3$ (%)	34.8	64.6	10.8	16	46	1.86	0.87	-0.260	-	0.345	0.253	-0.379
org.C (%)	0.72	1.43	0.01	0.4	53	1.99	0.99*	0.452	0.253	-0.501	-	0.488
Fe (‰)	14.3	26.5	7.4	5	38	1.85	0.72	-	-0.260	-0.976	0.452	0.981
Mn (ppm)	273	417	65	100	37	1.52	0.35	0.384	0.382	-0.320	0.691	0.413
Zn (ppm)	153	510	24	140	91	3.33	3.00**	-0.230	-0.263	0.116	0.434	-0.052
Cu (ppm)	48	227	4	60	125	4.70	2.40**	-0.462	-0.545	0.436	-0.325	-0.318
Pb (ppm)	232	908	5	270	116	3.91	11.60**	-0.391	-0.195	0.291	0.229	-0.217
Cr (ppm)	105	278	20	80	78	2.70	0.76	0.924	-0.426	-0.981	0.502	0.963
Ni (ppm)	22	47	11	12	52	2.10	0.24	-0.141	-0.695	0.187	-0.751	-0.101
Co (ppm)	5	10	0	3	58	2.10	0.42	0.849	-0.590	-0.805	0.032	0.836
mean only for the metals								0.133	-0.324	-0.256	0.158	0.313

* excluding Messolongi Lagoon

** excluding Kavala Bay

TABLE V: Concentrations and correlation coefficients the various parameters in Lesbos Island.

		mean	max.	min.	stand. deviat.	coef. var.	max. / mean	mean / gen.mean	Correlation coefficient Fe	$CaCO_3$	sand	org.C	clay
Sand	(%)	45.6	93.1	11.7	31	65	1.91	1.53	-0.914	0.555	-	-0.533	-0.972
Silt	(%)	34.6	55.3	4.8	20	58	1.60	0.74	0.847	-0.410	-0.985	0.650	0.917
Clay	(%)	16.8	35.9	2.1	12	73	2.14	0.76	0.963	-0.727	-0.972	0.344	-
$CaCO_3$	(%)	35.9	49.7	22.8	10	30	1.38	0.90	-0.725	-	0.555	0.282	-0.727
org.C	(%)	1.32	3.25	0.35	0.8	62	2.45	1.81*	0.162	0.282	-0.533	-	0.344
Fe	(‰)	11.7	21.0	3.2	6	48	1.79	0.59	-	-0.725	-0.914	0.162	0.963
Mn	(ppm)	447	1126	172	370	82	2.52	0.58	0.952	-0.885	-0.844	0.041	0.946
Zn	(ppm)	32	43	18	10	30	1.33	0.63**	0.894	-0.585	-0.991	0.545	0.974
Cu	(ppm)	8	12	3	3	40	1.52	0.40**	0.794	-0.163	-0.854	0.561	0.767
Pb	(ppm)	28	39	10	9	31	1.40	1.40**	0.686	-0.518	-0.859	0.638	0.845
Cr	(ppm)	155	247	40	90	57	1.59	1.12	0.812	-0.764	-0.552	-0.288	0.704
Ni	(ppm)	89	315	20	86	97	3.55	0.99	0.136	-0.010	0.221	-0.690	-0.137
Co	(ppm)	9	19	0	7	74	2.09	0.75	0.963	-0.843	-0.865	0.091	0.956
mean only for the metals									0.748	-0.561	-0.707	0.132	0.752

* excluding Messolongi Lagoon

** excluding Kavala Bay

*

* *

TABLE VI: Concentrations and correlation coefficients of the various parameters in East Aegean Sea.

		mean	max.	min.	stand. deviat.	coef. var.	max. / mean	mean / gen.mean	Correlation coefficient Fe	$CaCO_3$	sand	org.C	clay
Sand	(%)	10.9	45.0	0.2	14	127	4.1	0.54	-0.157	0.677	-	-0.802	-0.809
Silt	(%)	56.2	73.6	45.6	9	16	1.3	1.21	-0.780	0.171	-0.296	-0.255	-0.322
Clay	(%)	32.8	45.5	9.4	14	41	1.4	1.50	0.635	-0.777	-0.809	0.951	-
$CaCO_3$	(%)	59.0	77.0	45.1	10	18	1.3	1.48	-0.683	-	0.677	-0.863	-0.777
org.C	(%)	0.35	0.47	0.28	0.06	17	1.3	0.48*	0.623	-0.863	-0.802	-	0.951
Fe	(‰)	22.5	32.6	14.2	6	26	1.5	1.14	-	-0.683	-0.157	0.623	0.635
Mn	(ppm)	861	2640	280	720	84	3.1	1.10	0.746	-0.656	-0.535	0.810	0.773
Zn	(ppm)	38	55	25	10	27	1.5	0.74**	0.900	-0.784	-0.530	0.876	0.895
Cu	(ppm)	17	29	4	8	49	1.7	0.85**	0.664	-0.670	-0.739	0.911	0.979
Pb	(ppm)	17	22	11	4	25	1.3	0.85**	0.899	-0.568	-0.296	0.738	0.768
Cr	(ppm)	86	157	52	43	50	1.8	0.62	0.840	-0.896	-0.649	0.866	0.871
Ni	(ppm)	131	291	39	86	65	2.2	1.46	0.665	-0.891	-0.805	0.853	0.880
Co	(ppm)	15	24	8	5	36	1.6	1.25	0.868	-0.701	-0.527	0.831	0.874
mean only for the metals									0.797	-0.731	-0.530	0.813	0.834

* excluding Messolongi Lagoon

** excluding Kavala Bay

The ratio t, of q to its mean value for the metal, indicates even better that levels of the metals in the Patraikos and Pagassitikos Gulfs, as well as in the Messolongi Lagoon, are normal, while they are low in the East Aegean Sea.

TABLE VII: Ratio, q, of the concentration of a metal to that of Ni and ratio, t, of q to the mean value of q for the metal.

	Fe		Mn		Zn		Cu		Pb		Cr		Co	
	q	t	q	t	q	t	q	t	q	t	q	t	q	t
Patraikos G.	257	0.92	12.90	1.39	0.65	1.25	0.32	1.60	0.14	0.7	0.91	0.45	0.17	1.21
Messolongi L.	259	0.93	11.00	1.19	0.80	1.54	0.29	1.45	0.20	1.0	1.66	0.83	0.13	0.93
Pagassitikos G.	202	0.73	7.67	0.83	0.49	0.94	0.18	0.90	0.22	1.1	2.26	1.13	0.13	0.93
Kavala B.	650	2.34	12.40	1.34	6.95*	13.40	2.18*	10.90	10.54*	52.7	4.77	2.38	0.23	1.64
Lesbos I.	131	0.47	5.02	0.54	0.36	0.69	0.09	0.45	0.31	1.5	1.74	0.87	0.10	0.71
E. Aegean S.	172	0.62	6.57	0.71	0.29	0.56	0.15	0.65	0.13	0.6	0.66	0.33	0.11	0.78
mean of q	278		9.26		1.59		0.20		0.20		2.00		0.14	

* not included, when calculating the mean value of q.

A comparison with other unpolluted parts of the Mediterranean Sea indicates that the levels of metals in sediments are about the same as those reported here. Refer to the papers by SMITH and CRONAN (1975) for the Central part of the Aegean Sea, ANGELIDIS et al. (1981) for the South Evoikos Gulf, AMORE et al. (1983) round the southernmost tip of Sicily, BREDER et al. (1981) near the Southern Ligurian and Northern Ligurian Coasts, OBIOLS and PEIRO (1981) for the vicinity of the Delta of the Ebro River, DONAZZOLO et al. (1981) for an unsullied section of the Gulf of Venice. This is remarkable considering the wide assortment of analytical procedures, ranging from total extraction to weak acid digestion. On the contrary, other workers found metal contents in polluted sediments up to 30 times as high as those reported here, excluding the few polluted locations in the Kavala Bay. See papers by GRIMANIS et al. (1977) for the Upper Saronikos Gulf, MAJORI et al. (1977) for the Gulf ot Trieste, RINGOT (1983) for the vicinity of Cannes, ELSOKKARY (1979) for the area to the East of Alexandria, CENCIARINI et al. (1981) for the Gulf of Villefranche.

General remarks

1) Depth tends to increase the percentage of clay and reduce that of sand and organic carbon when dealing with different areas.

2) As shown by the correlation coefficient between sand and the metals, as the proportion of sand diminishes and that of clay rises. the levels of the metals go up. Calcium carbonate has a similar effect to that of sand, but only when it represents a large fraction of the sample (East Aegean Sea). Organic carbon content is related to that of the metals only in the East Aegean and the Pagassitikos Gulf.

3) The concentrations of the metals follow more closely that of iron than that of sand, as indicated by the correlation coefficient. Since the level of iron is in general quite high and easy to determine, calculating the ratio of the concentration of the metals to that of iron could be used to detect pollution.

4) Elevated ratios of metal content to mean content, as well as poor correlation coefficients between sand or clay and the metals, suggest pollution (Kavala Bay).

5) There appear to be marked eutrophication in the Messolongi Lagoon, a moderate one in the nearshore south east Lesbos Island area, as suggested by the high percentage of organic carbon in the sediments.

6) The oil-platforms and the fertilizer factory in the Bay of Kavala cause heavy lead, zinc and copper pollution.

Note that. when the texture of the sediment in an area does not vary very much, it is obvious that the correlation coefficient of the parameters with the metals (particularly sand and iron) will tend to be smaller. This is the case of the Patraikos Gulf, in which, except for 2 stations out of 21 the percentage of sand does not exceed 9 and therefore the levels of metals exhibit only slight variations. Conversely, when there are great differences in the grain composition of the sediments (Pagassitikos Gulf). the correlation coefficient is higher. resulting in great variations in the metal concentration.

Conclusion

Of all the investigated areas, significant metal pollution in sediments was detected only in the Kavala Bay under the oil-platforms or next to the fertilizer factory and that solely for lead, zinc and copper.

References

AMORE C., A. CASTAGNA, A. CURRAO, E GIUFFRIDA, F. SARRO and F. SINATRA, (1983). - Relationship between heavy metals and sediments in the southern Ionian continental shelf. Marine Pollution Bulletin, Vol. 14, n° 9, pp 352-356.

ANGELIDIS M., A.P. GRIMANIS, D. ZAFIROPOULOS and M. VASSILAKI-GRIMANI, (1981). - Trace elements in sediments of Evoikos Gulf, Greece. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M 413-418

BREDER R. H.W. NURNBERG and M. STOEPPLER, (1981). - Toxic trace metal levels in water and sediments from the estuaries of the southern Ligurian and northern Tyrrhenian coasts: a comparative study. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M 285-292.

BUCHANAN J.B., (1971). - Sediments. In methods for the study of marine benthos (N.A. Holme and A. McIntyre, eds.), pp 35-39. I.B.P. handbook n° 16. Oxford University Press, Oxford.

CENCIARINI J., F. FERNEX, R. PUCCI, F. RAPIN et R. VAISSIERE (1981). - Comparaison entre la répartition de différents polluants dans les sédiments marins superficiels du plateau continental le long de la Côte d'Azur. Différences et analogies dans les modalités de transport. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. 341-352.

DONAZZOLO R., H. MERLIN, L.M. VITTURI, A.A. ORIO, B. PAVONI, G. PERIN and S. RABITTI, (1981). - Heavy metal contamination in surface sediments from the Gulf of Venice, Italy. Marine Pollution Bulletin, Vol. 12, n° 12, pp. 417-425.

ELSOKKARY I.H., (1979). - Distribution and nature of heavy metals in some marine sediments of the Mediterranean Sea coast east of Alexandria, Egypt. IVes Journées Etud. Pollutions, Antalya, C.I.E.S.M 183-185.

GAUDETTE H., W. FLIGHT, I. TONES and D. FOLGER, (1974). - An inexpensive titration method for the determination of organic carbon in recent sediments. J. Sedim. Petrol., 44, 249-253.

GRIMANIS A.P., M. VASSILAKI-GRIMANI and G.B. GRIGGS, (1977). - Pollution studies of trace elements in sediments from the upper Saronikos Gulf, Greece. Journal of Radioanalytical Chemistry, Vol. 37, 761-773.

MAJORI L., G. NEDOCLAN, G.B. MODONUTTI and C. CAMPELLO, (1977). - Pollution par métaux dans la mer Adriatique du Nord. Note I: Etude sur les sédiments superficiels du Golfe de Trieste. IIIes Journées Etud. Pollutions, Split, C.I.E.S.M. 99-101.

OBIOLS J. and L. PEIRO, (1981). Heavy metals in marine sediments from the Delta del Ebro. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. 391-394.

RINGOT J.L., (1983). - Etude de la répartition et de l'origine de la contamination des sediments de la baie de Cannes-La Napoule par les métaux lourds. VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M. 283-292.

SATSMADJIS J. and F. VOUTSINOU-TALIADOURI, (in press). - An index of metal Pollution. Oceanologica Acta.

SCOULLOS M., N. MIMIKOS, M. DASSENAKIS and L. BACHAS, (1983). - Trace metals and petroleum aromatic hydrocarbons in the Gulf of Gera, Lesbos Island, Greece. VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M. 411-414.

SMITH P.A. and D.S. CRONAN, (1975). - Chemical composition of Aegean Sea sediments. Marine Geology, 18. M7-M11.

VARNAVAS S.P. and G. FERENTINOS, (1983). - Heavy metal distribution in the surface sediments of Patraikos Bay, Greece. VIIes Journées Etud. Pollutions, Cannes, C.I.E.S.M. 405-409.

VOUTSINOU-TALIADOURI F., (1981). - Metal pollution in the Saronikos Gulf. Marine Pollution Bulletin, Vol. 12, n° 5, pp 163-168.

VOUTSINOU-TALIADOURI F. and J. SATSMADJIS, (1983). - Metals in polluted sediments from the Thermaikos Gulf (Greece). Marine pollution Bulletin, Vol. 14, n° 6, pp 234-236.

MERCURY LEVELS IN THE FLESH GUT AND LIVER OF STRIPED BREAM (LITHOGNATHUS MORMYRUS) FROM POLLUTED AND UNPOLLUTED HABITATS

by

Hava HORNUNG*, Yuval COHEN** and Boris S. KRUMGALZ*
Israel Oceanographic & Limnological Research
P.O. Box 8030, Haifa 31080, (Israel)
** Environmental Protection Service
Ministry of the Interior
P.O. Box 6158, Jerusalem 91061 (Israel)

Abstract

Total mercury concentrations were determined in the flesh, guts and livers of over 100 specimens of Lithognathus mormyrus caught off the Mediterranean coast of Israel. In specimens from a polluted area, the mercury concentrations ranged from 0.168 to 0.471 µg/g wet wt. in the flesh, from 0.125 to 2.20 µg/g in the gut (including the contained food), and from 0.280 to 1.70 µg/g in the liver, while the corresponding values in specimens from an unpolluted area were from < 0.005 to 0.184 µg/g, from < 0.005 to 0.162 µg/g and from 0.023 to 0.392 µg/g, respectively. Body size-mercury level correlations were better for specimens from the unpolluted area when compared to specimens from the polluted area, reflecting the fact that in a polluted habitat, even small-size fish accumulate relatively high levels of mercury. It is concluded that mercury levels in L. mormyrus are a function of the levels in its habitat and that mercury accumulation by this species is associated with its feeding habits.

*
* *

Introduction

Mercury levels in edible fish are examined world-wide because of the associated potential for hazards to human health. HORNUNG et al. (1980) determined total mercury concentrations in 12 species of trawl fish caught off the Israeli Mediterranean coast. In most species mercury concentrations in the flesh were lower than 0.5 ug/g wet wt. irrespective of sampling locations. In a preliminary study with a few species of inshore fish from the Israeli coast, we found that fish from Haifa Bay, which is relatively contaminated with anthropogenic mercury, have higher mercury concentrations in the flesh than fish caught at unpolluted locations (HORNUNG et al., 1984). Subsequently, we initiated a comprehensive study of mercury levels and distribution in inshore fish species in relation to their ecology. This report presents the data obtained for over 100 specimens of Lithognathus mormyrus from polluted and unpolluted habitats. This species, which is commercially exploited, lives within a restricted area in the littoral zone.

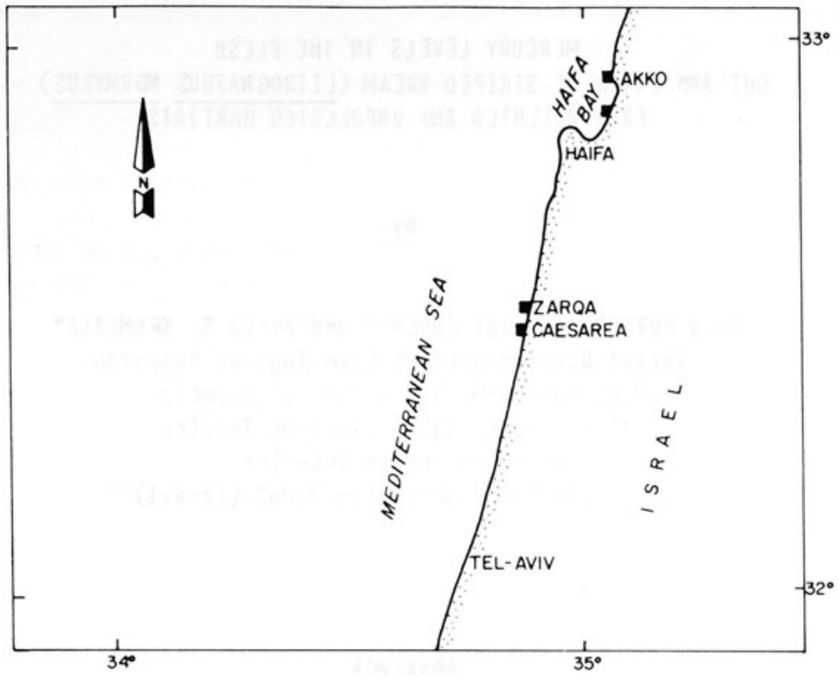


Fig. 1. - Sampling locations along the Israeli Mediterranean coastline.

Materials and methods

Sampling locations are shown in figure I. Haifa Bay (including Akko area) receives anthropogenic mercury, while there are no known pollution sources in the vicinity of the Zarqa and Caesarea stations. HORNUNG et al. (1984) found that shallow surficial sediments in Haifa Bay containing from ca. 0.06 to 0.99 µg-Hg/g dry wt. were up to ca. 160 times enriched in mercury relative to sediments south of the Bay. Mercury levels in benthic organisms from these locations correlated with the levels in the hsediments. It is reasonable to assume that the data previously collected south of Haifa Bay is indicative of the situation at the Zarqa and Caesarea stations.

Samples of L. mormyrus caught mainly with entangling nets at depths ranging from 3 to 10 m were obtained from local fishermen over a period of four years from 1979 to 1983. Sex, weight and length were determined for the freshly caught fish and then a piece of muscle tissue, the liver and the gut (gastro-intestinal tract including the contained food) were removed, placed separately in acid prewashed plastic bags and frozen. The gut content of selected samples was examined and identified under a microscope.

For analysis, the samples were thawed, homogenized and wet digested with concentrated nitric acid as described by HORNUNG et al. (1984). Total mercury concentrations were determined on a Coleman Mercury Analyser, MAS-50, with a detection limit of 0.005 µg Hg/g and an estimated precision (from replicate analyses) of 2.8 % . NBS reference material (Albacore tuna) was analysed with each batch of samples and the results were in good agreement with NBS value (0.95 ± 0.1 µg-Hg/g NBS value ; 0.95 ± 0.07 our value).

Results and discussion

Mercury concentrations in the flesh and internal organs of L. mormyrus were not significantly different for males and females from the same sampling station and did not vary systematically in

specimens sampled at a given station on different occasions. Consequently the data for males and females and for different sampling occasions have been amalgamated in the following presentation and discussion of the results. The mean values and ranges of mercury concentrations found in the flesh, livers and guts of the samples are listed in table 1 and it is evident that :

Table 1. - Mean values and ranges of mercury concentration (µg/g wet wt.) in the flesh, liver and gut of L. mormyrus (n = number of specimen analyzed).

Sampling Station			Body Length (cm) 10-15	16-20	21-24
Haifa Bay, Akko (Polluted)	Flesh	n	23	30	5
		mean	0.246 ± 0.051	0.267 ± 0.057	0.322 ± 0.088
		range	0.192 - 0.383	0.168 - 0.371	0.256 - 0.471
	Liver	n		29	3
		mean		0.724 ± 0.334	0.755 ± 0.147
		range		0.280 - 1.70	0.599 - 0.890
	Gut	n		25	2
		mean		0.660 ± 0.594	0.532 ± 0.257
		range		0.125 - 2.20	0.350 - 0.714
Zarqa, Caesarea (Unpolluted)	Flesh	n	8	29	11
		mean	0.035 ± 0.011	0.070 ± 0.034	0.090 ± 0.039
		range	0.022 - 0.054	0.031 - 0.184	0.039 - 0.145
	Liver	n	4	25	11
		mean	0.166 ± 0.091	0.152 ± 0.091	0.213 ± 0.011
		range	0.065 - 0.276	0.023 - 0.377	0.049 - 0.392
	Gut	n	5	25	7
		mean	0.034 ± 0.029	0.047 ± 0.038	0.056 ± 0.04
		range	0.007 - 0.071	BDL - 0.162	0.027 - 0.117

Note: Four small specimens from the unpolluted stations were analyzed. These are not included in Table 1 but are included in Figs. 2 & 3.

a. In all of the fish analysed the mercury levels in the flesh were lower than the maximum allowable concentrations in fish intented for sale around the Mediterranean (ranging from 0.5 to 1.0 ppm in different countries).

b. Fish from the polluted stations were enriched in mercury relative to fish from the unpollued stations.

c. In general, mercury levels in the flesh and the internal organs increased with body size. As illustrated in figure II for the mercury concentrations in the flesh, the correlations between mercury levels and body length and weight are better for specimens from the unpolluted stations than for specimens from the polluted stations. This reflects the fact that in a polluted habitat even small-size fish accumulate relatively high levels of mercury.

d. Mean mercury levels in the liver were always higher than the levels in the flesh and gut. However, in specimens from the polluted stations, the levels in the gut were higher than the levels in the flesh, while in the specimens from the unpolluted stations the levels in the flesh were higher only in specimens between 16 - 20 cm. The numerical ratios of mercury levels in the internal organs and flesh for specimens in the 16-20 cm. body size range are listed in table 2.

Table 2. - Ratios of mercury levels in the internal organs and flesh of specimens of L. mormyrus in the 16 - 20 cm body size range. (L = Liver; G = Gut; F = Flesh).

	L/F	G/F	L/G
Polluted stations	2.7	2.5	1.1
Unpolluted stations	2.2	0.7	3.2

	F	L	G
Polluted/Unpolluted	3.8	4.8	14.0

Note: The ratios were calculated only for specimens in the 16-20 cm body size range because the number of samples available from both the polluted and unpolluted stations were relatively large and comparable.

Fish can accumulate mercury compounds either by direct absorption from the surrounding water or through their food sources (OLSEN et al., 1973 : SINOVCIC et al., 1980 ; MACCRIMMON et al., 1983). Existing data on mercury distribution in various fish species reveal that in some cases mercury accumulates in the liver to a greater extent than in the flesh while in others the reverse is true (e.g. GREIG et al:, 1975 ; DOI and UI, 1975 : DENTON and BRECK, 1981). It has been demonstrated,

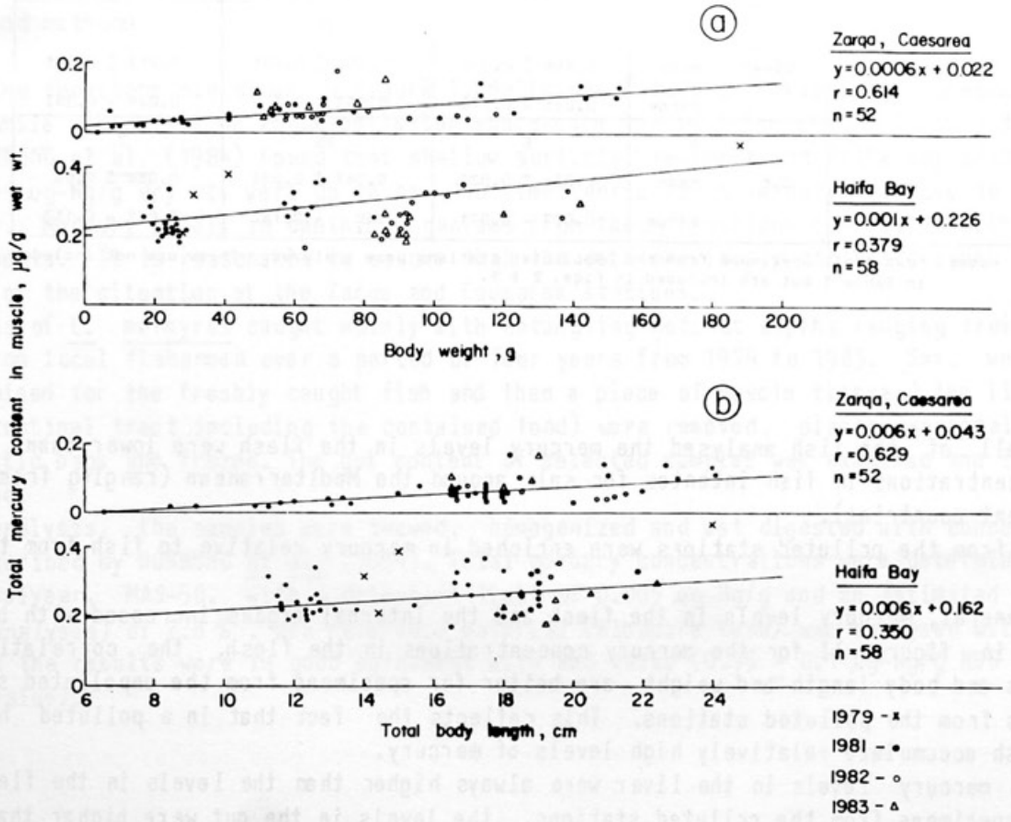


Figure 2. - Relationships among mercury levels in the flesh of L. mormyrus and body weight (a) and length (b). All correlation coefficients are significant at the 1 % levels.

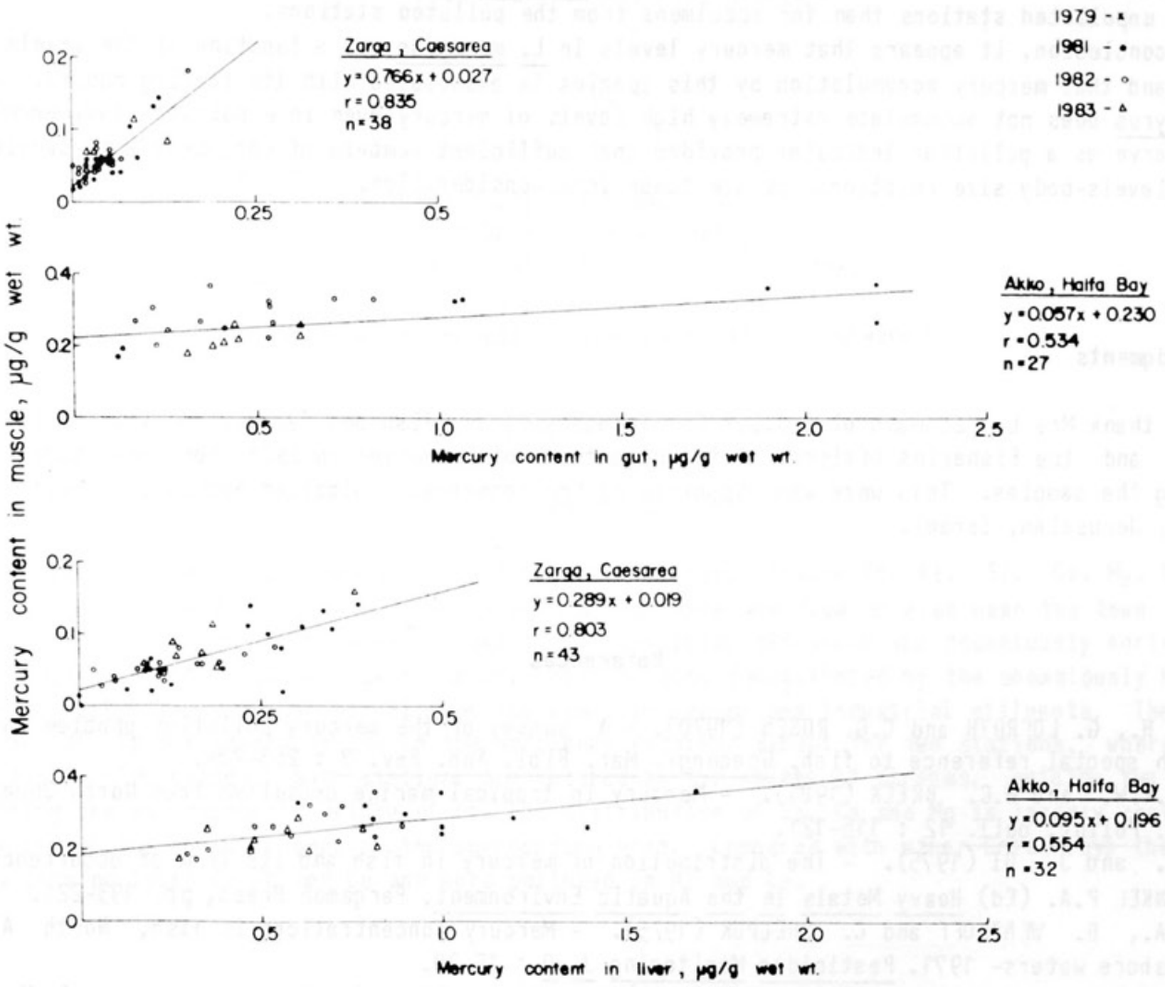


Fig. 3. - Relationships among mercury levels in the internal organs and flesh of L. mormyrus. All correlation coefficients are significant at the 1 % level.

however, that when food is the source of mercury, the greatest accumulation occurs in the kidneys, liver and spleen (BACKSTROM, 1967, 1969 cited in ACKEFORS et al., 1970). Our results indicate that the elevated levels of mercury in specimens of L. mormyrus which inhabit a polluted environment result primarily from uptake of mercury during feeding from contaminated food and sand particles. Thus, as shown in table 2, when the gut was enriched in mercury (polluted stations) the accumulation of mercury in the liver was enhanced (higher L/F ratio in the polluted stations) and the mercury levels in the liver and gut were similar.

From examination of the gut content of L. mormyrus it appears that this species feeds mainly on small crustaceans over a sandy bottom. The amount of sand in the gut content of selected samples varied from about 10 to 85 % of the total volume. Crustacean remains were mostly of small organisms such as copepods, amphipods, etc. Fragments of molluscan shells, bristles of polychaetes and birtlle stars and small fragments of algae were also present. The molluscan fragments in the gut were in general small and might have been swallowed with the sand. In a few samples almost entire shells were present suggesting that a whole mollusc was eaten. It can be expected that mercury levels in the sand

and the organisms which form the diet of L. mormyrus would be more variable in a polluted than in an unpolluted environment. Such variations could explain the observation that the correlations among the mercury levels in the internal organs and flesh of L. mormyrus (figure III) were better for specimens from the unpolluted stations than for specimens from the polluted stations.

In conclusion, it appears that mercury levels in L. mormyrus are a function of the levels in its habitat and that mercury accumulation by this species is associated with its feeding habits. Although L. mormyrus does not accumulate extremely high levels of mercury even in a polluted environment, it could serve as a pollution indicator provided that sufficient numbers of specimens are sampled and mercury levels-body size relationships are taken into consideration.

Acknowledgments

We thank Mrs L. Zismann of I.O.L.R for identifying the fish and for the analysis of their gut content, and the Fisheries Officers of the Department of Fisheries in Haifa for their assistance in obtaining the samples. This work was supported by Environmental Protection Service, Ministry of the Interior, Jerusalem, Israel.

References

ACKEFORS H., G. LOFROTH and C.G. ROSEN (1970). - A survey of the mercury pollution problem in Sweden with special reference to fish. Oceanogr. Mar. Biol. Ann. Rev. 8 : 203-224.

DENTON G.R.W. and W.C. BRECK (1981). - Mercury in tropical marine organisms from North Queensland. Mar. Pollut. bull. 12 : 116-121.

DOI R.S. and J. UI (1975). - The distribution of mercury in fish and its form of occurrence. In : KRENKEL P.A. (Ed) Heavy Metals in the Aquatic Environment, Pergamon Press, pp. 193-221.

GREIG R.A., D. WENZLOFF and C. SHELPUK (1975). - Mercury concentrations in fish, North Atlantic Offshore waters- 1971. Pesticides Monitoring J. 9 : 15-20.

HORNUNG H., L. ZISMANN and O.H. OREN (1980). - Mercury in twelve Mediterranean trawl fishes of Israel. Environ. Intern. 3 : 243-248.

HORNUNG H., B.S. KRUMGALZ and Y. COHEN (1984). - Mercury pollution in sediments, benthic organisms and inshore fishes of Haifa Bay, Israel. Mar. Environ. Res. 12, 191-208.

MACCRIMMON H.R., C.D. WREN and B.L. GOTS (1983). - Mercury uptake by lake trout Salvelinus namaycush, relative to age growth and diet in Tadenac Lake with comparative data from other Precambrian Shield Lakes. Can. J. Fish. Aquat. Sci. 40 : 114-120.

OLSEN K.R., H.C. BERGMAN and P.O. FROMM (1973). - Uptake of methylmercuric chloride and mercuric chloride by trout. J. Fish. Res. Board Can. 30 : 1.293-1.299.

SINOVCIC G., M. GUTIERREZ and R. ESTABLIER (1980). - On the accumulation of mercury in the blood, liver, spleen and kidney of Halobatrachus didctylus (Schneider) and resulting haematologic, cytohaematologic and histopathologic alterations. Acta Adriat. 21 : 219-225.

HEAVY METAL DISTRIBUTION IN SURFACE SEDIMENTS FROM THE KALAMATA BAY, GREECE

by

S.P. VARNAVAS*, A.G.PANAGOS and G. LAIOS
* Department of Geology
University of Patras, Patras (Greece)
Department of Geology
National Technical University of Athens (Greece)

Abstract

Surface sediments have been analysed from Kalamata bay, Greece for Al, Si, Ca, Mg, Pb, Co, Ni and Cu. The sediments analysed from the port of Kalamata and from an area near the town which is affected by the discharge of domestic sewage and industrial effluents are anomalously enriched in Co and Cu suggesting their anthropogenic input. This is also demonstrated by the anomalously high Cu/Al and Co/Al ratios found at the outfalls of the domestic sewage and industrial effluents. The concentrations of Pb are generally low in the sediments examined except for two stations, where Pb has anomalously high values. Both stations are located at the mouths of streams, outside two villages suggesting the anthropogenic origin of Pb. The distribution of Si, Ca and Mg is largely controlled by the nature of the rocks present in the surrounding area. Compared with other Greek bays the Kalamata bay is less polluted in Pb and Cu and more polluted in Ni and Co.

*
* *

Introduction

The gulf of Kalamata is an open gulf with a maximum depth of 345 m (at its southern part). On the northern and western coasts of the bay the seafloor dips quite gently, whereas on its eastern coasts the seafloor slope is very steep (see figure I). A number of major rivers run into the bay transporting large quantities of fresh water and sediments.

The major pollution sources of the bay include domestic sewage and industrial effluents. The major contributor of domestic sewage is the town of Kalamata and a number of villages built either on the coasts or on the banks of the rivers which flow into the bay. Olive oil, olive kernel factories and slaughterhouses existing in the surroundings area (see figure I) also contribute to the pollution of the bay with their own effluents wich discharge untreated into the bay.

The aim of this paper delete is: a) To determine the distribution of some trace metals in the surface sediments of the gulf of Kalamata b) to deduce their source and mode of incorporation and c) to determine the level of metal pollution in the gulf compared whit other Greek bays.

Material and methods

For this study 31 sediment samples from the gulf of Kalamata were analysed for Al, Si, Ca, Mg, Pb, Cu, Co and Ni. The sediment samples were collected using a 30 cm3 Van Veen grab Figure I shows the location of the samples. Subsamples for analysis were taken from the upper 5 cm of the original sample and were stored in deep freeze. They were then dried using a Chemical Laboratory Instruments L.t.d. freeze-drier. When dry, the samples were ground to fine powder and stored in plastic phials.

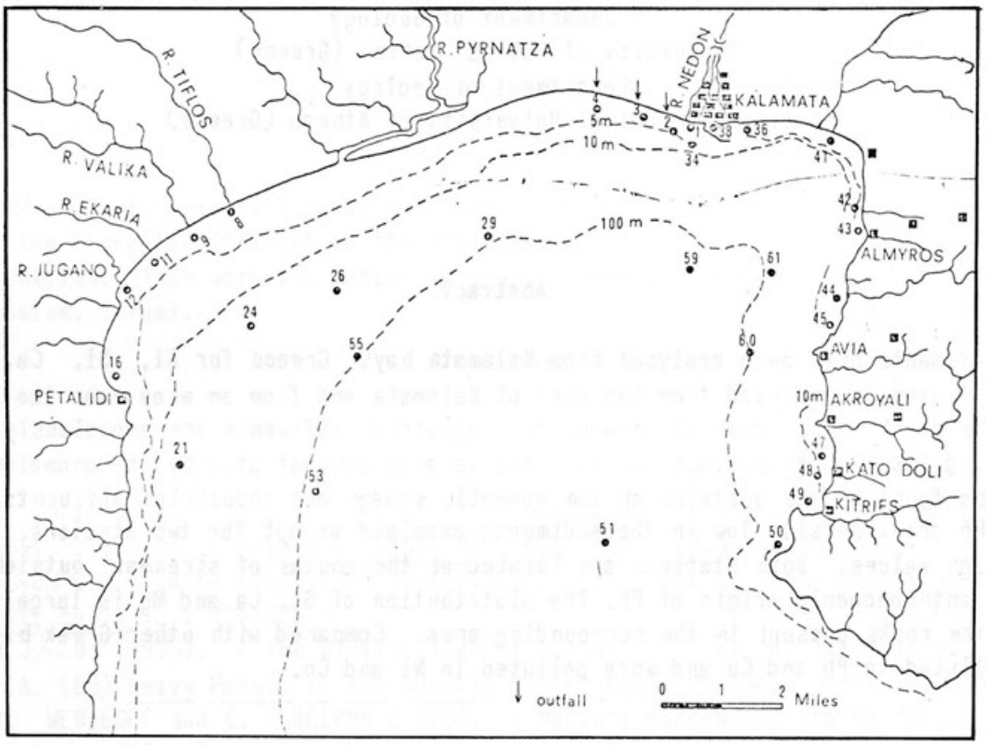


Fig. 1. - Area of study: bathymetry, location of grab samples and location of outfalls.

Chemical analysis was carried out on a Perkin-Elmer 403 atomic absorption spectrograph. For the determination of Si the samples were subjected to an HCl-HF-HBO3 attack, while for the other elements the samples were first subjected to an HF/HNO3/HClO4 attack and taken into 1M HCl. All samples were analysed in duplicates. Analytical precision was better than ± 10 per cent on the basis of replicate analyses. The accuracy was checked by analyses of U.S Geological Survey Standard rocks and internal Standards of red clay.

Results and discussion

The distribution of trace metals in marine sediments is largely controlled by the presence of some major element phases (i.e. clay minerals). Thus, before we examine the distribution of trace metals in sediments from the gulf of Kalamata, the distribution of some major elements, such as Al, Si, Ca and Mg is defined. Table I shows the concentrations of Al, Si, Ca, Mg, Pb, Cu, Co and Ni in the sediments analysed for this study.

SAMPLE No	Al %	SiO_2 %	Ca %	Mg %	Pb (ppm)	Co (ppm)	Ni (ppm)	Cu (ppm)
1	5.92	51.32	4.60	0.51	18	42	123	56
2	5.04	56.85	3.49	0.84	9	40	121	54
3	5.26	51.65	2.80	0.49	13	43	125	48
4	4.20	67.18	3.13	0	8	34	113	52
8	1.60	37.26	4.89	0	8	10	173	17
9	3.01	52.82	8.78	0	15	19	129	28
11	2.61	50.01	8.10	0	13	14	157	11
12	2.53	47.00	7.15	0	14	14	161	19
16	1.82	43.83	7.66	0	13	12	133	37
21	5.04	41.44	7.69	1.38	20	29	142	42
24	3.79	40.98	7.63	0.24	23	18	144	40
26	1.80	44.81	6.08	0	11	12	140	37
29	4.49	42.18	3.73	0.28	16	28	145	41
34	4.69	45.90	2.07	0	14	27	136	45
36	4.54	44.44	2.17	0	12	30	142	42
38	5.73	34.22	2.38	0	14	29	156	38
41	4.18	40.06	9.51	1.87	14	21	127	40
42	4.58	36.43	11.33	2.20	30	21	144	41
43	2.03	26.07	11.38	0.05	15	12	163	35
44	2.72	37.80	15.63	1.89	18	16	128	40
45	2.55	31.84	12.65	1.01	23	15	142	36
47	5.26	39.08	12.28	2.22	21	24	144	39
48	4.67	35.38	14.58	1.78	40	20	136	40
49	5.21	44.17	12.13	2.11	12	26	134	44
50	3.30	27.14	20.38	2.27	19	15	143	40
51A	6.28	40.91	9.49	2.76	12	30	142	38
53	6.62	43.41	6.01	2.14	14	37	144	45
55	6.26	47.71	6.28	2.01	15	36	141	46
59	7.92	44.14	4.14	2.18	10	37	148	51
60	7.08	41.19	5.71	2.50	11	33	145	47
61	7.31	44.00	6.14	2.45	12	33	147	47

Table 1. - Chemical analyses of Kalamata Bay surface sediments.

Aluminium, silica, calcium and magnesium

Aluminium varies between 1.60 and 7.92 per cent, its lowest concentrations being found along the coastline, while they increase towards the centre and deeper parts of the bays (see figure II). This distribution pattern suggests the incorporation of Al in the fine fraction of the sediments (the clays).

Silica varies between 26.07 and 67.18 per cent. Its highest values are found along the north and north-western coasts while its lowest values are found along the eastern coasts. Towards the centre of the bay intermediate values of SiO2 are observed.

The distribution of Ca and Mg is generally similar suggesting a common lithological derivation. They are both probably incorportted in carbonates. The zone with the highest content SiO2 is characterized by the lowest contents of Ca and Mg whereas the lowest contents of Ca and Mg are found along the eastern coast of the bay, where SiO2 shows its lowest values. An examination of the geology of the surrounding area justifies the distribution of the above elements, since east of the bay carbonate rocks (limestones) prevail while north and west of the bay silicate rocks (cherts and sandstones) prevail. The distribution patterns of Si, Ca and Mg show that these elements are associated with the coarse fraction of the sediments, being incorporated in detrital minerals which are derived from the weathering of the above-mentioned rocks.

Lead

Lead varies between 8 and 40 ppm, its highest values being found at station 48 at the mouth of a stream outside the village Kato Doli (see figure I). Similarly, a relatively high concentration of Pb

Table 2. - Low and upper levels of some metals in surface sediments of various Greek bays and other Mediterranean coasts.

			Pb ppm	Ni ppm	Cu ppm	Co ppm	Al %	Fraction	Reference
GREEK BAYS	KALAMATA BAY	Lower	8	113	11	10	1.60	Total	1
		Upper	40	173	55	43	7.92		
	NAVARINO BAY	Lower	9	115	30	9	1.21	Total	2
		Upper	59	151	66	27	6.31		
	SARONIKOS BAY	Lower			1	5		Total except for Cu which is the 0.05 NHCl + 0.025 NH_2SO_4-soluble	3.4
		Upper							
		Piraeus harbor			364	140			
		Athens Outfall			58	18			
		Elefsis bay			8	4			
	THERMAICOS BAY	Lower	13	35	4	7		0.5 NHCl-soluble	5
		Upper	228	160	37	20			
	PAGASITICOS BAY	Lower	5	3	0.5	2			
		Upper	28	114	16	14			
	PATRAICOS BAY	Lower	10	69	23	14	1.90	Total	6
		Upper	40	168	101	33	7.20		
OTHER MEDITERRANEAN COASTS	TRIESTE BAY	Lower	3	12				?	7
		Upper	67	59					
	ISRAEL COAST	Lower	4	2					8
		Upper	19	9					
	EGYPT COAST	Lower		13	5			Total	9
		Upper		99	77				
Average shale			20	68	45	19	8.0	Total	10

1:This study; 2:Varnavas and others 1984; 3: Grimanis and others (1977); 4: Voutsinou-Taliadouri (1981); 5:Chester and Voutsinou (1981); 6:Varnavas and Ferentinos (1983); 7: Majori and others (1976); 8: Roth and Hornung (1977); 9: Moussa (1982); 10: Turekian and Wedepohl (1961).

(30 ppm), compared with the rest of the bay, is found at station 42 at the mouth of a stream traversing the village Kato Verga. It is highly probable that the relatively high concentrations of Pb found at the above stations are due to anthropogenic input. In the rest of the bay, including the port and the area near the town of Kalamata which is affected by the discharge of domestic sewage and industrial effluents, the concentrations of Pb are very low.

Copper

Copper varies between 11 and 56 ppm. The highest concentrations of Cu are found in the port of Kalamata and in a zone near this town which is affected by the discharge of domestic sewage and industrial effluents. This would suggest anthropogenic input of Cu in the bay. The lowest concentrations of Cu are observed at the north-western coasts of the bay. at the mouths of a number of rivers showing that these rivers do not play any important role in transporting Cu into the bay. In the rest of the bay intermediate values of Cu are observed (see figure III).

An examination of the relationship between Cu and Al shows that there is generally positive correlation between these two elements, suggesting the association of Cu with the clays (see figure IV). It is highly probable that Cu is adsorbed on to the surface of clay minerals. The adsorption capacity of clays for metals and especially for Cu is known from elsewhere (VARNAVAS, 1979). Interestingly the Cu/Al ratios at stations 1,2 and 4 (at outfalls of domestic sewage and industrial effluents) are anomalously high compared with the rest of the stations confirming the imput of Cu from these outfalls.

Cobalt

Cobalt varies between 10 and 43 ppm. Generally its concentrations increase towards the centre of the bay, except for stations 1, 2, 3 and 4 where Co shows its highest concentrations (see figure V). As in the case of Cu there is a positive correlation between Co and Al, showing the association of Co with the clays (see figure IV). Similarly the anomalously high Co/Al ratios found at stations 1, 2, 3 and 4 suggest anthropogenic input of Co.

Nickel

Nickel varies between 113 ppm and 173 ppm. Its highest concentrations are found at stations 8 (mouth of river Tiflos), 11 (mouth of river Ekaria), 12 (mouth of river Jugano), 38 (port of Kalamata) and 43 (mouth of stream near the village Almiros where an olive oil factory exists). In the rest of the bay, there are no significant variations in the concentrations of Ni. Stations 1, 2, 3 and 4 which are characterized by elevated concentrations of Cu and Co have relatively low contents of Ni. There is no clear relationship between Ni and Al suggesting the association of Ni with other than clay minerals.

Conclusions

1) - The distribution of Si, Ca and Mg in the Kalamata bay sediments is controlled by the presence of silicate and carbonate rocks in the surrounding area The highest concentrations of Si are found along the north and north-western coast of the bay, while the highest contents of Ca and Mg

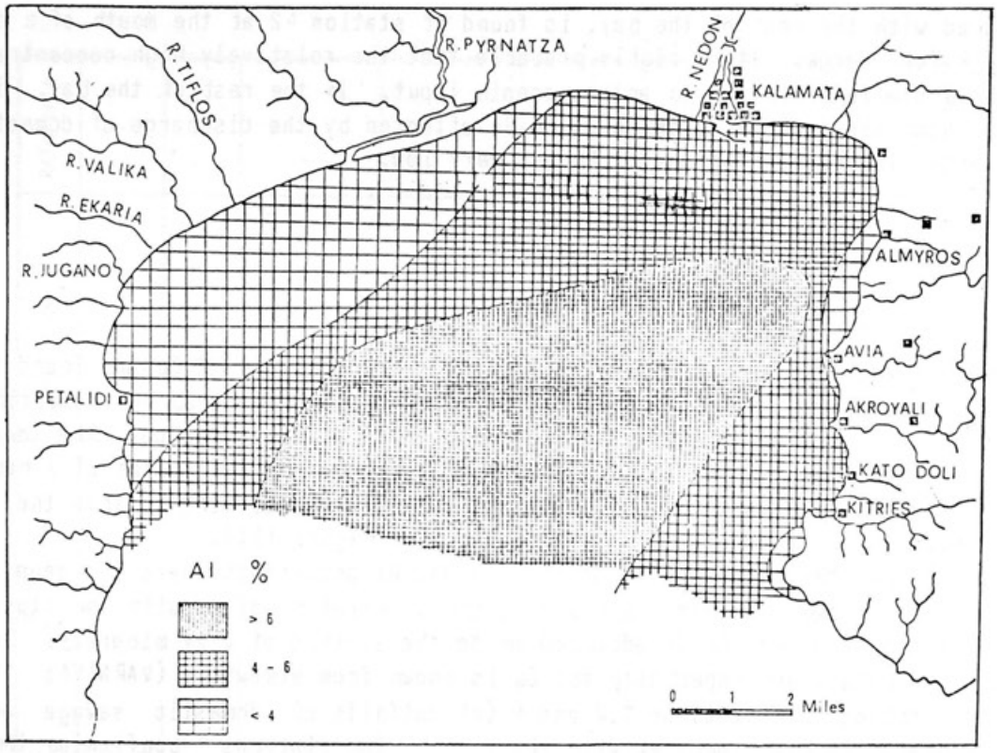


Fig. 2.- Distribution of Al in the Kalamata Bay surface sediments.

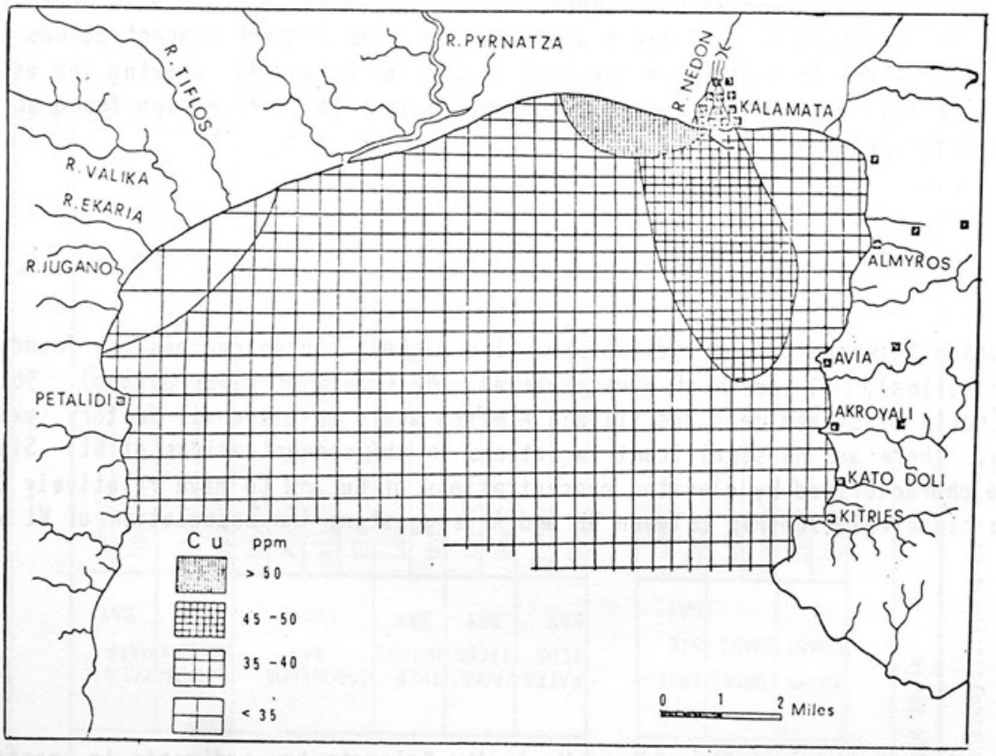


Fig. 3. - Distribution of Cu in the Kalamata Bay surface sediments.

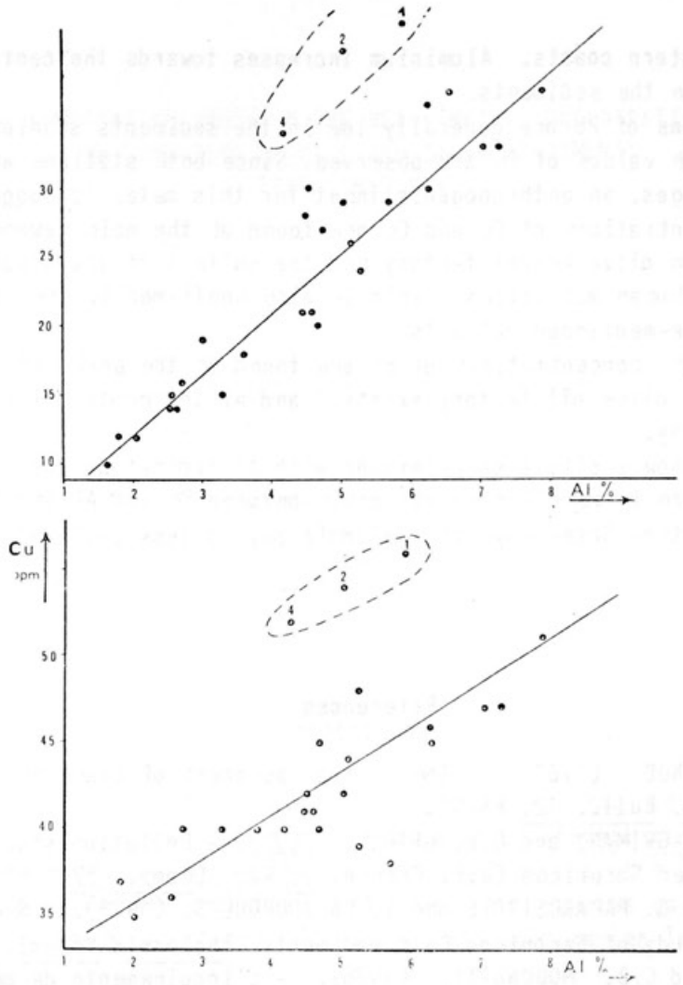


Fig. 4. - Correlation between Cu-Al and Co-Al in the Kalamata Bay surface sediments.

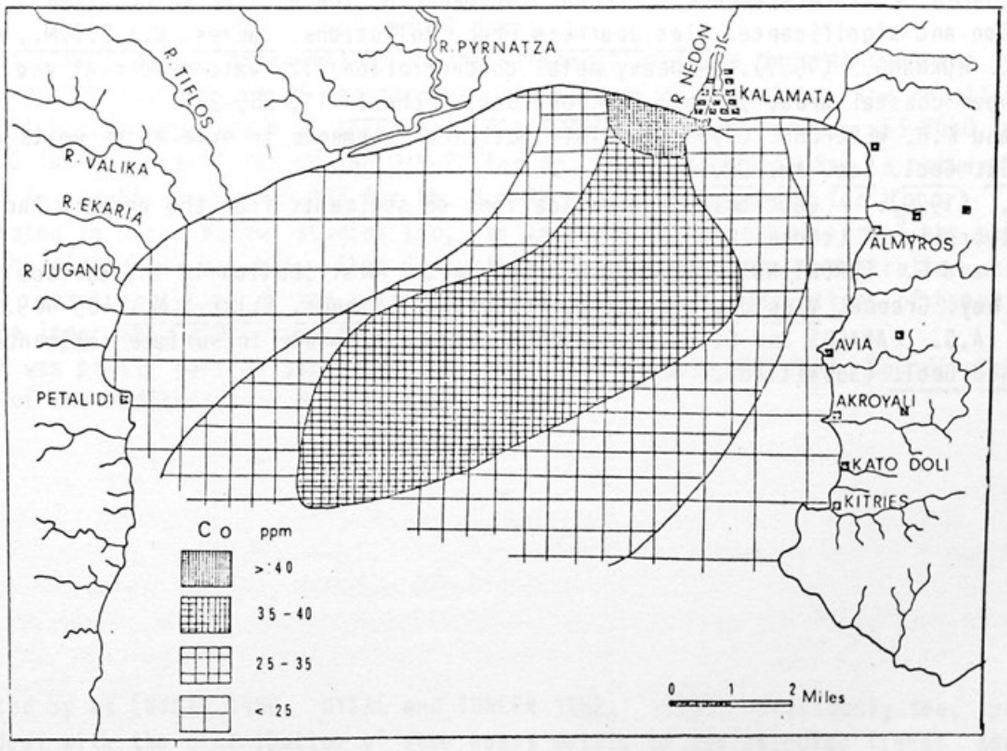


Fig 5. - Distribution of Co in the Kalamata Bay surface sediments.

are observed along the eastern coasts. Aluminium increases towards the centre of the bay reflecting the distribution of clays in the sediments.

2) - The concentrations of Pb are generally low in the sediments studied, except for two stations where anomalously high values of Pb are observed. Since both stations are located at the mouths of streams traversing villages, an anthropogenic input for this metal is suggested here.

3) - The highest concentrations of Cu and Co are found at the main sewage outfall of the town of Kalamata, the outfall of an olive kernel factory and the outfall of the slaughterhouses, suggesting input of these metals by human activities. This is also confirmed by the anomalously high Cu/Al ratios observed at the above-mentioned outfalls.

4) - Anomalously high concentrations of Ni are found at the port of Kalamata outside the village Almyros where an olive oil factory exists, and at the mouths of rivers flowing into the north-western part of the bay.

5) - Copper and Co show positive correlations with Al indicating their association with the clays minerals, whereas there is no clear relationship between Pb and Al and Ni and Al.

6) - Compared with other Greek bays the Kalamata bay is less polluted with Pb and Cu and more polluted with Ni and Co.

References

CHESTER R. and F. VOUTSINOU (1981). - The initial assement of trace metal pollution in coastal sediments. Mar. Pollut. Bull., 12, 84-91.

GRIMANIS A.P., M. VASSILAKI-GRIMANI and G.B. GRIGGS. (1977). - Pollution studies of trace elements in sediments from the upper Saronicos Gulf, Greece. J. Rad. Chemi., 37, 761-773.

HOPKINS T., A.P. GRIMANIS, G. PAPAKOSTIDIS and T. PAPADOPOULOS. (1973). - Neutron activation techniques in a pollution study of Saronicos Gulf sediments. Thalassia Yougsl. 9, 218-226.

MAJORI L., G. NEDOCLAN and G.B. MODONUTTI, (1976). - L'inguinamento da metalliacque, sedimenti, miltili e pesci dell'Alto Adriatico. Int. Rept., 1st. Ig. Univ. Trieste, p 1-103.

MOUSSA A.A., (1982). - Trace elements in recent sediments of the Nile delta continental shelf their accumulation and significance. VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M., 401-404.

ROTH I. and H. HORNUNG, (1977). - Heavy metal concentration ??? water sediment and fish from the Mediterranean coastal area, Israel. Environ. Sci. Technol 11, 265-269.

TUREKIAN K.K. and K.H. WEDEPOHL. (1961). - Distribution of elements in some major units of the earths crust. Bull. Geol. Soc. Am. 72, 175-191.

VARNAVAS S.P., (1979). - Geochemical investigations on sediments from the eastern Pacific Ph. D. Thesis University of London, 429 p.

VARNAVAS S.P. and G. FERENTINOS, (1983). - Heavy metal distribution in the surface sediments of Patraicas bay, Greece. VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M., 405-409.

VARNAVAS S.P., A.G. PANAGOS and G. LATOS. (1984). - Trace elements in surface sediments of Navarino bay, Greece, Env. Geol. (Submitted).

A COMPARATIVE STUDY ON THE HEAVY METAL CONCENTRATIONS IN SOME FISH SPECIES AND IN THE SEDIMENTS FROM IZMIR BAY

by

Hüseyin UYSAL and Sezginer TUNCER*
Ege University, Science Faculty, Biology Dept. Hydrobiology Div.
Bornova/Izmir
* Ege University, Hydrobiology and Water Resources Research Center
Iskele-Urla/Izmir
(Turkey)

Abstract

In this study some heavy metal concentrations (Cu, Zn, Fe, Pb, Cd, H_{gt}) in the common edible fish species: such as Serranus scriba L., Diplodus annularis L., Smaris alcedo Risso, Mullus barbatus L., Dentex gibbosus L., Pagellus erythrimus L., caught from polluted (Inner Bay) and unpolluted (Gülbahçe Bay) waters: and their environmental bottom sediment samples have been determined. Samples were analysed monthly from March to October 1983 using "AAS 1250 Varian Techtron Atomic Absorption Flame Spectrophotometer".

According to our results present metal concentrations in the above mentioned species varied in between: Cu 0,014-2,798 µg/g, Zn 0,887-10,207 µg/g, Fe 0,702-17,896 µg/g, Pb 0,0-3,152 µg/g, Cd 0,0-0,430 µg/g, H_{gt} 0,022-0,463 µg/g, on wet weight basis.

Levels of heavy metal concentration in the sediments are more important in the Inner Bay than Gülbahçe Bay due to a higher rate of uncontrolled discharges in Inner Bay. The results are given in ppm (dry weight basis) and varied in Inner Bay in between 2100-4400 for Fe, 53-8660 for Zn, 33-866 for Cu, 40-280 for Pb, 0,2-40 for Cd and 0,4-22 for Hg, in the Gülbahçe Bay in between 770-32000 for Fe, 16-135 for Zn, 12-56 for Cu, 35-183 for Pb, 1,4-14 for Cd and 0,4-1,9 for H_{gt}.

As indicated in our previous studies too, an increase in the urbanisation has resulted in an increase in the discharges as well. This is further strengtned by the uncontrolled waste disposal. Due to heavy metal pollution of the waters of the bay higher concentrations of heavy metals were observed in the Inner Bay, where the industry has developed more.

This work was partly carried out under the frame work of both UNEP/FAO Coordinated Programme on the Pollution of the Mediterranean MED-POL Phase II.

*
* *

Introduction

As reported by us (UYSAL 1980, UYSAL and TUNCER 1982, 1983), previously too, present studies once again deal with the distribution of some heavy metals in the selected fishes adding now the bottom sediments of the specimens from different polluted areas of the Izmir Bay.

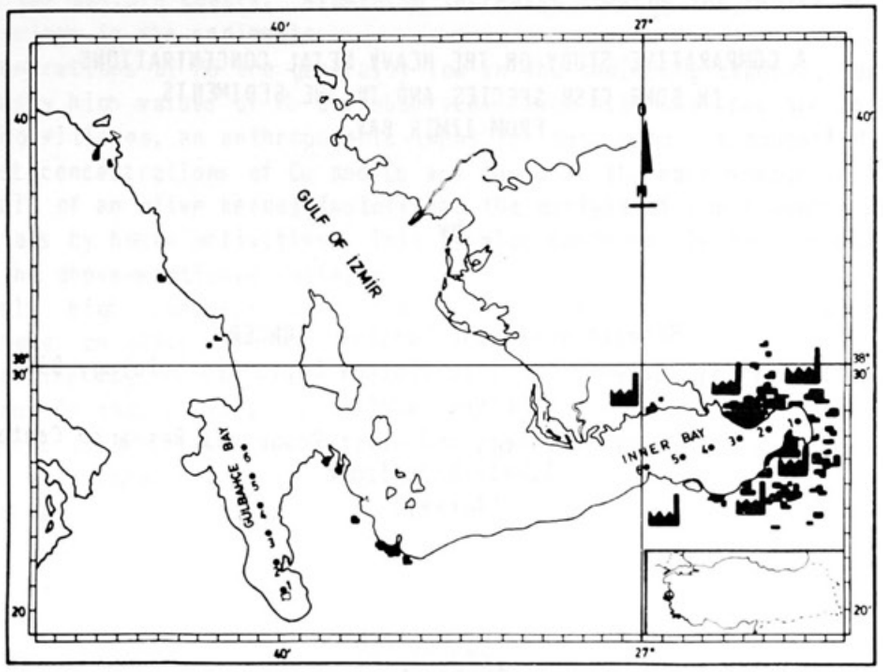


Fig. 1. - Localisations of stations in the Izmir Bay.

Table I. - Variation of heavy metals in some fish from the Izmir Bay (µg/g F.W.)

Species	Nomb. of Species	T. Length (mm)	Localite	Cu	Zn	Fe	Pb	Cd	Hgt
	8	210		0,690	5,140	11,950	1,550	0,090	0,130
S. scriba	4	180	Gülbahçe	0,680	5,310	24,100	2,300	0,050	0,320
	8	150	bay	0,700	5,600	11,600	1,920	0,090	0,270
D. gibbossus	3	800	Gülbahçe bay	0,880	4,500	31,430	2,360	0,170	0,375
P. erythrinus	2	195	Gülbahçe bay	0,670	3,500	4,100	0,660	0,080	0,185
	3	140	Gülbahçe	0,550	6,150	10,470	2,200	0,160	0,200
S. alcedo	4	160	bay	1,260	7,220	10,200	2,000	0,090	0,190
	6	135	Gülbahçe	0,160	6,030	11,600	2,090	0,180	0,145
D. annularis	8	170	bay	0,670	9,970	12,560	2,260	0,190	0,235
	2	130	Inner.	2,105	16,970	15,350	1,640	0,430	0,440
D. annularis	3	160	bay	1,030	7,443	12,640	2,510	0,200	0,110
M. barbatus	3	155	Gülbahçe bay	0,950	5,980	5,800	1,180	0,100	0,060
	3	130	Inner	0,480	3,740	10,168	1,900	0,130	0,200
M. barbatus	4	160	bay	0,600	5,900	11,675	1,310	0,050	0,040

Due to a gradual increase of the industrial effluents and urban, touristic agricultural etc. sewages, important quantities of the chemicals are being discharged especially in to the Inner Bay waters.

The chemicals and the metals are being transported to the marine environment in the particulate or soluble states.

The marine organisms accumulate the chemicals as well as the heavy metals in large quantities in their body as compared to their surroundings in the sea water (UYSAL and TUNCER, 1982).

This results in an increase in the heavy metal levels of these organisms as well as in the fish through feeding, adsorption and absorption, however, accumulation quantity depends upon the age, habitat and feeding behaviour (UYSAL and TUNCER, 1982).

Heavy metal concentrations in fish are of vital importance for public health in their locality. However, as indicated in aur previous studies to the recent observation showed that, the resident demersal fish species reflect heavy metal level better than the pelagic fish, especially pelagic fish do not necessarily reflect the increased metal input in the environment (GRIMANIS et al., 1978; UYSAL 1980). On the other hand sediments can play an important role in the distribution of toxic substances in the marine biota (BALKAS et al., 1978). Because, levels of heavy metals in the sediment, water and biota serve as an indication of the quality of life fauna. As such, the aim of this study was to investigate the present status of the distribution of heavy metals in the economically important fish populations and their environmental water and bottom sediments in different ways and compare with those of the other coastal areas of Turkey and the Mediterranean.

The data obtained depicts that, it is a must to continue this type of work, because of its importance in the human food consumption. We have thus persuid this work in two different places in our bay.

This work was carried out in the frame work of FAO (GFCM)/UNEP Co-ordinated programme on the MED-POL Phase II.

Material and method

Fishing was carried out in two areas namely Inner Bay and Gülbahçe bay as indicated in the figure 1, the sediment samples were also collected from these areas at the same time.

The species of Serranus scriba L., Dentex gibbosus L., Pagellus erythrinus L., Smaris alcedo Risso, Diplodus annularis L., Mullus barbatus L. were caught by fisherman's nets during the months from March till October 1983 as transported daily to the laboratory. These were kept in a deep freeze (-21° C) until analysis and samples prepared according to (BERNHARD 1976) as given in FAO FIRI/T158 report. The composite edible fillet samples of fish were weighed and digested with conc. HNO_3:$HClO_4$(5:1)(Merck) under reflux and filtered.

Sediments were collected every month with a benne "Orange peel" of capacity 4,5l. and with a benthic benne from the different stations and depths, then stocked in the plastic bags (Fig. 1). These were acidified with HNO_3(Merck) and stocked (-25° C) on the vessel "Hippocampus". Each sample was ovendried at 60° C for 24 h. and then sieved, using a mesh 160 μ. From the dried sediment samples a quantity of 0,1-0,3 g(<160µm) was oxidized with conc. HCl:HNO_3(3:1) under the reflux and then filtered.

All samples were diluted with bidistilled water and assayed using a AAS 1250 Varian Techtron Atomic Absorption Flame Spectrophotometer. Mercury analysis was carried out by flameless cold vapour technique using Model 65 As/Se/Hg analyses kit (BRODIE, 1979).

Intercalibration fish homogenate samples (MA-A-2 obtained from the IAEA, Monaco Laboratory) were used as a control for the analytical methods. Results found were in good agreement with those cited in the literature

Results and discussion

In fish species:

The concentrations of some heavy metals (Cu, Zn, Fe, Pb, Cd, H_{gt}) in the tissues of the mentioned species were determined separately from Gülbahçe and Inner Bay. Heavy metal analysis was done using specimens with different total lenghts (in mm).

The variations of heavy metals in different species and average concentrations are given in Tables I and II respectively.

As indicated in our previous studies too (UYSAL, 1980; UYSAL and TUNCER, 1982), it can be seen from the tables that there are differences in the metal concentrations according to the lenght of the species and localities.

The mean concentrations given in figure 2 show that, average concentrations of the metals in the above mentioned fish from these two bays. The heavy metal concentrations of D. annularis is slightly higher than M. Barbatus (VASILIKIOTIS et al., 1982). On the other hand according to our present results in the both species from the Inner Bay are higher than the Gülbahçe Bay.

Table II. - Average heavy metal concentrations in some fish of the Izmir Bay (µg/g F.W.)

Species	Localite	Cu	Zn	Fe	Pb	Cd	H_{gt}
S. scriba	Gülbahçe bay	0,700	5,300	15,900	1,900	0,080	0,240
D. gibbossus	Gülbahçe bay	0,880	4,500	31,430	2,360	0,170	0,375
P. Erythrinus	Gülbahçe bay	0,670	3,500	4,100	0,660	0,080	0,185
S. alcedo	Gülbahçe bay	0,900	6,700	10,300	2,100	0,130	0,200
D. annularis	Gülbahçe bay	0,400	8,000	12,000	2,180	0,190	0,190
	Inner bay	1,570	12,200	14,000	2,080	0,320	0,280
M. barbatus	Gülbahçe bay	0,950	5,980	5,800	1,180	0,100	0,060
	Inner bay	0,540	4,800	5,670	1,600	0,090	0,120

However all the species analysed are not caught from both the bays, because of increased pollution in the Inner Bay. But in the species caught from two sides a difference in the resistance to the pollution effects was observed and species like M.barbatus and D.annularis can be accepted as species resistant to pollution. Because these species as well as Engraulis encrasicholus seem to be much more adapted to the environmental conditions of the polluted bay waters than the others species studied (UYSAL and TUNCER, 1983). It has been reported that the amount of vital eggs in anchovy captured from the Inner Bay is 98.8 per cent (MATER, 1979).

The correlation between the concentration of the heavy metals and the total lenght was studied applying standard statistical methods on the available data for S.scriba. No correlation was found as regards the mentioned parameters for Cd, Cu, Pb, Zn, Fe (Fig. 3), but significant negative correlation was observed for mercury ($r = -0,607$). In this studies the equation of $Y = -0,038 + 0,935$ has been used for correlation statistics (Fig. 4).

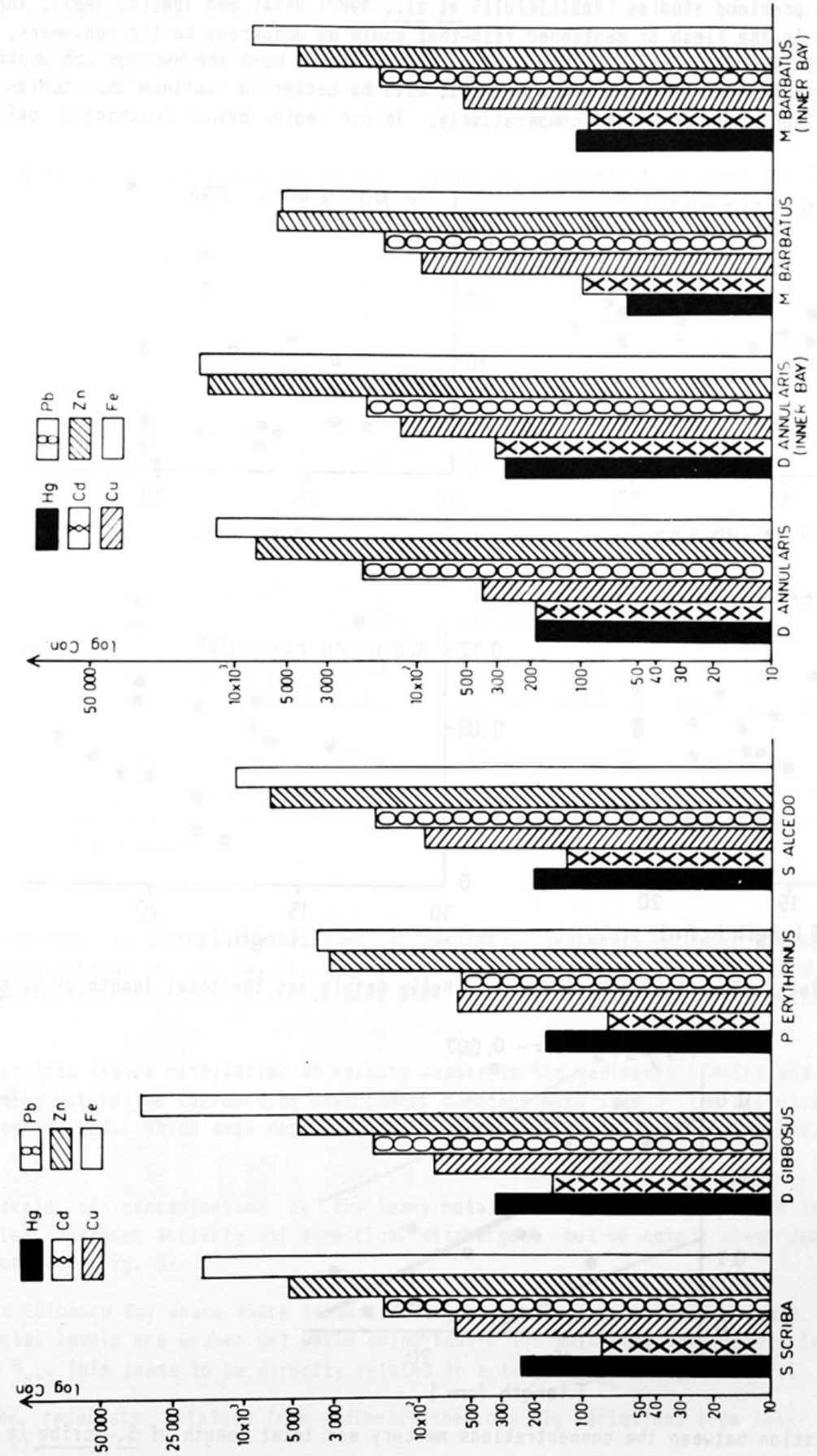


Figure 2. - Mean heavy metal concentration in some fish of the Izmir Bay (µg/kg F.W.).

As indicated the previous studies (VASILIKIOTIS et al., 1982: UYSAL and TUNCER, 1982), there are still no toxic levels in the flesh of mentioned fish-that could be dangerous to the consumers, as the values lie just within the range given by WHO (1973). On the other hand the average consumption of this fish in our region is generally low. Therefore it will be better to continue the studies on the pollution effects on food chain organisms comperatively. In our region before reaching a definitive conclusion.

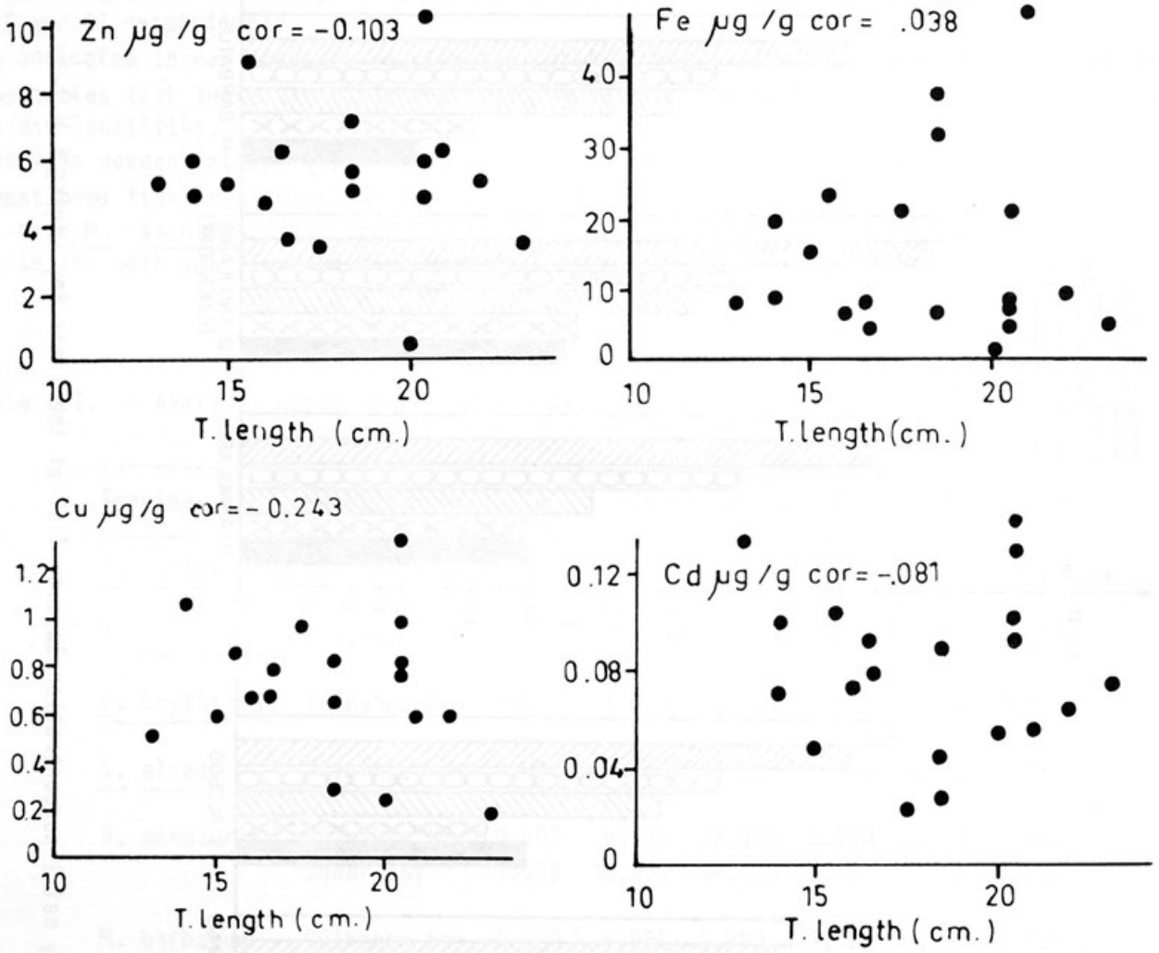


Figure 3. - Correlation between the concentration of heavy metals and the total length of S. scriba.

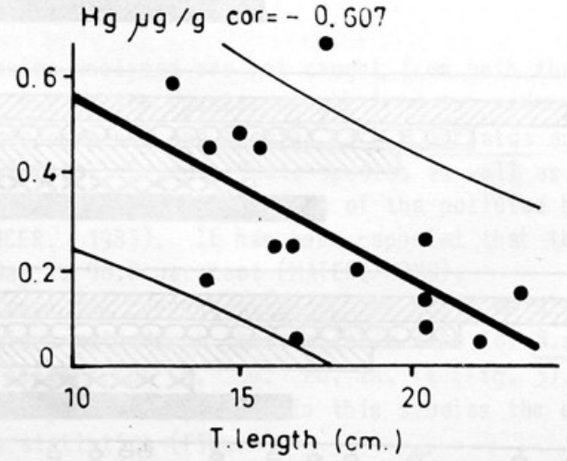


Figure 4. - Correlation between the concentrations mercury and total length of S. scriba (n = 2)

In sediments:

Table III presents the values obtained from the superficial sediments of Inner Bay and Table IV from the Gülbahçe Bay. The average concentrations into stations are shown in Fig. 5.

Table III. - Concentrations of heavy metals in sediments from Inner Bay (ppm/dry weight)

St.	Depth (m)	Fe(10^2)	Zn	Cu	Pb	Cd	H_{gt}
1	8	257±69* (130-390)	363±55 (242-477)	78±25 (51-152)	135±53 (58-280)	10-10,7 (0,2-40)	5±5,5 (0,5-22)
2	11	274±63 (130-300)	315±89 (118-477)	206±214 (33-866)	147±37 (107-245)	4,4±2,4 (2,3-11)	2,36±1,0 (0,6-4,1)
3	15	270±85 (190-330)	368±162 (250-860)	158±150 (50-620)	123±31 (69-216)	7,5±8,3 (1,8-33)	1,40±0,6 (0,40-2,5)
4	17	289±63 (140-420)	259±27 (220-305)	71±12 (40-88)	110±40 (45-234)	3,5±1,3 (1,3-6,4)	1,9±1,6 (0,4-6,8)
5	13	230±99 (21-440)	220±19 (169-242)	78±21 (50-139)	115±41 (83-234)	4,0±3,0 (0,9-12)	1,9±1,7 (0,4-6,8)
6	12	193±47 (130-297)	135±47 (53-238)	37±9 (14-52)	75±31 (40-143)	2,4±0,5 (0,6-3)	1,3±0,6 (0,4-3,0)

* $p < 0,005$

According to the results obtained (96 samples), sediments of Inner Bay show a contamination of the metals such as Fe, Zn, Cu, Pb, Cd and H_{gt}. The level decreases from Fe to H_{gt}. We have noted that in deeper waters dissolved oxygen is very weak and often almost null, particularly during the summer time.

For this reason methylation of mercury occurs in the sediments (TUNCER and UYSAL, 1983). The H_2S gas coming out in the surrounding environment combines with some of the dissolved metals particularly Hg, forming HgS, which gets deposited on the surfaced deeper layers (JERNELOV, 1972; YIGIT et al. 1982).

Levels of contamination by the heavy metals in the Inner Bay is more important due to the factories, harbour activity and domestical discharges, but we note a clear decrease at the exit of the same area (Fig. 5).

In Gülbahçe Bay where there is no industry and contamination is the least. At the bottom of the bay metal levels are weaken but while going toward the main sea, the levels increase except for the Cd and H_{gt}. This seems to be directly related to a transfer of the pollutants.

The resultats obtained from sediments showed a big variations from Inner Bay to Gülbahçe Bay (Table V).

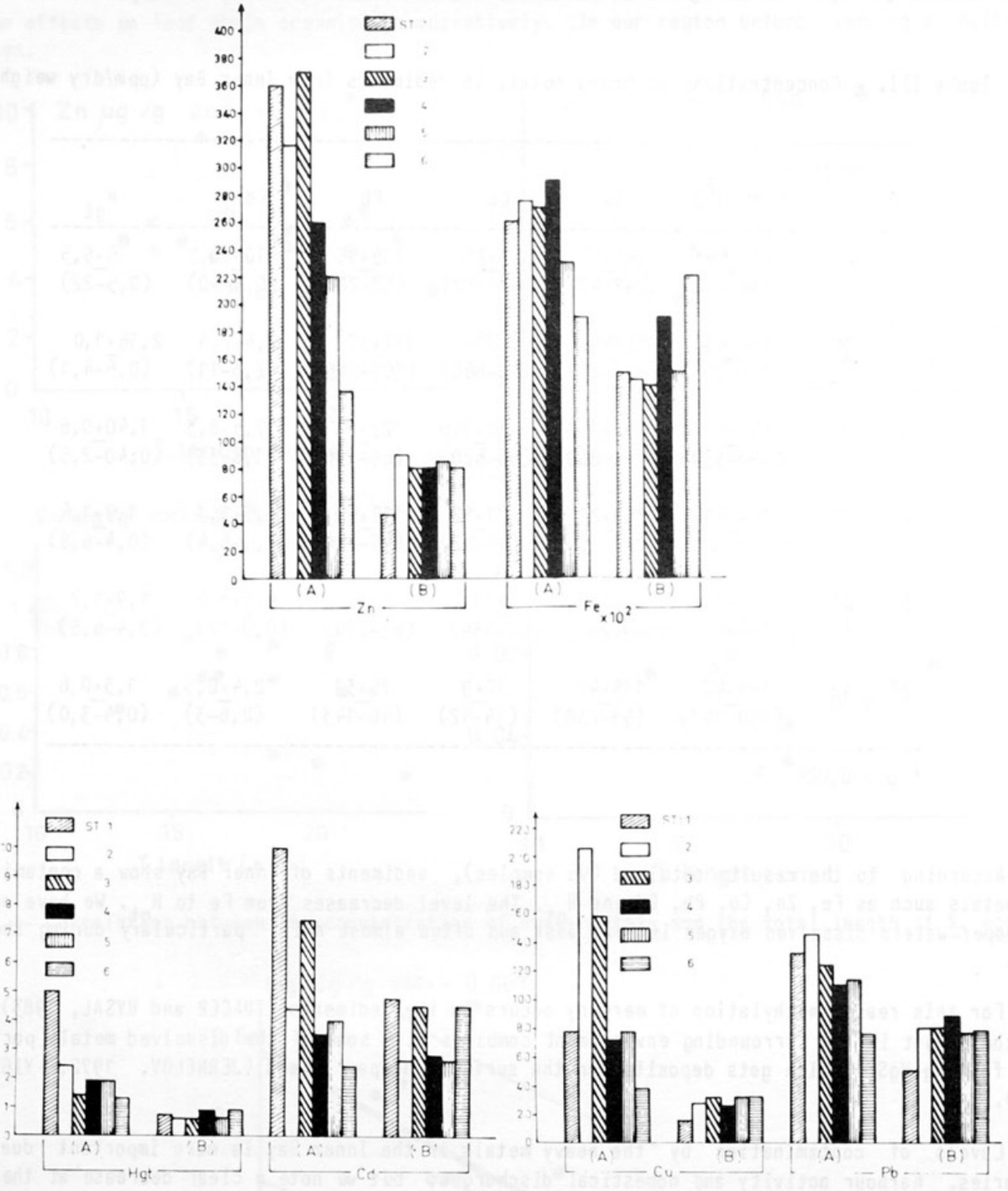


Figure 5. - Average concentrations of heavy metals in sediments ppm (dry weight)
(A) Inner bay
(B) Gulbahçe bay.

Table IV. - Concentrations of heavy metals in sediments from Gülbahce Bay (ppm/dry weight).

St.	Depth (m)	Fe(10^2)	Zn	Cu	Pb	Cd	H_{gt}
1	11	153±50* (99-240)	44±19 (16-70)	16±3 (12-19)	50±9 (35-60)	4,8±5,0 (1,4-14)	0,5±0,1 (0,3-0,7)
2	14	146±56 (83-260)	88±25 (63-135)	28±15 (14-54)	80±37 (47-155)	2,6±0,6 (1,8-3,4)	0,6±0,2 (0,4-0,9)
3	15	143±64 (10-240)	80±8 (65-94)	32±8 (24-40)	80±29 (45-145)	4,5±3,5 (1,7-13)	0,6±0,5 (0,2-1,9)
4	22	189±49 (13-220)	79±22 (23-95)	27±14 (13-56)	87±42 (43-183)	2,8±0,6 (1,8-3,7)	0,9±0,3 (0,4-1,3)
5	27	150±63 (7,7-230)	85±13 (67-106)	32±13 (13-55)	75±26 (39-128)	2,6±0,8 (1,8-4,2)	0,6±0,2 (0,4-1,0)
6	28	223±66 (150-320)	79±21 (46-110)	33±4 (35-48)	78±24 (55-132)	4,5±1,4 (1,9-6,1)	0,9±0,3 (0,4-1,4)

* $p < 0,005$

Table V. - Average concentrations of heavy metals from Izmir Bay (ppm/dry weight).

Localite	H_{gt}	Cd	Pb	Cu	Zn	Fe(10^2)
Inrer Bay	2,31	5,23	117,5	104,7	276,7	252,2
Gülbahçe Bay	0,70	3,63	75,0	28,0	75,8	167,3
Gulf of Trieste (MAJORI et al. 1978)	8,74	2,53	126,5	43,7	205,2	180,0
Patraikos Bay (VARNAVAS and FERENTINOS, 1982)	-	-	(10-40)	(23-101)	(275-430)	-
Thermaikos Gulf (VOUTSINOU-TALIADOURI, 1982 (< 0,45 µm))	-	(0,45-8,5)	(35-310)	(40-60)	(90-170)	(350-550)

This study allows us to follow the evolution of the pollution by the metals in the sediments, but it is necessary to complete the study by determining the organic carbon, amount of metals in the water and the organisms. In addition a detailed study of the contamination in the sediments in relation to their granulometric nature should also be under taken.

References

BALKAS I.T., I. SALIHOGLU, G. TUNCEL, S. TUGRUL, and G. RAMELOW (1978). - Trace metals and Organochlorine residue content of Mullidae Family Fishes and Sediments in the Vicinity of Erdemli (Içel), Turkey IVes Journées Etud. Poll. Antalya, C.I.E.S.M. 159-163.

BERNHARD M. (1976). - FAO Fisheries Technical Paper No : FIRI/T158, Rome.

BRODIE K.G. (1979). - Analysis of arsenic and other trace elements by vapour generation. Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, March 5-9, 1979.

GRIMANIS A.P., C. PAPDOPOULOU, D. ZAFIROPOULUS, M. VASSILAKI-GRIMANI and N. TSIMENIDIS (1978). - Pollution Monitoring of Eleven Trace elements in three Marine Organisms from Saronikos Gulf, Greece. IVes Journées Etud. Poll. Antalya C.I.E.S.M. 233-234.

JERNELOV A. (1972). - "Factors in the Transformation of Hg to CH_3Hg", in "Environmental Hg contamination", Ann Arbor Sc. Publ. Ann Arbor, Michigan.

MATER S. (1979). - Pollusyonun Izmir Korfezinde teleost baliklarin yumurtalari üzerine etkileri, TUJJB Bülteni, 11. 71-76.

MAJORI L., G. NEDOCLAN, G.B. MODONUTTI and F. DARIS (1978). - Levels of metal pollutants in sediments and biotaa of the Gulf of Trieste: a long term survey. IVes Journées Etud. Poll. Antalya, C.I.E.S.M. 237-243.

TUNCER S., H. UYSAL (1983). - The distribution of total mercury in sediment samples of Izmir Bay and its accumulation in soles (Solea vulgaris) Mabahiss/John Murray International Symposium on Marine Science of the North West Indian Ocean and adjacent Waters, 3-7 Sept. 1983, Alexandria (in press).

UYSAL H. (1980). - Levels trace elements in some foodchain organisms from the Aegean Coasts. Ves Etud. Poll., Cagliari C.I.E.S.M. 503-512.

UYSAL H., S. TUNCER (1982). - Levels of Heavy Metals in Some Commercial Food Species in the Bay of Izmir (Turkey). VIes Journées Etud. Poll. Cannes, C.I.E.S.M. 323-327.

UYSAL H., S. TUNCER (1983). - Accumulation of Some Heavy Metals (Cu, Mn, Zn, Fe, Cd, H_{gt}) in certain organ and tissues of Scad (Trachurus trachurus L.) and (Anchovy Engraulis encrasicholus L.) in the Bay of Izmir. Mabahis/John Murray International Symposium on Marine Science of the North West Indian Ocean and Adjecent Waters. 3-7 September 1983, Alexandria. (in press).

VASILIKIOTIS G., K. FYTIANOS, A. ZOUTOU (1982). - Heavy metals in marine organism of the North Aegean Sea, Greece. VIes Journées Etud. Poll. Cannes, C.I.E.S.M. 303-306.

VARNAVAS S.P., G. FERENTINOS (1982). - Heavy metal distribution in the surface sediments of Patraikos Bay, Greece. VIes Journées Etud. Poll. Cannes, C.I.E.S.M. 405-409.

VOUTSINOU-TALIADOURI, F. (1982). - Metal concentration in polluted and unpolluted Greek sediments: a comperative study. VIes Journées Etud. Poll., Cannes C.I.E.S.M. 245-259.

WHO (1973). - Health Hazards of the Human Environment, Geneva, Switzerland.

YIGIT V., H. OZBAL, A. CERITOGLU, N. MUFTIGIL, F. AKSIRAY (1982). - Izmit Korfezindeki agir metal kirliliginin su ve dip çamuru ortamindaki dagilimi ile bazi canlilardaki birikimi. TUBITAK Marmara Bilimsel ve Endüstriyel Arastirma Enstitüsü, 62.1-49.

THE APPLICATION OF DIFFERENT ANALYTICAL EXTRACTION METHODS IN THE STUDY OF SEDIMENTS IN A POLLUTED LAGOON

by

A. CONTU, G. SARRITZU and M. SCHINTU
Istituto di Igiene - Università degli Studi di Cagliari
Via Porcell, 4 - 09100 Cagliari (Italy)

Abstract

The S. Gilla lagoon (South Sardinia) receives high levels of heavy metals especially Hg, Pb and Cr, originating from a chloro-alkali plant, a treatment plant for lead and zinc minerals and urban wastes from Cagliari and its surroundings.

In an effort to further investigate heavy metal pollution the sediments were treated with 4.0N HNO3-0.7N NCl; 0.5N HCl; 1 N NH2OH.HCl+25 % CH3COOH; 0.05N EDTA, to show the affinity of the metals to the different components of the sediment.

The results showed that some metals (Pb, Cr, Cu) can be remobilized in the environment.

The 0.5N HCl leachable concentrations can also be used as an index of anthropogenic inputs of metals into the lagoon.

*
* *

Introduction

Previous investigations on total levels of some heavy metals (Pb, Hg, Zn, Cr, Cu, Cd) (G. SARRITZU et al., 1982: CONTU et al., 1983) in the sediments of S. Gilla lagoon have shown that area is generally heavily polluted.

a) antropogenic inputs: combined untreated domestic and industrial wastes from the Cagliari zone are still discharged into the laggon;

b) natural inputs: the Mannu and Cixerri rivers, which drain a wide catchment area have transported a large quantity of metals into the lagoon.

The aim of our study is to investigate the association of some metals (Pb, Hg, Cr, Cu) with the main components of sediments, by means of the different extraction methods proposed by AGEMIAN and CHAU (1977) in order to measure the antropogenic and mineralogical fractions in the sediments and therefore the extent of the metals in the biological cycles.

Materials and methods

Fourteen samples of sediment were taken by a gravity corer from seven sampling stations (figure I) in January and April 1981, within a wider research programme aimed at defining the level of chemical microbiological and toxicological pollution in the lagoon (A. CONTU et al., 1979). The superficial layer of sediments (0-1 cm) was analysed. Samples were air-dried, ground in an agate

mortar and sieved to 100 mesh to normalize all samples, as the particle size of the sediments strongly influences the metal content (OLIVER and AGEMIAN, 1973).

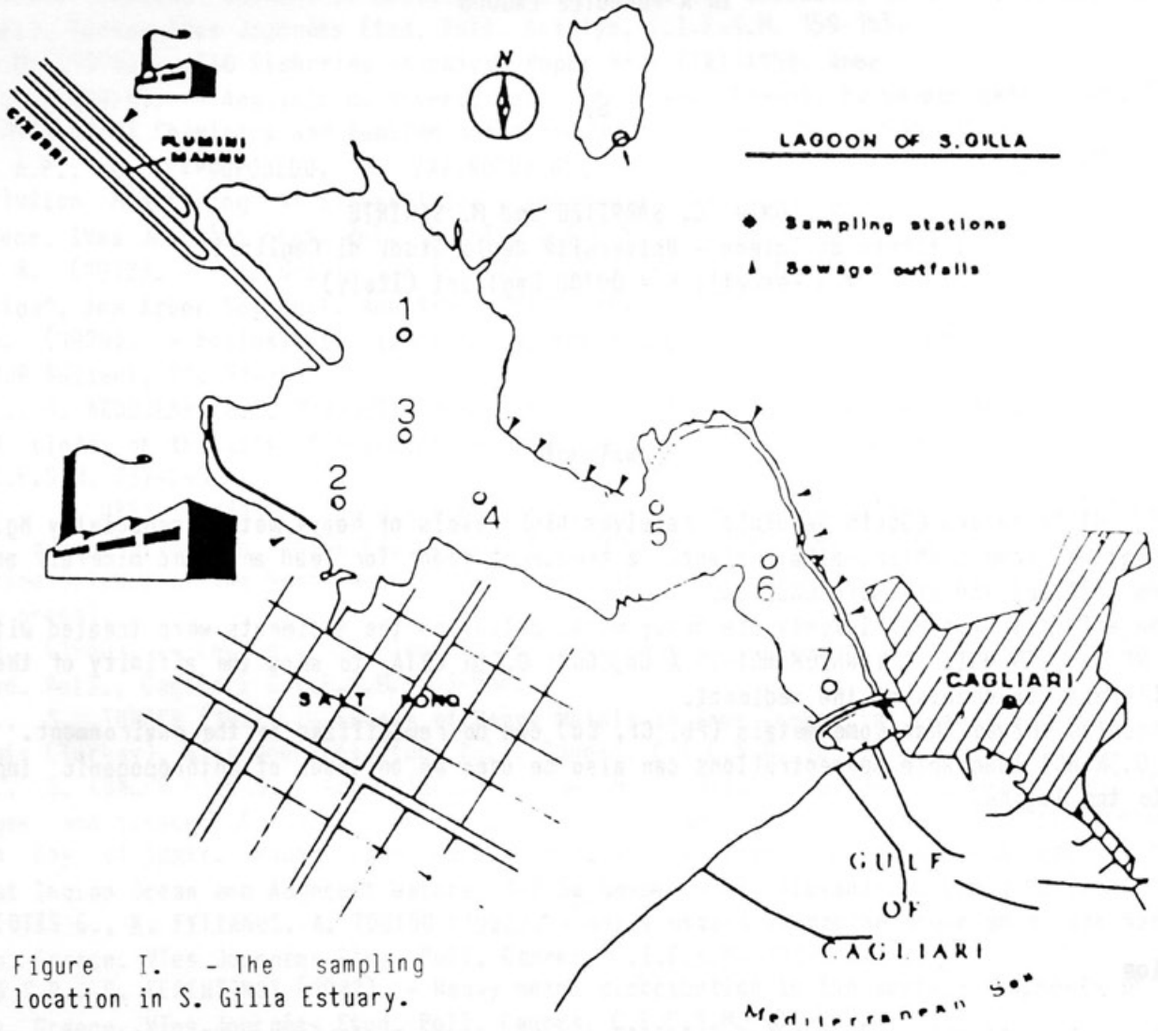


Figure I. - The sampling location in S. Gilla Estuary.

Subsamples of the 100 mesh fraction were treated according to AGEMIAN and CHAU (1977) with the following five reagents:

A) a mixture of HF/HClO4/HNO3 in an acid digestion bomb, to liberate the total content of metals in the sediment;

B) 4.0N nitric-0.7N hydrochloric acid solution. This mixture would be extracted adsorbed metals, precipitated salts, oxides and hydroxydes of Fe and Mn, and most organically complexed metals; it may attack some silicates;

C) 0.5N hydrochloric acid solution (cold attack at room temperature for 24 hours). This should remove weakly-bonded and adsorbed metals as well as the metals complexed at the organic matrix;

D) 1N hydroxilamine hydrochloride + 25 % acetic acid solution. This reducing mixture extracted ferromanganese minerals, carbonate minerals and adsorbed trace metals from sediments. This technique does not dissolve sulphide minerals or organic complexes;

E) 0.05 EDTA solution at pH 4.8. Some authors (BRADSHAW et al., 1974; MAYNARD and FLETCHER, 1973; BEAVINTON, 1975) used this strong complexing solution to extract Cu from polluted samples of soil and sediment.

A Perkin Elmer 450 atomic absorption spectrophotometer with air acetylene flame was used to determine concentration of Pb, Cr, Cu. Levels of Hg were determined by the cold vapour method.

We have checked our procedures for trace metal determination by analysing a NBS standard reference "River Sediment". We carried out five replicates of the extractions on some samples of sediments. Employing all reagents, relative standard deviation is within 10 per cent for various ions.

Metal	Reagents *	Sample													
		1		2		3		4		5		6		7	
		a **	b ***	a	b	a	b	a	b	a	b	a	b	a	b
Hg	A	7.00	6.00	95.00	12.40	6.50	9.00	9.40	12.00	5.50	9.20	12.00	8.60	8.70	6.50
	B	4.09	1.01	85.50	10.40	1.63	6.68	1.73	1.36	1.36	4.54	6.59	1.23	2.95	1.70
	C	0.33	0.39	7.64	0.59	0.10	0.52	0.08	0.14	0.13	0.61	0.21	0.21	0.23	0.23
	D	0.13	0.12	3.81	0.11	0.16	0.31	0.72	0.45	0.63	0.68	0.45	0.23	0.54	0.21
	E	0.07	0.08	0.14	0.08	0.20	0.23	0.09	0.12	0.10	0.15	0.14	0.12	0.13	0.07
Pb	A	330	120	1100	630	110	320	160	220	195	750	760	105	630	290
	B	295	90	860	230	90	250	110	160	130	550	560	90	430	200
	C	241	82	696	196	68	194	96	140	104	470	490	64	374	182
	D	310	100	1060	300	100	300	150	90	160	730	710	70	565	260
	E	210	90	730	200	80	230	80	150	190	560	570	50	450	190
Cr	A	60	50	500	120	75	100	90	60	65	140	145	80	180	75
	B	22	15	330	38	18	40	12	20	20	89	68	18	158	28
	C	28	8.0	320	26	6.0	28	6.5	11	10	72	66	7.0	76	23
	D	31	13	390	50	8	40	11	15	20	95	90	14	110	32
	E	10	6.2	184	32	6.0	21	6.0	9.2	9.0	61	20	46	60	25
Cu	A	70	30	160	110	60	100	120	60	120	140	140	30	75	35
	B	37	23	108	40	54	65	36	35	44	49	48	24	50	31
	C	22	12	71	27	13	34	18	18	13	28	27	10	25	21
	D	42	14	138	43	17	72	27	22	15	20	50	14	30	31
	E	24	18	102	34	19	56	22	23	27	40	35	14	33	28

Tab. I. Mean Concentration of Metals. All Values in µg/g)

* A) $HF/HNO_3/HClO_4$ B) 4.0N HNO_3/0.7N HCl C) 0.5N HCl D) 1N $NH_2OH.HCl$+25%CH_3COOH E)0.05N EDTA

** Sampling of January

*** Sampling of April

Results and discussion

The results of the chemical analyses and the percentage extraction of the reagents in proportion to the total level are shown in tables 1 and 2.

Metal	Reagents *	Sample													
		1		2		3		4		5		6		7	
		a **	b ***	a	b	a	b	a	b	a	b	a	b	a	b
Hg	B	58,4	16,8	90,1	84,3	24.9	74.2	14.5	10.3	24.7	49.3	54.9	14.3	33.9	26.6
	C	4.7	6.5	8.1	4.8	1.5	5.8	0.9	1.1	2.4	6.6	1.8	2.4	2.6	3.7
	D	1.9	2.0	4.0	0.9	2.5	3.4	7.7	3.8	11.5	7.4	3.8	2.7	6.2	3.2
	E	1.0	1.3	0.1	0.6	3.1	2.6	1.0	1.0	1.8	1.6	1.2	1.4	1.5	1.1
Pb	B	89.4	75.0	78.2	69.7	81.8	78.1	68.8	72.7	66.7	73.3	73.7	85.7	68.3	69.0
	C	73.0	68.3	63.3	59.4	61.8	60.6	60.0	63.6	53.3	62.7	73.7	61.0	59.4	62.8
	D	93.9	83.3	96.4	90.9	90.9	93.8	93.8	86.4	82.1	97.3	93.4	66.7	89.7	89.7
	E	63.6	75.0	66.4	60.6	72.7	71.9	50.0	68.2	97.4	74.7	75.0	47.6	71.4	65.5
Cr	B	36.7	30.0	66.0	31.7	24.0	40.0	13.3	33.3	30.8	63.6	46.9	22.5	87.8	37.3
	C	46.7	16.0	64.0	21.7	8.0	28.0	6.7	18.3	15.4	51.4	45.5	8.7	42.2	37.0
	D	35.0	26.0	78.0	41.7	10.7	40.0	12.2	25.0	30.8	67.9	62.1	17.5	61.4	42.7
	E	16.7	12.0	36.8	26.7	8.0	21.0	6.7	15.0	13.0	43.6	13.8	57.5	33.3	33.3
Cu	B	52.9	76.7	67.5	36.4	90.0	65.0	30.0	58.3	36.7	35.0	34.3	80.0	66.7	88.6
	C	31.4	40.0	44.4	24.5	21.7	34.0	15.0	30.0	10.8	20.0	19.3	33.3	33.3	60.0
	D	60.0	46.7	86.3	39.1	28.3	72.0	22.5	36.7	12.5	28.6	35.7	46.7	40.0	91.4
	E	34.3	60.0	63.8	30.9	31.5	56.0	18.3	38.3	22.5	28.6	25.0	46.7	44.0	80.0

Tab. II. Mean Recovery of Metals from Samples Relative to Total Concentration All Values in Percent)

*, **, *** :.see Tab. I.

Mercury. Very high levels were found at station 2, in the vicinity of a waste outfall from a chlor-alkali plant. At the other stations too the levels of mercury were high, with higher values in the samples collected in January. Reagent B extracted percentages of the element ranging from 10 to 90 per cent, while C, D, and E extracted not more than 11 per cent. The high extraction values of Hg by reagent B suggested that Hg was present in the sediment under the prevailing form of sulphide and organic complexes, and had a more limited mobility in the environment.

Lead. For Pb too the highest levels were found in the samples from station 2, followed by samples 5b, 6a, 7a. For this reason we considered that the sources of contamination might be twofold: a petrochemical industry and a treatment plant for lead and zinc minerals. The reagent D extracted the highest quantity of Pb from all samples, showing a significant association of Pb to ferromanganese oxides. All the reagents, however, liberated amounts of Pb ranging from 50 to 90 per cent of the total levels.

In all stations the sequence of efficiency of extraction of the reagents was D>B>E>C.

Chromium. In the sediments, Cr showed a pattern of distribution similar to that of Pb in that the origin of both was to be attributed to one source. The fractions of metals leachable with all reagents was lower than that of Pb (7 to 87 per cent). Moreover the cold attack with 0.5N.HCl extracted higher amounts of Cr than of Pb. The sequence of reagent efficiency was D>B>C>E>. Like Hg, Cr was in a more leachable form in the more contaminated stations.

Copper. This metal also showed very high concentrations at all points in the lagoon. All the reagents liberated amounts of Cu not exceeding 30 per cent of the total, with the highest percentages in sample 7b.

We noted that reagents C and E extracted higher quantities of metal than the others: this confirmed the hypothesis that EDTA and HCl liberated organically bound Cu. Moreover the efficiency of extraction of reagent D showed the presence of a strong inorganic component.

CONCENTRATION RANGE OF HEAVY METALS IN SEDIMENTS FROM OTHER MEDITERRANEAN AREAS

AREA	METHODS OF EXTRACTION	GRAIN SIZE	Pb	Cu	Cr	Hg
BAY of HAIFA, ISRAEL (Roth and Hornung, 1977)	1N $NH_2OH.HCl$-25%CH_3COOH	70 mesh	8.4	1.6	4,2	–
GULF of POZZUOLI, ITALY (De Rosa et Al., 1983)	HCl 37% HNO_3 conc.	63 u	327 -	63.5 -	63.9 -	- 0.69
LIGURIAN COAST, ITALY (Cosma et Al., 1981)	HF/$HClO_4$/HCl 1N $NH_2OH.HCl$-25%CH_3COOH 0.5N HCl 0.05N EDTA	unsieved samples	144/228* 69.2/143* 69.0/156* 45.5/89.3*	38.7/85.9* 11.8/12.9* 19.9/51.9* 7.52/17.2*	166/252* 17.6/66.4* 22.7/56.9* - -	- - - -
BAY of ANGES, FRANCE (Cenciarini and Fernex, 1980)	HNO_3/$HClO_4$/HF CH_3COOH $CHCl_3$	63 u	29-90 16-49%** 0.3-8	19-66 5-17 5-15%**	- - -	- - -
PRESENT WORK ***	HF/$HClO_4$/HCl 4N HNO_3-0.7N HCl 1N $NH_2OH.HCl$-25%CH_3COOH 0.5N HCl 0.05N EDTA	100 mesh	408 289 242 350 270	89.3 46 24 38 34	124 62 49 65 35	14.8 9.34 0.81 0.61 0.12

* Samples collected in front of Genoa; ** % of the total; *** Mean

Conclusions

The application of different analytical extraction methods has permitted us to investigate the availability of some pollutants in the S. Gilla biotope. Some metals (Pb, Cr, Cu) in fact associate with the various components of the sediment in different ways and therefore they can be remobilized in the environment. 1N $NH_2OH.HCl$-25 % acetic acid solution, in our case, seems to be preferable in the extraction of Cr and Pb, in that it liberates them in higher concentration values even than those of the 4N HNO_3-0.7N HCl mixture, which is considered capable of attacking an aluminosilicate crystal lattice, and is therefore unsuitable for environmental studies. Moreover the results obtained by cold attack of 0.5N HCl confirm its validity in studying environmental pollution as an index of antropogenic fraction.

References

AGEMIAN H. and A.S.Y. CHAU, (1977). - A study of different analytical extraction methods for nondetrital heavy metals in aquatic sediments. Arch. Environm. Contam. Toxicol., 6, 69-82.

BEAVINGTON F., (1975). - Heavy metal and contamination of vegetables and soil in domestic gardens around a smelting complex. Environ. Pollut., 9, 211.

BRADSHAW P.M.D., B.W. THOMSON, SMEE and I.O. LARSON, (1974). - The application of different analytical extractions and soil profile sampling in exploration geochemistry J. Geochem. Explor., 3, 209.

CENCIARINI J. and F. FERNEX (1980). - Dosage de métaux associés à diverses fractions chimiques des sédiments marins du plateau continental devant Nice et Villefranche. Actes 105 Congrès Nat. Soc. Savantes, Caen, Sciences. fasc. II, pp. 351-362.

CONTU A., G. SARRITZU, M. SAU, M.G. ENNAS, (1979). - Laguna di S. Gilla: situazione attuale e programmi di risanamento. Igiene Moderna, 7, 741-755.

CONTU A., P. MULAS, G. SARRITZU and M. SCHINTU, (1983). - Heavy metals in the superficial sediments of contaminated estuary. Rev. Int. Oceanogr. Med., LXX-LXXI, 79-86.

COSMA B., R. FRACHE, F. BAFFI and A. DADONE, (1981). - Trace metals in sediments from Ligurian coastal area, Italy. Mar. Chem., 8, 125.

MAYNARD D.E. and W.K. FLETCHER (1973). - Comparison of total and partial extractable copper in anomalous and background peat samples. J. Geochem. Explor., 2, 19.

DE ROSA S., V. DAMIANI and F. SERENA. - Studio dei sedimenti del golfo di Pozzuoli: 2) Livelli di contaminazione da metalli pesanti. Atti 5 Congresso A.I.O.L., Stresa, 1982, pp. 437-449.

OLIVER B.G. and H. AGEMIAN, (1973). - Further studies on the heavy metal levels in Ottawa and Rideau river sediments. Environ. Sci. Technol., 7, 135.

ROTH I. and H. HORNUNG, (1977). - Heavy metal concentrations in water, sediments, and fish from Mediterranean coastal area, Israel. Environ. Sci. Technol., 11, 265.

SARRITZU G., A. CONTU, M. SCHINTU and P. MULAS, (1982). - Les teneurs en mercure dans les sédiments de la lagune de S. Gilla, Sardaigne, Italie. Actes VIes Journées Etud. Pollution, Cannes, C.I.E.S.M., 415-419.

ETUDE RADIOTOXICOLOGIQUE DE MYTILUS SP. PRELEVEE SUR LE LITTORAL NORD-OCCIDENTAL MEDITERRANEEN

par

D. CALMET, M.L. DABURON et J.M. WILLEMOT
Commissariat à l'Energie Atomique
Institut de Protection et de Sûreté Nucléaire
DPS/Service d'Etudes Appliquées de Protection Sanitaire
B.P. n° 6 - 92260 Fontenay-aux Roses (France)

Résumé

Les résultats des mesures radiologiques du réseau d'observation du littoral nord occidental méditerranéen mis en place par le Commissariat à l'Energie Atomique (IPSN-SEAPS) mettent en évidence la présence de radionucléides artificiels d'origine industrielle comme le ^{106}Ru et de Radionucléides principalement dus aux retombées atmosphériques comme le ^{137}Cs et le $^{239+240}Pu$, en particulier au sein d'échantillons de Mytilus sp., qui apparaît être un excellent bio-indicateur des variations spatio-temporelles des contaminations radioactives. L'estimation des conséquences sanitaires pour les populations humaines et riveraines concernées par l'ingestion de mollusques contaminés par ces 3 radionucléides, a conduit à une fraction (10^{-9}) de la limite de dose annuelle à l'organisme entier recommandée par le CIPR 26.

Abstract

The result of radiological measurements of the north west mediterranean observation network of the Commissariat à l'Energie Atomique (IPSN-SEAPS) outline the level of artificial radionuclides coming from industrial sewages as ^{106}Ru, and from atmospheric fall out as ^{137}Cs and $^{239+240}Pu$ measured on samples of Mytilus sp., which is quite a perfect bioindicator of spatio-temporal variations of radionuclides contaminations. The sanitary consequences for the waterside population involved by contaminated molluscs ingestion lead to a fraction (10^{-9}) of annual dose limit recommanded by IRCP 26.

*
* *

Introduction

La production croissante d'électricité nucléaire en France a été réalisée par un développement de la totalité des installations impliquées dans le cycle du combustible nucléaire. La surveillance des conséquences des rejets d'effluents liquides contenant des radionucléides issus du fonctionnement de ces installations est réalisée grâce à différents réseaux d'observation d'indicateurs biologiques.

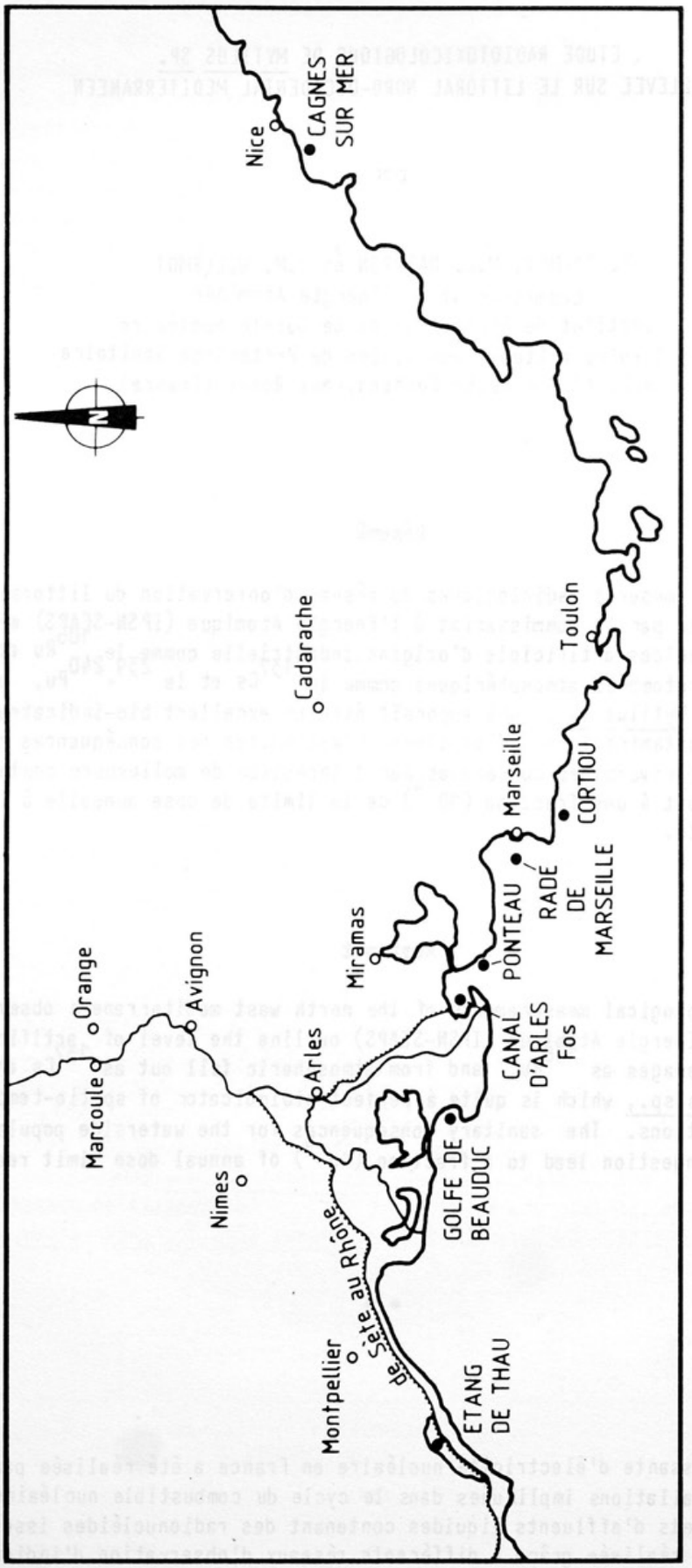


Figure 1. - Localisation des stations de prélèvements, du réseau d'observation radiologique CEA (IPSN-SEAPS), sur le littoral méditerranéen nord-occidental.

Compte tenu du nombre d'installations nucléaires sur les cours du Rhône, il nous a paru intéressant de suivre l'évolution temporelle des concentrations de radionucléides artificiels au sein des différents organismes marins susceptibles d'accumuler ces éléments, et ce en plusieurs stations du littoral réparties de part et d'autre de l'estuaire du Rhône. Les rejets liquides des installations nucléaires situées sur le cours du Rhône et la circulation des eaux littorales étant connus, il est alors possible de relier les variations observées sur les organismes échantillonnés à celles des effluents rejetés. Dans une deuxième approche, une évaluation des conséquences sanitaires a été menée en fonction des régimes alimentaires régionaux.

Matériel et méthode

1. - Le réseau d'observation du littoral méditerranéen

Il est articulé autour de différents bio-indicateurs marins dont Mytilus sp., espèce bien représentée géographiquement et qui présente une capacité élevée d'accumulation des radionucléides [1, 2] susceptibles d'être présents dans le milieu [3].

Les stations de prélèvements, sur le littoral méditerranéen, ont été choisies d'une part en tenant compte de l'existence d'un courant géostrophique orienté d'Est en Ouest, et d'autre part du fait que l'aire de dilution des eaux du Rhône, vectrices des radionucléides cherchés, ne s'étend pas à l'Est, au delà de la Rade Marseille [4, 5]. Les stations retenues sont, d'Est en Ouest, celles : de Cagnes-sur-Mer comme station de référence, la calanque de Cortiou, la rade de Marseille, Ponteau, le débouché du canal d'Arles, Beauduc et l'étang de Thau (figure n° 1).

2. - Conditionnement et protocole de mesure des échantillons

Les échantillons prélevés dans les différentes stations sont acheminés dans les meilleurs délais au laboratoires d'Etudes Sanitaires du Centre d'Etudes Nucléaires de Fontenay-aux-Roses. Dans un premier temps la chair des moules est isolée des coquilles puis les échantillons subissent une déssiccation dans une étuve à 80° C avant d'être réduits en cendres à 550° C et d'être conditionnés dans des géométries utiles de 360 ml.

L'analyse spectrométrique et le comptage sont effectués sur des installations à bas mouvement propre équipées de semi-conducteur en germanium-lithium. Le traitement chimique des cendres pour déterminer l'activité en ^{239}Pu et ^{240}Pu est réalisé suivant un protocole décrit par BALLADA [6].

Seuls les échantillons de Mytilus sp. issus de la station du golfe de Fos ont fait l'oblet d'un dosage de plutonium. Les principaux émetteurs gamma artificiels rejetés par l'industrie nucléaire ont été recherchés sur l'ensemble des échantillons.

Résultats et discussion

Les résultats des mesures réalisées au cours du 2e semestre 1983 sur des échantillons de Mytilus sp. ont été reportés sur la figure n° 2. Il apparaît que parmi l'ensemble des radionucléides artificiels susceptibles de se trouver dans les eaux du Rhône, seuls le césium 137, le ruthénium 106 et les Isotopes 239 et 240 plutomium ont été détectés.

Le césium 137 est détecté à des niveaux d'activité compris entre 1 et 4 $Bq.kg^{-1}$ de cendres pour l'ensemble des stations. Cette homogénéité spatiotemporelle s'explique par le fait que le césium 137 est l'un des principaux produits des retombées atmosphériques d'essais d'armes nucléaires, dans l'atmosphère de l'hémisphère nord, principalement entre 1962 et 1980 [7].

En ce qui concerne les résultats des mesures de ruthénium 106, il apparaît un gradient spatial décroissant de part et d'autre de la station de Ponteau qui est caractérisée par les valeurs mensuel-

	ETANG de THAU	BEAUDUC	CANAL D'ARLES	PONTEAU	RADE DE MARSEILLE	CORTIOU	CAGNES-sur-MER
NB.$^{137}_{Cs}$	7	2	7	7	7	7	6
MINIMUM	LD	2	2	LD	–	LD	LD
MOYENNE	3	3	3	2	–	1	3
MAXIMUM	4	4	4	4	LD	3	4
NB.$^{106}_{Ru}$	7	2	7	7	7	7	6
MINIMUM	LD	159	30	56	LD	–	–
MOYENNE	16	178	67	138	10	–	–
MAXIMUM	48	196	118	233	26	LD	LD
NB.$^{239+240}_{Pu}$	0	0	7	0	0	0	0
MINIMUM	–	–	0.17	–	–	–	–
MOYENNE	–	–	0.47	–	–	–	–
MAXIMUM	–	–	1.03	–	–	–	–

Figure 2. Moyenne des teneurs en ^{137}Cs, ^{106}Ru et $^{239-240}Pu$ dans les échantillons de Mytillus sp. récoltés au cours du 2e semestre 1983 en 7 stations du littoral méditerranéen. Les valeurs sont exprimées en $Bq.kg^{-1}$ de cendres. LD : Limite de Détection.

les les plus élevées. Ce gradient est limité vers l'Est à la station située dans la rade de Marseille conformément au schéma courantologique régional. En fait, une corrélation positive apparaît entre l'évolution des rejets liquides de Marcoule et les niveaux de ^{106}Ru contenu dans les échantillons de Mytilus sp. récoltés aux stations de Ponteau et au débouché du canal d'Arles dans le golfe de Fos (figure n° 3). Par contre aucune corrélation ne semble lier ces effluents aux mesures concernant les

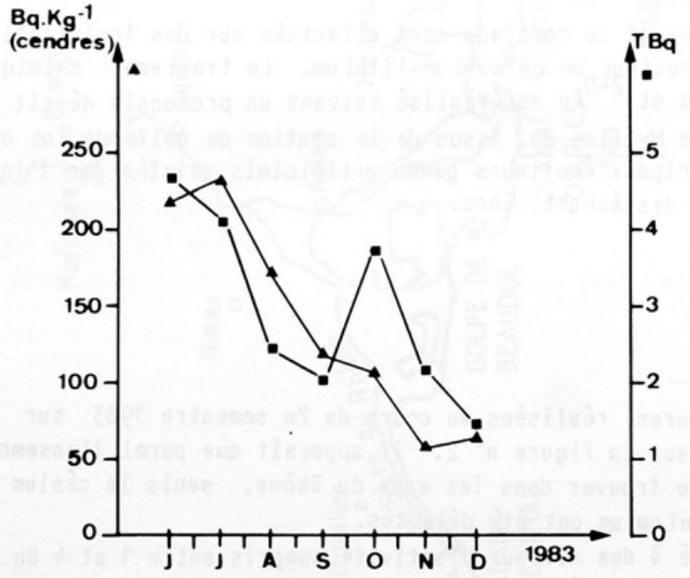


Figure 3. - Tracés chronologiques de l'évolution des mesures du ^{106}Ru dans les effluents liquides de Marcoule exprimés en TBq (■), dans les échantillons de Mytilus sp. des stations de Ponteau (▲) exprimées en $Bq.kg^{-1}$ de cendres d'organisme.

stations plus éloignées, pour lesquels des processus complexes de dispersion des radionucléides doivent intervenir. Les teneurs en plutonium 239 et 240 des échantillons de la station au débouché du canal d'Arles restent constantes au cours du 2e semestre 1983 et comparables à celles que l'on peut attendre des retombées atmosphériques aux mêmes latitudes.

L'évaluation des conséquences sanitaires à la suite de l'ingestion des radionucléides artificiels mis en évidence dans la chair de Mytilus sp., coquillage susceptible d'être consommé localement, a été établie pour une consommation annuelle moyenne d'une personne vivant dans la région méditerranéenne [8]. Ainsi pour les niveaux de radioactivité en ^{137}Cs, ^{106}Ru et $^{239+240}Pu$ observés à la station de Ponteau, il apparaît que l'équivalent de dose engagée est de l'ordre de 10^{-9} Sv représentant environ 10^{-6} de la limite de dose recommandée par la Commission International de Protection Radiologique pour les individus du public [9].

Conclusion

Le suivi radiologique d'un mollusque filtreur comme Mytilus sp. a permis de distinguer les différentes sources de contamination radioactive en Méditerranée nord-occidentale. Ainsi il a été établi que la présence de ^{137}Cs et de $^{239+240}Pu$ est due principalement aux retombées atmosphériques suite aux essais d'armes nucléaires dans l'hémisphère nord, tandis que celle du ^{106}Ru dans les organismes s'explique par les rejets industriels dans les eaux du Rhône.

L'évaluation des conséquences sanitaires pour les populations riveraines concernées par la consommation de coquillages prélevés à proximité de l'embouchure du Rhône est négligeable et représente dans le cas le plus défavorable une fraction de l'ordre de 10e de la limite de dose recommandée par le CIPR 26.

Références

[1] E.D. GOLDBERG, (1985). - The mussel watch a first step in global marine monitoring. Mar. Pollut. Bull., 6, 111.

[2] E.D. GOLDBERG, N. KOIDE, V. HODGE, A.R. FLEGAL, J. MARTIN, (1983). - US mussel watch : 1977-1978. Results on trace metals and radionuclides. Est. Coast. and Shelf Sci., 69-93.

[3] R. FUKAI, S. BALLESTRA, M. THEIM, J. GUION, (1981). - Input of transuranic elements through rivers into the Mediterranean sea. In "Impacts of radionuclide releases into the marine environment", IAEA, Vienne, 3-14.

[4] M. LACOMBE, P. TEHERNIA, (1972). - Caractères hydrologiques et circulation des eaux en Méditerranée. In "The Mediterranean sea, a natural sedimentation laboratory", D.J. STANLEY Edit. 25-36.

[5] M.J. MINAS, (1971). - Observations hydrologiques dans le golfe de Marseille (années 1961 à 1965). Thethys, 3, 447-457.

[6] J. BALLADA, (1967). - Détermination analytique du plutonium dans l'environment. Rapport CEA-R 3320, 157 p.

[7] UNSCEAR, (1982). - Ionizing radiation : sources and biological effects. United Nations Scientific Commitee on the effet of atomic radiation, 772 p.

[8] M.A. MERCIER, (1982). - Consommation et lieux d'achat des produits alimentaires en 1979. Les collections de l'INSEE, M(95), 198 p.

[9] CIPR Publication CIPR 26, (1980). - Recommandations de la Commission Internationale de Protection Radiologique. Pergamon Press, Oxford, 63 p.

SEASONAL VARIATIONS OF ZINC COPPER AND NUTRIENTS IN COASTAL SEA-WATER OF THE LIGURIAN SEA

by

André VEGLIA and Raymond VAISSIERE
Centre Scientifique de Monaco
16, boulevard de Suisse
MC 98000 Monaco (Pincipauté)

Abstract

Samples of sea-water collected at 5 metres depth twice a month from March 1982 to June 1983, at four coastal stations in the vicinity of Monaco have been analysed for their zinc and copper contents (AAS) as well as their nitrate, phosphate and silicate concentrations (Technicon). Mean values for each parameter and for each day of sampling were plotted after a 3-step smoothing procedure. A co-variation of zinc and nitrate concentrations appears with maximum in summer and minimum in winter. The correlation is significant and the two parameters are bound by the relation Zn ($\mu g.l^{-1}$) = 2.268 NO3 - ($\mu atg.l^{-1}$) + 1.446. For the same period the sea-water density has been plotted. The curve shows that a decrease in metal and nitrate concentrations happens in the homogenization period of the water column. The possible grounds for the observed increase in metals and nutrients in summer are discussed. The surface layer seems to act like a reservoir for these substances. The thermal stratification of the water column in summer. combined with a seasonal increase of anthropogenic pollution, appears to be the main cause of the enhanced concentration of nutrients and metals in the surface layer observed during this period.

*
* *

Introduction

Recent works have brought to light that metals, e.g., cadmium, zinc and nickel, are involved in the internal biogeochemical cycle of organic matter in the oceans (BRULAND, 1980) while copper, showing a more complex distribution, seems to be governed by surface water removal, deep water scavenging and bottom water enrichment from the sediment (BOYLE et al., 1977 : BRULAND, 1980).

With regard to surface coastal waters, large differences are noticed amongst results given by various investigators with levels usually higher than those observed in the open sea (FRACHE et al., 1976 ; BAFFI et al., BRULAND and FRANKS, 1983). In the vicinity of the coast the biogeochemical cycle is , of course, disturbed by terrigenous and/or anthropogenic inputs, by diagenetic remobilization from shelf sediments and probably complicated by the seasonal changes of other factors like salinity and temperature.

This paper deals with the variation of Cu and Zn concentrations at 5 metres depth in relation to the upper layer hydrographic conditions in coastal waters near the Principality of Monaco (Ligurian - Mediterranean sea) during a 16 months' survey.

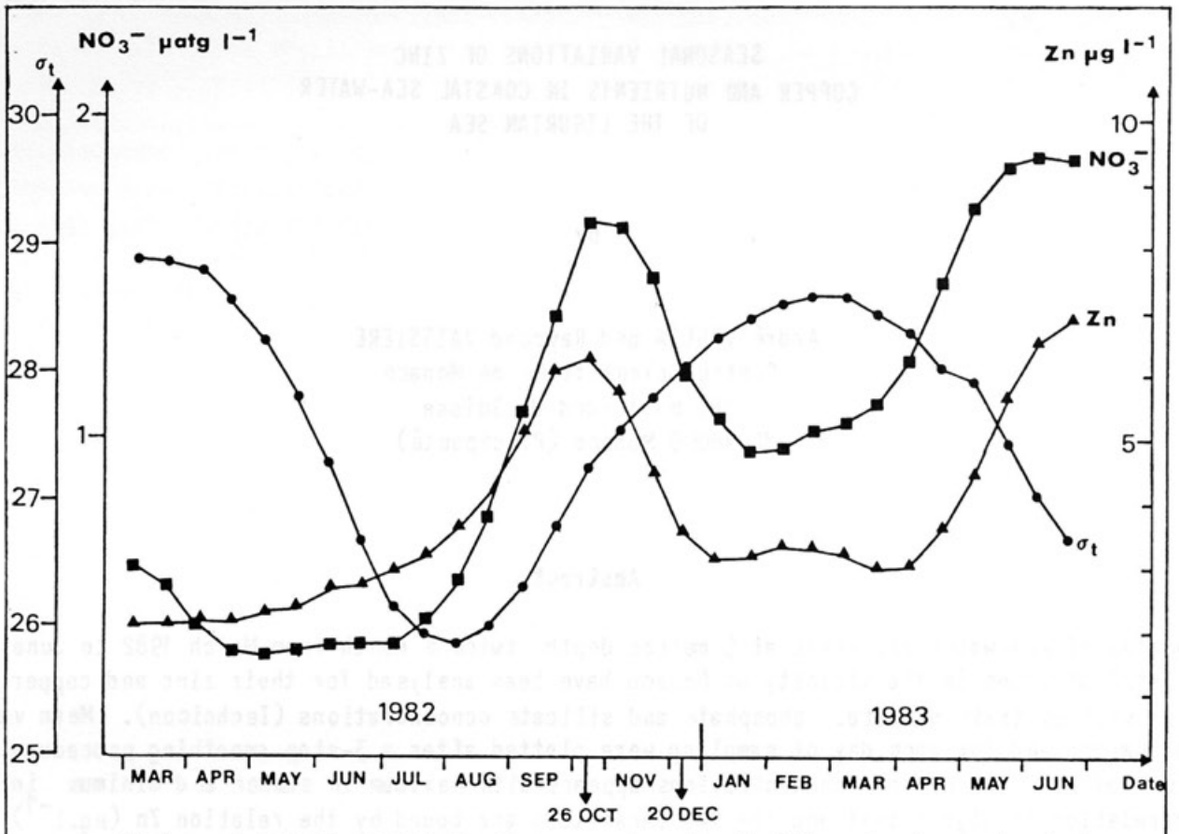


Fig. 1 – Seasonal variations of sea-water density (σ_t) and zinc and nitrate concentrations.

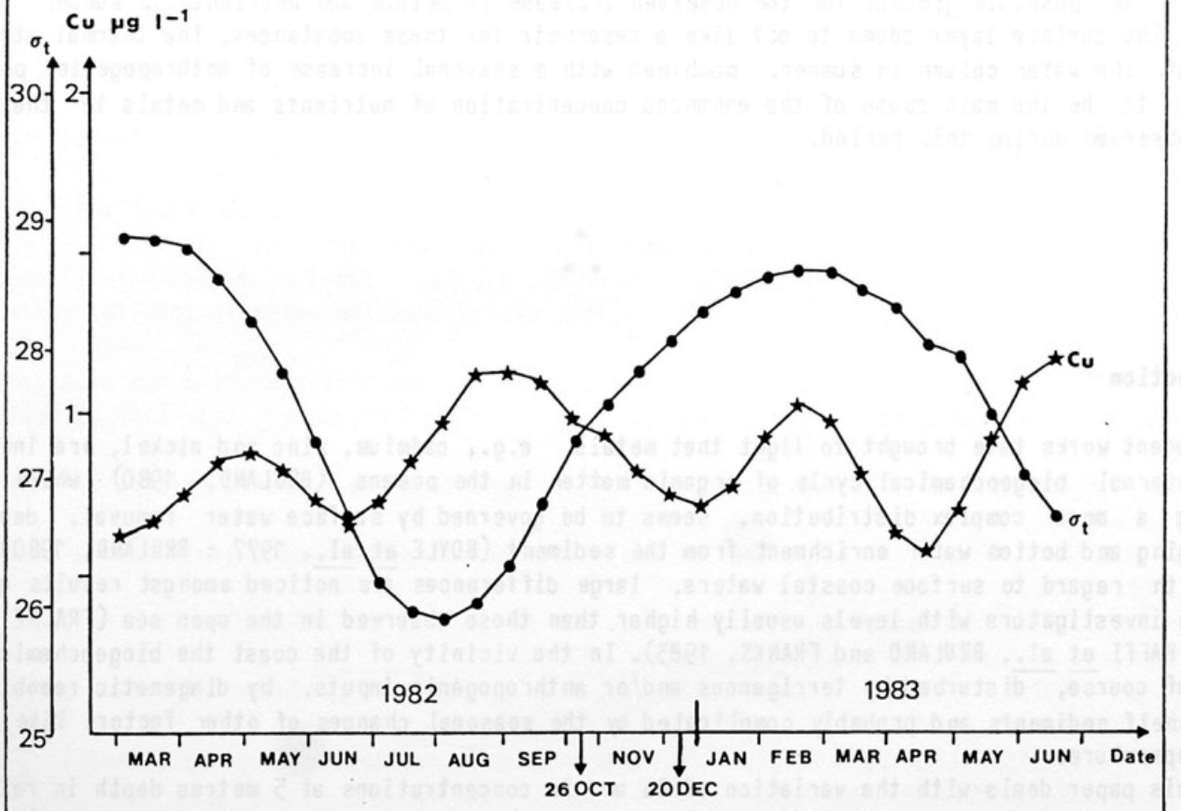


Fig. 2 – Seasonal variations of sea-water density (σ_t) and copper concentration.

Materials and methods

Samples of sea-water were collected every fortnight from March 1982 to June 1983 at four stations near Monaco, using the facilities of the R.V. Ramoge. The stations were all located within six miles of the coast.

Sampling operations were carefully conducted to avoid contaminations from the boat and the sea-atmosphere interface. Samples for metal analysis were collected at 5 m under the surface by means of a N.I.O. 1.2.1. plastic bottle fixed to a stainless steel hydroline. The bottle contents were transfered to polyethylene flasks previously acid-washed and sea-water conditioned and immediately frozen at -20 °C on receipt in the laboratory (within two hours after sampling).

Sampling done at the beginning of the survey by using a nylon hydroline or by collecting samples from a small raft at 50 m from the ship directly in clean polyethylene flasks did not produce lower results for zinc and copper than those obtained by the system N.I.O. sampler - stainless steel hydroline. It was decided, therefore, to continue to use this system.

Unfiltered sea-water samples were analysed by atomic absorption spectroscopy for their trace metal contents (KINRADE and VAN LOON, 1974 ; A.F.N.O.R., 1976). 400 ml of sea-water were extracted at pH 4 by 20 ml of MIBK after chelation of metals by APDC and DDDC. Organic extracts were aspirated into the air-acetylene flame of an atomic absorption spectrophotometer (Instrumentation Laboratory Model 251). Automatic background correction was made by means of a deuterium hollow cathode lamp. Metal concentrations in the samples were determined by calibration with standards prepared by adding known amounts of Zn and Cu to NaCl solution (38 g/l) and extracted in the same way. All reagents were purified before use by extraction whit MIBK. All the glassware was conditioned by 10 % nitric acid and washed with high purity demineralized water before use. All chemical operations were done under a laminar flow hood to avoid contaminations. The limit of detection of the method as estimated from the variation of the absorbance of pure MIBK was about 0.5 µg/l for zinc and 0.3 ug/l for copper (2σ). The reproducibility of the method was checked by repeating twice the analysis of a large number of samples collected at the beginning of the survey. Correlation coefficients between both determinations were found to be about 0.95 for zinc and 0.90 for copper, wich may be estimated as satisfactory at this concentration level (about 1 µg/l).

Sampling for nutrient analysis and salinity measurements was done by means of a 1.7 l Niskin bottle at two levels, surface and 10 m. A Technicon Autoanalyzer was used for nutrient content determination (TREGUER and LE CORRE, 1974). The analysis began within two hours after sampling.

The sea-water temperature was measured in situ by means of Richter and Wiese reversing thermometers. The salinometer used was an Autosal Guildline.

Data processing

First, the mean value of every parameter was calculated for the day of sampling from the four results corresponding to the four stations. These mean values were then plotted according to the date of sampling and the curves were smoothed in a classical way (i.e. each value was replaced by the average of itself and the two neighbouring points). The latter procedure was repeated twice. By doing so, overall variations of the parameters under survey could be described while small occasional concentration variations would in theory be eliminated, the smoothing procedure removing or at least minimizing the influence of eventual results which were too high due to contamination.

It should be recalled that sampling for metal content was made at 5 m depth while sampling for nutrients and salinity as well as temperature measurements were made at the sea-surface and at 10 m depth. Hence the mean value for each of these three types of parameters was calculated using the results from the two levels.

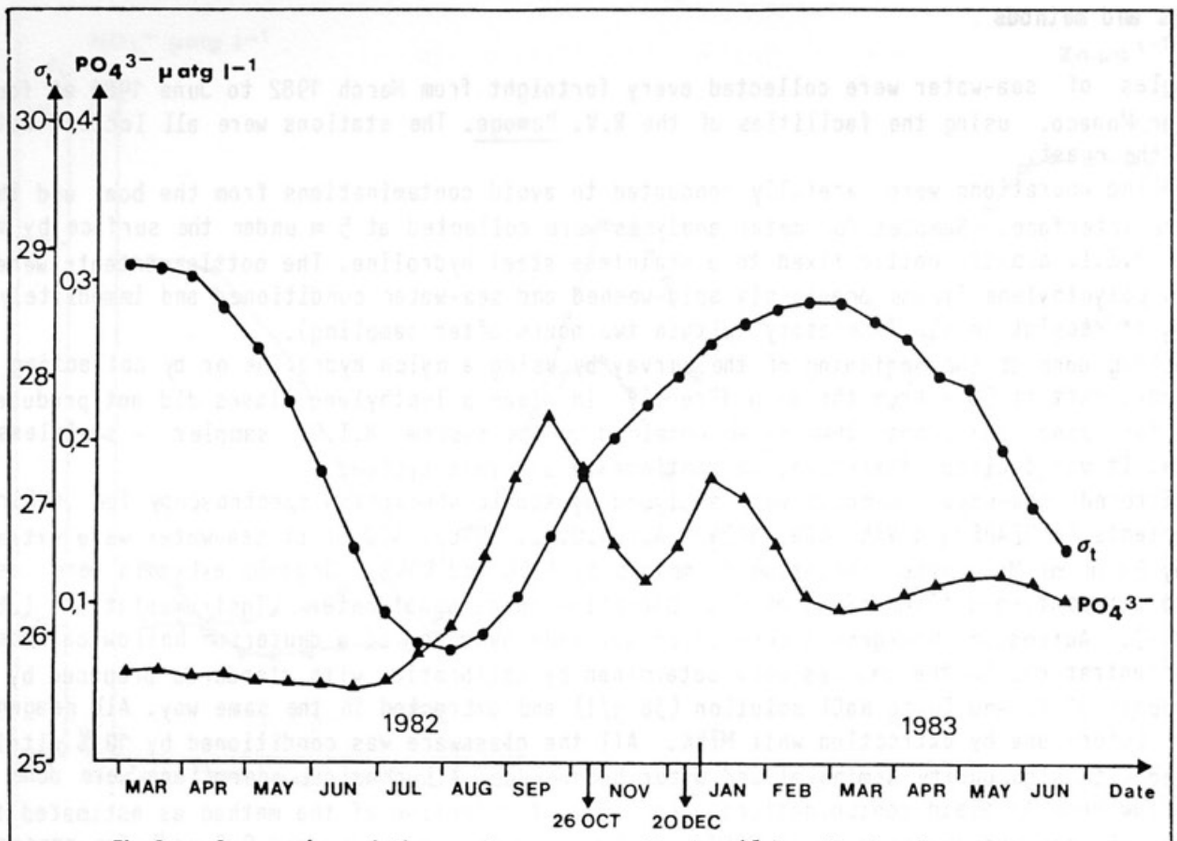


Fig.3 – Seasonal variations of sea-water density (σ_t) and phosphate concentration.

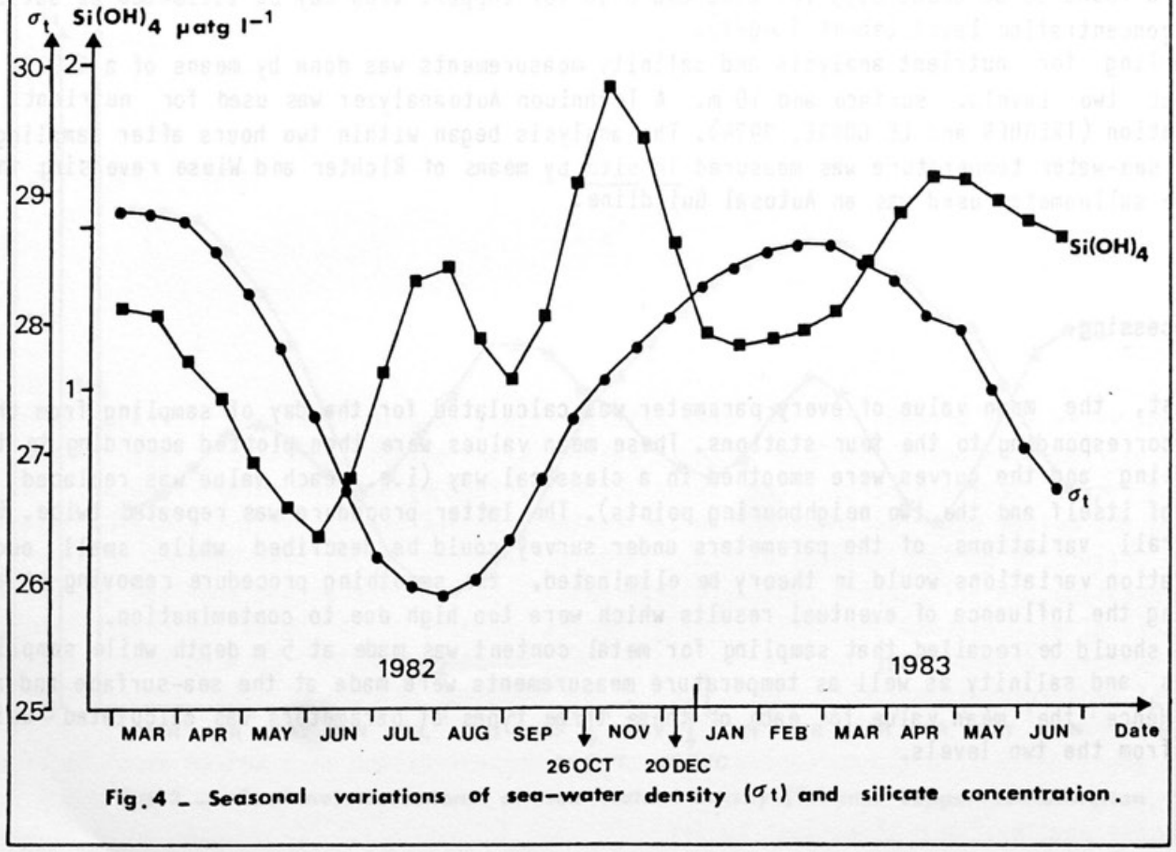


Fig.4 – Seasonal variations of sea-water density (σ_t) and silicate concentration.

Results

Metals : The most frequent concentration values were found between 1 and 2 $\mu g.l^{-1}$ for zinc and 0.4 and 0.8 $ug.l^{-1}$ for copper. These results are of the same order of magnitude as those found by other authors in the same geographical area (BAFFI et al., 1983 : FRACHE et al., 1976, 1980 : R.N.O., 1982). Higher values were, however, quite often noticed.

The variation of Zn concentration is plotted on figure I. Two peaks can be seen, one in October 1982 and the other one at the end of June 1983. A depletion is observed during cold periods with an earlier and faster increase of the metal concentration in spring 1983.

Copper concentration variations (figure II) appear with four peaks. Two of them, the highest ones, take place roughly at the same time as zinc but the two others , of lower magnitude, can be seen in mid-spring 1982 and late winter 1983.

Nutrients : The highest nitrate concentrations are observed in late summer 1982 and late spring/early summer 1983 (figure I).

With regard to phosphate and silicate concentrations, the variations are more or less similar to nitrates but not so regular with peaks appearing in September 1982 and January 1983 for phosphates (figure III) and in August and November 1982 for silicates (figure IV).

Sea-water density : The seasonal variations of σ_t are normal. An increase for cold periods and a decrease for warm periods are observed with a slight shift to earlier times in the year for 1983 (figures I-IV).

Discussion

The variations of the concentrations of metals, especially Zn, are obviously related, if not entirely, at least partly, to the seasonal variations of the sea-water density. It appears that during the warmer months the surface water layer which has poor exchange with the deeper waters would concentrate Zn and Cu - and most certainly other substances-from various sources. On the contrary, with the mixing process starting in autumn the metal content of the surface layer would then be diluted in the water column and the concentration in the upper layer would decrease.

The behaviour of Cu is similar to that of Zn but with two additional peaks in May and February for which it is difficult to give an explanation. They could be the result of contamination or have some other origin.

If one compares the plots in figure I it is obvious that there is a zinc-nitrate co-variation, and a linear regression equation (30 points) can be written as follows:

$$Zn\ (\mu g.l^{-1}) = 2.268\ N\text{-}NO3\ (\mu atg.l^{-1}) + 1.446$$

with a correlation coefficient $r = 0.843$ ($p < 0.001$).

To a certain extent the Zn-phosphate and Zn-silicate co-variations are similar with one depletion of silicate in September and one peak of phosphate in January.

In open-sea conditions, correlations Zn-silicate, Cu-silicate (BRULAND, 1980 ; DANIELSSON, 1980) and Cu-nitrate (BOYLE and EDMOND, 1975) have been observed and elements like Zn are said to have a nutrient type distribution in the oceans (BRULAND and FRANKS, 1983).

At first sight one could conclude that the same type of distribution exists in a coastal area. In open-sea conditions, however, the nutrient concentration decreases in summer in the upper layer above the seasonal thermocline after exhaustive use by phytoplankton. In early autumn from the beginning of the vertical mixing process and until the end of winter, the water column is enriched with nutrients, especially nitrate, of deeper water origin.

The results reported here for a Mediterranean coastal area do not fit the theory at least for the 10 m deep surface layer. One can see that nitrate (and metal) concentrations increase from spring to late summer and decrease with the seasonal thermocline erosion. In other words the upper layer looks like a source of nutrients to deeper layers.

In the particular case of this coastal water system, one could suggest, therefore, that the relative isolation of the surface layer from deeper water masses causes an accumulation of metals and nutrients in this layer. It is difficult, of course, to identify with certainty the sources of these substances. One must notice, however, that from early spring to late summer, the population of the "Côte d'Azur" is increased by a factor of two or three. This may result in a significant increase in sewage and waste waters rejected into the coastal waters. The organic matter transferred to the sea in this way may rise in summer and be rapidly oxidized to nitrate. The augmentation of phosphate may be due to an increased consumption of washing powders containing mineral phosphates. Studies carried out some years ago in the same area showed, for instance, a direct relationship between detergents and phosphates in summer (BOISSON et al., 1980). No satisfactory explanation, however, can be found for the concentration increase of silicate in summer (except perhaps dry deposition of aeolian dust).

We conclude, therefore, that the enhanced concentration of nutrients and metals in surface coastal waters observed in summer is mainly due to the thermal stratification of the water column combined with a seasonal increase of anthropogenic pollution during this period.

Acknowledgment

The authors wish to thank the crew of the R.V. Ramoge for assistance during the sampling operations and the scientific team of the C.S.M. for nutrient and metal determinations, salinity measurements and data processing.

References

A.F.N.O.R. (Association Française de Normalisation) (1976). - Essais des eaux Norme expérimentale, T. 90-112.

BAFFI F., A. DADONE, R. FRACHE (1983). - Heavy metals in inshore waters near Genoa. Mar. Pollut. Bull., 14, pp. 469-471.

BOISSON M., J-L. RAPAIRE, R. VAISSIERE (1980). - Les surfactants anioniques en baie de Monaco, corrélations avec divers paramètres du milieu marin. 5th Workshop on pollution of the Mediterranean, Cagliari, C.I.E.S.M., 175-182.

BOYLE E., J-M EDMOND (1975). - Copper in surface waters south of New Zealand. Nature (Lond.) 253, 107-109.

BOYLE E.A., F.R. SCLATER, J.M. EDMOND (1977). - The distribution of dissolved copper in the Pacific. Earth and Planetary Science Letters, 37, 38-54.

BRULAND K.W. (1980). - Oceanographic distributions of cadmium, zinc, nickel and copper in the North Pacific. Earth and Planetary Science Letters, 47, 176-198.

BRULAND K.W., R.P. FRANKS (1983). - Mn, Ni, Cu, Zn and Cd in the Western North Atlantic. Trace metals in Sea-Water, NATO Conference Series, Plenum Press, New York, 395-414.

DANIELSSON L.G. (1980). - Cadmium, cobalt, copper, iron, lead, nickel and zinc in Indian Ocean water. Mar. Chem.,8 199-215.

FRACHE R., F. BAFFI, A. DADONE, G. ZANICCHI (1976). - The determination of heavy metals in the Ligurian Sea. I, The distribution of Cu, Co, Ni and Cd in surface waters. Mar. Chem., 4, 365-375.

FRACHE R., F. BAFFI, A. DADONE, G. SCARPONI, I. DAGNINO (1980). - The determination of heavy metals in the Ligurian Sea. II, The geographical and vertical distribution of Cd, Cu and Ni. Deep-Sea Res., 27A, 1079-1082.

KINRADE J.D., J.C. VAN LOON (1974). - Solvent extraction for use with flame atomic absorption spectrometry. Anal. Chem., 46, 1894-1898.

R.N.O. (Réseau National d'Observation de la Qualité du Milieu Marin). - Centre National pour l'Exploitation des Océans. Bulletin n° 20 (2nd semester, 1982).

TREGUER P., P. LE CORRE, (1974) . - Manuel d'analyse des sels nutritifs dans l'eau de mer, adaptation au flux continu. Laboratoire d'Océanologie Chimique, Université de Bretagne Occidentale.

COMPARISON OF 210PB TRACE METALS (HG, PB, CU, CR) PROFILES AND RIVER DISCHARGE IN A CORE OFF THE PO DELLA PILA RIVER MOUTH (ITALY)

by

Stefano GUERZONI*, Mariangela RAVAIOLI*
Gabriella ROVATTI* and Oscar Daniel SUMAN **
* Istituto di Geologia Marina/CNR/via Zamboni
n° 65/40127 Bologna (Italy)
** Scripps Institution of Oceanography/UCSD/ La Jolla
CA 92093 (U.S.A.)

Introduction

Rivers carry large quantities of heavy metals into the marine environment, some of which are natural and some of which are derived from pollution. It is often difficult to assign quantitative estimates of the riverine flux of pollutant metals to the sea because of the lack of data for the heavy metal chemistry of rivers prior to the era of significant pollution Careful measurements of the composition of the marine sediment cores combined whit independent indicators of the "age" of particular sediment layers. can help to provide estimates of riverine metal fluxes in the past. We report here new analytical data for a sediment core just off-shore of the Po River discharge which should be representative of the fine sediments derived from this river over the past few decades. These data, examined in conjunction with pre-industrial metal concentrations from other similar types of detrital sediment deposits, indicate the proportion of metals accumulating in riverine sediments near the Po which were derived from pollution. The proportion of pollutant metals in these nearshore marine sediments, combined with measured present-day fluxes of metals can provide a good indication of the natural and pollutant metal fluxes of the Po to the northern Adriatic Sea.

Study area

The geographical area discussed here has been extensively described by many authors. Morphology, hydrodynamics, sedimentology, mineralogy and sediment pollution have been discussed by NELSON (1970), FRIGNANI and RAVAIOLI (1982), DAL CIN (1983), COLANTONI et al. (1979), DONAZZOLO et al. (1984) and GUERZONI et al., (1984). The sediment core analysed here was obtained directly in front of the mouth of the Po della Pila (figure I), 4 km offshore at a water depth of 26 metres. This branch of the Po, which is currently responsible for about one half of the total water discharge of the river, debouches into a zone of accretion, with more rapid advance of the subaqueous part of the delta compared with the subaerial part. Regional and local subsidence is accompanied by continued fine sediment accumulation on the scale of cms per year. The nearshore bottom profile is very steep, in the order of 10 m/km, to a depth of about 30 metres. Although recent data (DAL CIN, 1983) demonstrate the historic reduction of particle discharge by the Po, due to removal of river channel sands for construction materials, and the resulting retreat of delta shorelines in some areas, the immediate

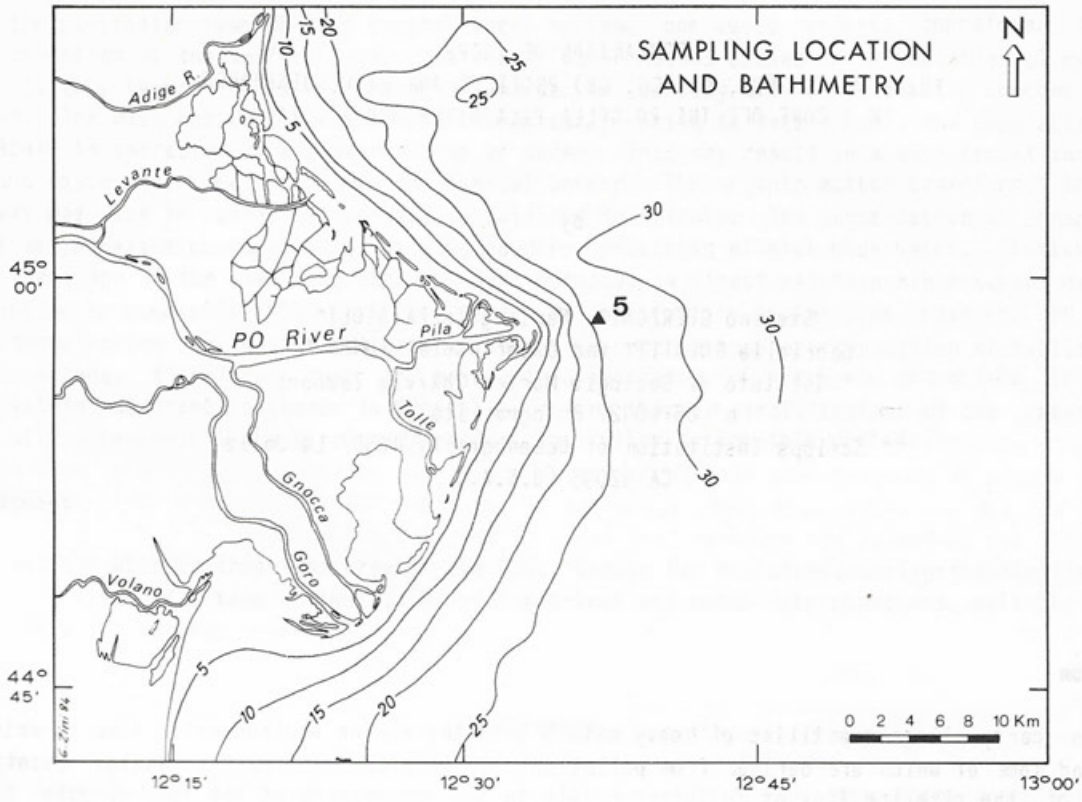


Figure I

vicinity of the principal discharge channel of the Po still receives sufficient new riverine particles to be a zone of active deposition.

Materials and Methods

We collected a sediment core in August. 1979, 4 km from the mouth of the Po della Pila (44 58.2'N, 12 35.0'E) using a modified (BUSATTI et al., 1980) Benthos gravity corer. Immediately after raising the core, it was quick-frozen. The core (6 cm diameter, 147 cm long) was then cut into sections, 15 of which were analysed for trace metals. Extraction of the sediments for metals was made with hot HNO3 (CARMODY et al., 1973), using procedures documented in detail elsewhere (FRIGNANI et al., 1978). Grain-size measurements were made with Sedigraph 5.000 D after wet sieving at 63 microns (MENEGAZZO Vitturi and RABITTI, 1980). Lead-210 was measured by low-background beta counting techniques (KOIDE and BRULAND, 1975) at the Scripps Institute of Oceanography.

Results and discussion

Concentrations of copper, chromium, mercury and lead with depth in the sediments core are listed in table 1, along with the fractions of sand (> 63 μ), silt (2- 63 μ) and clay (< 2 μ) in each sample. In addition total activities of 210 Pb are reported for 10 samples. The entire 150 cm of the

sediment core were predominantly fine particles (< 63 μ), with 98 per cent or more by weight of silt plus clay in all samples. Total 210 Pb activities were relatively high (2.5 to 5.8 dpm/g) compared with estimates of 210 Pb activities supported by decay of 226 Ra in similar environments (~ 1.5 dpm/g) (DONAZZOLO et al., 1982 : DECONINCK et al., 1983) . Based on these 210 Pb activities, and 137 Cs measurements on samples from the same core (TASSI PELATI et al., 1981), the apparent mean sedimentation rate was of the order of 2-4 cm/year. Thus all the sediments in this core probably accumulated in the last three to five decades, and should be representative of the fine sediments derived from the Po during that period.

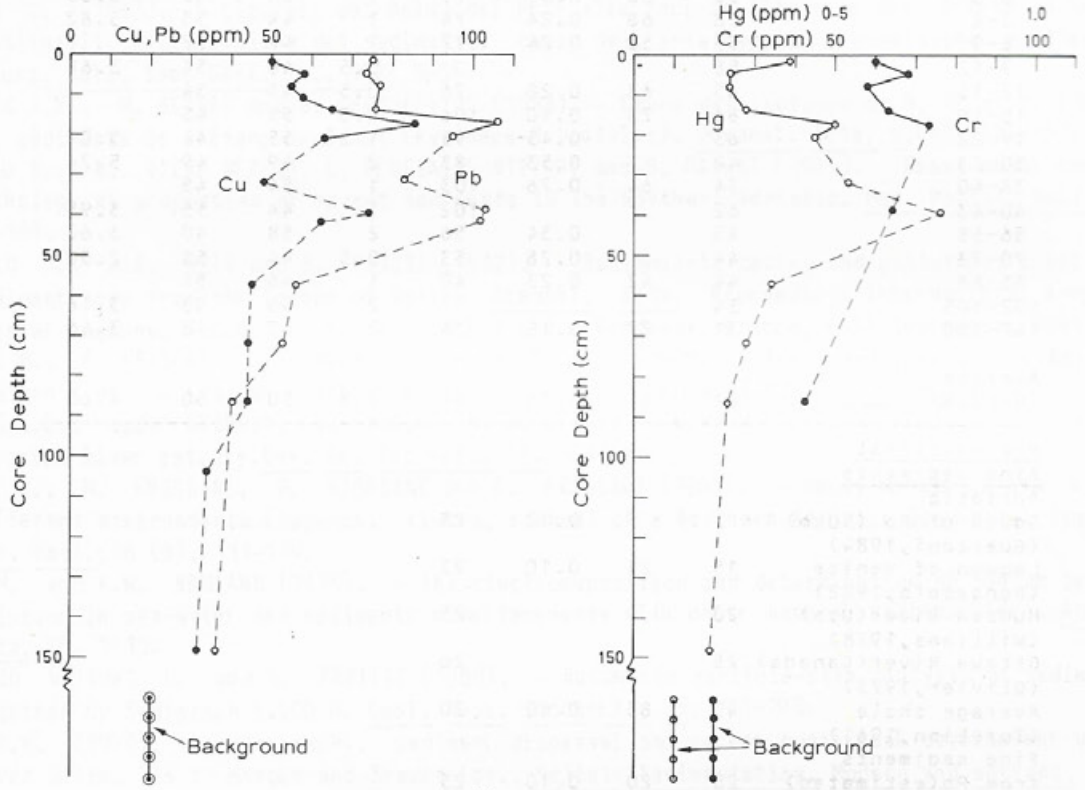


Figure II.

Trace metal concentrations at the bottom of the core (figure II) were lower for all four metals (Cu, Cr, Hg, Pb) than the upper sediments layer, but did not reach values as low as those which appear to be reasonable estimates for background concentrations in this area. Averaging the individual core segment metal data from 0 to 22 cm, as representative of the fine sediments from the Po which were accumulated in the five to ten years prior to collection of the core, and dividing by our estimate of the background metal concentrations in the fine Po-derived sediments (table 1), the present day sediments have approximately three times the natural levels of Cu, Cr, Hg and Pb. Thus about two thirds of those metals in currently accumulating fine sediments near the mouth of the Po were probably derived from "pollution" rather than natural sources.

It is interesting to compare the sediment metal concentrations reported here to trace metal flux data reported for the Po River from the period 1977-1978. The arithmetic mean water discharge (2.19×10^3 x 10^3/sec) and arithmetic mean suspended solids concentration (1.22×10^2 mg/l), based on the average of 69 individual values over a period of 18 months, indicate a mean annual particle discharge of 8.4×10^6/year over that period (PROVINI et al., 1980). Note that this value is considerably less than the annual particle discharge amount (27×10^6 T/y) suggested by NELSON (1970) and is reasonably consistent with the particle discharge estimates for the Po River during the last few decades (DAL CIN, 1983). Over the same period for which water discharge and suspended particle concentrations were

measured for the Po, total concentrations of several metals were analysed. These concentration data were then translated into total metal fluxes for the period of sampling (PROVINI and PACCHETTI, 1982). The 1977 and 1978 metal fluxes for Cu, Cr and Pb were: Cu (1.211 T/y, 734 T/y), Cr (944 T/y, 581 T/y) and Pb (1.312 T/y, 841 T/y). Averaging those two annual values for each metal, Cu (760 T/y),

depth in core(cm)	Cu ppm	Cr ppm	Hg ppm	Pb ppm	sand %	silt %	clay %	210Pb dpm
0-3	50	60	0.39	75	1	56	43	5.83
3-6	58	68	0.24	74	1	44	55	3.82
6-9	55	58	0.24	77	1	47	52	
9-12	58				0.5	46	54	3.61
12-15	65	63	0.28	76	0.5	46	54	
15-19	86	73	0.50	106	0.5	55	45	
19-22	63		0.45	95	1	55	44	5.07
30-33	48		0.53	83	2	49	49	5.24
38-40	74	64	0.76	103	1	54	45	
40-43	62			102	1	44	55	3.95
56-58	45		0.34	56	2	58	40	3.60
70-73	44		0.28	53	0.5	44	55	2.52
85-88	44	42	0.23	40	1	48	51	
102-105	34				2	45	53	3.16
147-150	31	27	0.19	36	2	49	48	3.46
Average (0-22cm)	62	64	0.35	84	0.8	50	50	4.60
Preindustrial Fine sediments								
Adriatic South of Po (50km) (Guerzoni,1984)			0.12	23				
Lagoon of Venice (Donazzolo,1982)	15	21	0.10	23				
Hudson River(Usa) (Williams,1978)	20			25				
Ottawa River(Canada) (Olivier,1973)	28			26				
Average shale (Turekian,1961)	45	83	0.40	20				
Fine sediments from Po(estimated)	20	20	0.10	25				
Po suspended particles (1977-78)	120	90		130				

Tab.1 Heavy metals; grain-size fraction; 210Pb data with depth and average concentrations in core n.5, compared with other study areas.

Cr (970 T/y) and Pb (1.080 T/y) and dividing by the total annual particle discharge (8.4×10^6 T/y), we obtain average metal concentrations on the Po suspended particles assuming that all the trace metals are associated with the particles : Cu = 120 mg/l, Cr = 90 mg/l, and Pb = 130 mg/l. These concentration values are 1.5 to 2 times the marine sediment metal concentrations derived from our core at the mouth of the Po (table 1). Assuming the ratios of pollutant to natural metal fluxes in the Po are the same as the pollutant to background concentration ratios in the fine marine sediment near the mouth of the Po, we estimate that the total annual natural metal fluxes from the Po are about one third of the present day fluxes listed above : Cu (250 T/y) : Cr (320 T/y) and Pb (360 T/y), and the current pollutant fluxes to the Adriatic from the Po are twice these values.

References

BUSATTI G., A. MAGAGNOLI and M. MENGOLI (1980). - Carotiere a gravita 1.2 T. Rapporto tecnico n.13, Ist. Geol. Mar., CNR, Bologna, 1980.

CARMODY D.J., J.B. PEARCE and W.E. YASSO (1973). - Trace metals in sediments of New York Bight. Mar. pollut.. Bull., 4, 132-135.

COLANTONI P., P. GALLIGNANI and R. LENAZ (1979). - Late Pleistocene and Holocene evolution of the north Adriatic Continental Shelf (Italy). Mar. Geol., 33, M41-M50.

DAL CIN R, (1983). I Litorali del Delta del Po e alle foci dell'Adige e del Brenta : caratteri tessiturali e dispersione dei sedimenti, cause dell'arretramento e previsione sull'evoluzione futura. Boll. Soc. Geol. It., 102, 9-56.

DECONINCK J.N., M. BERNAT and B. CHASSEFIERE (1983). - Etude des isotopes de U, Th et du 210 Pb dans les sédiments de l'étang du Canet (Pyrénées-Orientales). Oceanol. Acta, 6,(2), 131-137.

DONAZZOLO R., O. HIEKE MERLIN, L. MENEGAZZO VITTURI and B. PAVONI (1984). Heavy metal content and lithological properties of recent sediments in the Northern Adriatic. Mar. Pollut. Bull., 15(3), 93-101.

DONAZZOLO R., A.A. ORIO and B. PAVONI (1982). - Radiometric dating and pollutants profiles in a sediment core from the lagoon of Venice. Oceanol. Acta, Proceedings International Symposium on coastal Lagoons, S.C.O.R./I.A.B.O./U.N.E.S.C.O., Bordeaux, France, 8-14 September, 1981, 101-106.

FRIGNANI M., F. FRASCARI, G. QUARANTOTTO and R. POLETTI (1978). - Trace heavy metals in Adriatic Sea sediments of the italian coast from Pesaro to the Po delta. Giorn. Geol., 43, 21-45.

GOLDBERG E.D., J.J. GRIFFIN, V. HODGE, M. KOIDE and H. WINDOM (1979). - Pollution history of the Savannah River estuary.Env. Sc. Technol., 13, 588-594.

GUERZONI S., M. FRIGNANI, P. GIORDANI and F. FRASCARI (1984). - Heavy metals in sediments from different environments (Lagoons, rivers, seabed) of a Northern Adriatic Sea, area, Italy. Environ. Geol., 6 (8), 111-119.

KOIDE M. and K.W. BRULAND (1975). - The electrodeposition and determination of radium by isotopic dilution in sea-water and sediments simultaneously with other natural radionuclides. Anal. Chim. Acta, 75, 1-19.

MENEGAZZO VITTURI L. and S. RABITTI (1980). - Automatic particle-size analysis of sediment fine fraction by Sedigraph 5.000 D. Geol. Appl. Idrogeol., 15, 101-108.

NELSON B.W. (1970). - Hydrography, sediment dispersal and recent hystorical development of the Po River delta. In : Morgan and Shaver Eds., Deltaic Sedimentation. Modern and ancient, S.E.P.M. Spec. Publ., 15, 152-184.

OLIVER B.G. (1973). - Heavy metal levels at Ottawa River sediments. Environ. Sci. Technol., 7, 135-137.

PROVINI A., G.F. GAGGINO, S. GALASSI (1980). - Po e Adige : valutazione statistica della frequenza di campionamento in un programma di monitoraggio. Ingegneria Ambientale. 9 (5).

PROVINI A., G. PACCHETTI (1982). - Po e Adige : Valutazione del carico trofico e tossico convogliato nell'Alto Adriatico. Ingegneria Ambientale, 11, (3).

TASSI PELATI L., C. TRIULZI and S. ALBERTAZZI (1981). - Risultati preliminari sulla radioattività dei campioni biologici ed ambientali prelevati nell'Alto Adriatico. Atti Conv. "Risorse biologiche e inquinamento Marino", Roma 10-11 novembre 1981, 875-890.

TUREKIAN K.K. and K.H. WEDEPHOL (1961). - Distribution of elements in the earth's crust. Geol. Soc. Am. Bull., 72, 174-192.

WILLIAMS S.C., H.J. SIMPSON, C.R. OLSEN and R.F. BOPP (1978). - Sources of heavy metals in sediments of the Hudson River estuary. Mar. Chem., 6, 195-213.

MERCURY AND CADMIUM CONCENTRATIONS IN THREE MARINE BENTHIC ORGANISMS. VARIATIONS ACCORDING TO THE SAMPLING AREAS IN THE CANAL DE CORSE.

by

Dominique BEZARD, André VEGLIA and Raymond VAISSIERE
Centre Scientifique de Monaco
16, boulevard de Suisse
98000 Monte-Carlo (Principauté de Monaco)

Abstract

Calocaris macandreae (Crustacea Decapoda), Molpadia musculus (Holothuria) and Gryphus vitreus (Brachiopoda) have been collected four times per year from 1981 to 1983. at three stations in the Canal de Corse : I1 in the canal, I2 south of the canal, and L north of the canal. The mercury and cadmium contents were measured (A.A.S.), the accuracy of the methods being checked with reference material (I.A.E.A. M.A.-A-1).

For C. macandreae the mercury concentration in the abdomen is roughly twice that in the cephalothorax, while the latter contains significantly more cadmium than the abdomen.

The teguments of M. musculus contain a higher amount of mercury and cadmium than the guts : in the case of mercury, the teguments/guts ratio seems to vary with the origin of the Holothuria : it is 3.4 at station L, 6 at I2, and 17.8 at I1.

G. vitreus appears only at station I1. Mercury and cadmium concentrations are about ten times lower in the shell than in tissues.

The origin of these variations is discussed.

*
* *

Introduction

In the framework of a general research programme on heavy metal dynamics in Mediterranean ecosystems, mercury and cadmium concentrations have been measured in some organisms living in an area between northern Corsica and Capraia Island.

This paper deals with the distribution of these two metals in three benthic animals which are known to have different feeding behaviour : Calocaris macandreae (Crustacea Decapoda), Molpadia musculus (Echinodermata Holothuria), and Gryphus vitreus (Brachiopoda Articulata).

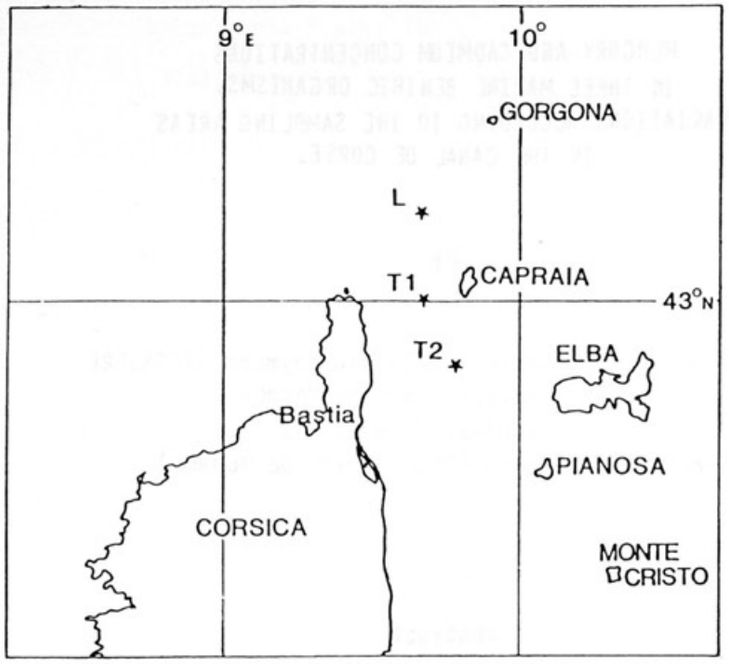


Figure 1. - Location of the sampling sites.

Materials and method

The stations were chosen according to hydrographic conditions, under-water features, and geographical location (figure I). The Tyrrhenian and Ligurian basins communicate through the Canal de Corse, which is narrow, with the sea-bed rising fairly rapidly towards Capraia (East) and Cap Corse (West).

One knows that the Levantine Mediterranean water, which is found between 200 and about 400 metres depth, runs south to north along the Tuscan archipelago apron in the Tyrrhenian Sea, but it is not certain that it always crosses the Canal de Corse to enter the Ligurian Basin. In the middle of the channel, the maximum depth is 370 m and in addition the current sometimes rotates gently in a cyclonic way (KRIVOSHEYA and OVCHINNIKOV, 1973, in VAISSIERE and SEGUIN, 1980). Hence, even if some eastern sea-water contributes to the hydrographic system of the Ligurian Sea in the studied area, there is a certain isolation of the two basins, for at least the deepest layer.

C. macandreae digs short horizontal galleries as it burrows through the first three to five centimetres of the sediment (CARPINE, 1970), which is slightly sandy and seems to contain a fair amount of interstitial water · the animal feeds on small particles and probably also on small preys. M. musculus, like almost all holothurians, lives on the sediment/water interface but it can burrow in the sediment : it is a detritus feeder, scooping up the substratum, pushing it into its mouth and ingesting the surface sediment to digest the organic contents G. vitreus is a true suspension feeder: living on the sediment, it opens its shell and displays its lophophore to capture particles in the water, or it feeds by means of pinocytosis mechanisms.

Individuals of the three species were collected by using a beam-trawl at different periods of the year from November 1981 to October 1983. They were rapidly stored and deep-frozen in small polyethylene flasks previously conditioned with HNO_3 and washed with high-purity demineralized water. For mineralization, the samples were wet-ashed by adding small amounts of 65 % HNO_3 and 35 % H_2O_2. The solutions were analysed by atomic absorption spectroscopy. A graphite furnace was used for the determination of cadmium, while the cold vapour technique was applied to the determination of mercury. The accuracy of the method was estimated by analysing a biological reference material (International Atomic Energy Agency, M.A.-A-1 dried copepods).

Sampling station	Sampling date	Sample	Mercury concentration (µg/g FW)*	Cadmium concentration (µg/g FW)*
T1	03/82 (5 indiv. pooled)	Soft parts	0.13 ± 0.03**	0.08 ± 0.01**
		Shell	0.03 ± 0.001	0.025 ± 0.005
	04/82 (1 indiv. pooled)	Whole	0.03 ± 0.001	0.02 ± 0.004
	12/82 (17 indiv. pooled)	Soft parts	0.10 ± 0.03	0.08 ± 0.01
		Shell	0.01 ± 0.001	0.03 ± 0.005

TABLE 1 - Mercury and cadmium concentrations for *G. vitreus*. (Samples have been pooled to get 1 to 2 g of fresh material)

Sampling station	Sampling date	Sample	Mercury concentration (µg/g DW)*	Cadmium concentration (µg/g DW)*
L	04/82 (1 indiv. pooled)	Tegument	0.78 ± 0.04**	1.31 ± 0.23**
		Viscera	0.23 ± 0.01	0.48 ± 0.08
T1	03/82 (1 indiv. pooled)	Tegument	4.77 ± 0.32	0.63 ± 0.08
		Viscera	0.27 ± 0.01	0.34 ± 0.05
T2	11/81 (13 indiv. pooled)	Tegument	1.32 ± 0.07	0.39 ± 0.05
		Viscera	0.42 ± 0.02	0.24 ± 0.04
	03/82 (1 indiv. pooled)	Tegument	6.61 ± 0.36	2.84 ± 0.68
		Viscera	0.73 ± 0.04	0.45 ± 0.06
	04/82 (9 indiv. pooled)	Tegument	3.73 ± 0.20	0.38 ± 0.04
		Viscera	0.67 ± 0.04	0.23 ± 0.03

TABLE 2 - Mercury and cadmium concentrations for *M. musculus*. (Samples have been pooled to get about 1 g of dry matter)

Dates	Sampling areas	Tegument/viscera ratio Mercury	Cadmium
04/82	L	3.4	2.7
03/82	T1	17.7	1.9
11/81	T2	3.1	1.6
03/82		9.0	6.3
04/82		5.6	1.6

TABLE 3 - Tegument/viscera ratio for *M. musculus* at different stations.

Sampling station	Sampling date	Sample	Mercury concentration (µg/g FW)*	Cadmium concentration (µg/g FW)*
L	11/81 (3 ind.)	Whole	0.29 ± 0.06**	0.16 ± 0.04**
	03/82 (4 ind.)	Whole	0.37 ± 0.08	0.15 ± 0.04
	(5 ind.)	Abdomen	0.49 ± 0.09	0.12 ± 0.03
		Cephalothorax	0.31 ± 0.06	0.17 ± 0.04
	04/82 (3 ind.)	Whole	0.56 ± 0.06	0.12 ± 0.03
	(6 ind.)	Abdomen	0.25 ± 0.07	0.11 ± 0.01
		Cephalothorax	0.14 ± 0.04	0.18 ± 0.03
	09/82 (4 ind.)	Whole	0.29 ± 0.06	2.38 ± 0.27
	(7 ind.)	Abdomen	0.21 ± 0.06	0.54 ± 0.12
		Cephalothorax	0.12 ± 0.03	0.83 ± 0.08
	12/82 (3 ind.)	Whole	0.32 ± 0.09	0.28 ± 0.05
	(4 ind.)	Abdomen	0.42 ± 0.11	0.17 ± 0.04
		Cephalothorax	0.17 ± 0.05	0.29 ± 0.05
T2	11/81 (2 ind.)	Whole	0.21 ± 0.06	0.20 ± 0.04
	(8 ind.)	Abdomen	0.35 ± 0.09	0.24 ± 0.04
		Cephalothorax	0.21 ± 0.06	0.30 ± 0.06
	03/82 (6 ind.)	Whole	0.17 ± 0.04	0.24 ± 0.06
	04/82 (4 ind.)	Whole	0.18 ± 0.04	0.20 ± 0.04
	09/82 (4 ind.)	Whole	0.27 ± 0.06	0.25 ± 0.03
	(7 ind.)	Abdomen	0.31 ± 0.07	0.12 ± 0.03
		Cephalothorax	0.13 ± 0.03	0.27 ± 0.03
	12/82 (3 ind.)	Whole	0.33 ± 0.07	0.36 ± 0.03
	(6 ind.)	Abdomen		0.26 ± 0.03
		Cephalothorax		0.32 ± 0.06

TABLE 4 - Mercury and cadmium concentrations for *C. macandreae*. (Animal length > 30 mm). (Samples have been pooled to get 1 to 2 g of fresh material)

* FW = fresh weight - DW = dry weight.
** The given uncertainties correspond to a confidence level equal to 0.95.

Results

Gryphus vitreus. Table 1 shows that whatever the season, mercury and cadmium accumulations are always higher in the soft parts (0.13 µg Hg/g and 0.08 µg Cd/g FW) than in the shells (0.01 µg Hg/g and 0.03 µg Cd/g FW).

Molpadia musculus. Table 2 shows that no apparent relation exists between metal concentrations in the tegument and the viscera, though they are always higher in the former. For mercury the tegument/viscera ratio is greatly increased at station T1, and to a lesser extent at station T2, whereas for cadmium this ratio was roughly constant at all stations, except at T2 in March 1982 (table 3).

Calocaris macandreae. Table 4 shows that mercury concentration in the abdomen is always higher (roughly double) than in the cephalothorax, with a relation expressed by the following linear regression equation :

$$y = - 0.017 + 0.581 x \quad (r = 0.85)$$

where y is the mercury concentration (ug/g FW) in the cephalothorax and x is the concentration (ug/g FW) in the abdomen (figure II). The cadmium concentration gives the opposite relation between the abdomen and the cephalothorax, which is expressed by the following linear regression equation :

$$y = 0.017 + 1.435 x \quad (r = 0.97)$$

where y is the cadmium concentration (µg/g FW) in the cephalothorax and x is the concentration (µg/g FW) in the abdomen.

The results are given for individuals with a length equal to or over 30 mm. Differences can be noticed inside or between the stations. For the whole body, mercury concentrations are about twice as high at station L as at station T2 in spring and they are about the same in September and in December. Inversely cadmium concentrations (whole body) are higher at station T2 than at station L in all seasons, except for a very high value in September 1982.

Discussion

Gryphus vitreus. Since these animals are well known to be suspension feeders one may expect the uptake of metal by these organisms to take place mainly by filtration and subsequent ingestion of small suspended particles (HYMAN, 1959). However, another important way of uptake may be pinocytosis. FOWLER et al. (1974, 1978) found for the Mediterranean mussel Mytilus galloprovincialis a concentration distribution of cadmium and mercury similar to our results with G. vitreus. According to these authors, the uptake of cadmium by adsorption appears to be a relatively slow process : in that case, the major part of the cadmium body burden would be achieved through the food-chain, but in the case of G. vitreus there are some other ways (e.g. pinocytosis) that should be taken into consideration as metal accumulation mechanisms in tissues. Animals living in the vicinity of the sediment could be exposed to substances released by the sediment. FOWLER et al. (1978) also showed that methylmercury is accumulated in mussels from both food and water to a greater extent than inorganic mercury. Our analysis did not give us any information as to the chemical form of this metal.

Molpadia musculus. The holothurian viscera analysis shows concentration values of cadmium and mercury that may be considered in some way as representative of the uppermost layer of the sediment, since these animals are simply feeding by ingesting the sediment around them. Station T2 samples seem to exhibit higher mercury concentrations than stations L and T1 samples : 0.42 to 0.73 µg/g for T2 against 0.23 µg/g for L and 0.27 µg/g for T1 (DW). The tegument of the Holothuria was subject to important variations in metal concentration, particularly for mercury in April 1982 at stations L and

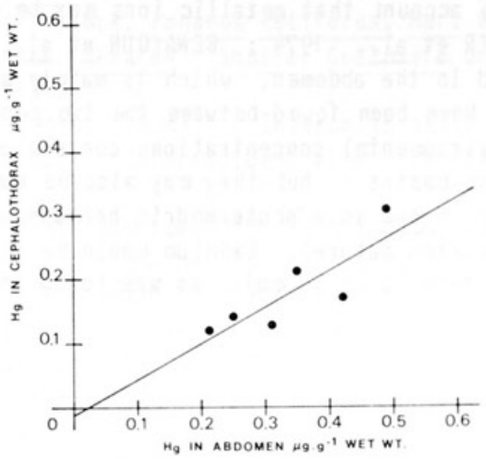


Figure 2. - Cephalothorax/abdomen linear regression equation for Mercury in Calocaris macandreae.

T2. The cadmium values were also high in March 1982 for T2 and in April 1982 for L. However, we have to keep in mind that the part analysed as tegument is not completely separated from the other organs (genital glands, muscles, etc.) which - as shown by THOMPSON et al., (1978) in EISLER (1981), who found values of 1.7 µg/g of cadmium (DW) in the muscles of Molpadia intermedia - may contribute to the high values observed . EISLER (1981) determined mercury levels of the order of 0.9 µg/g (DW) in echinoderms from non polluted areas. Many echinoderms, and especially holothurians, are prime movers of sediment and probably have an important place in the cycling of trace metals in the sea.

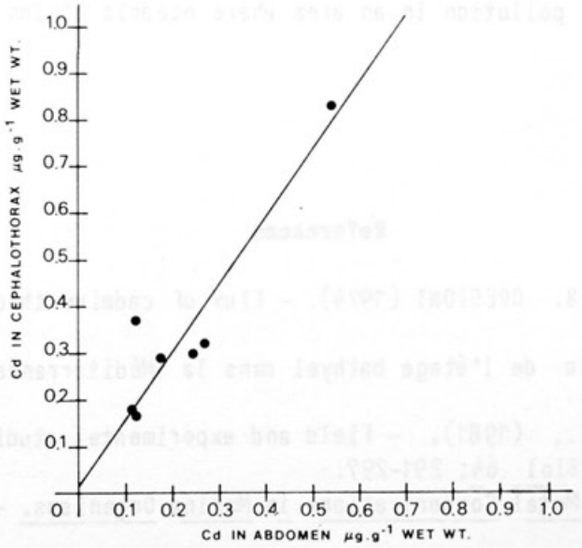


Figure 3. - Cephalothorax/abdomen linear regression equation for Cadmium in Calocaris macandreae.

Calocaris macandreae. Our results show that cadmium and mercury accumulations have different localizations in this decapod. NIMMO et al., (1977), FOWLER et al., (1978) and DAVIES et al. (1981) have shown that cadmium is selectively localized in decapod crustacean tissues in the following decreasing order : hepatopancreas, exoskeleton, muscle. In C. macandreae cadmium shows greater concentration in the cephalothorax, most certainly at the level of the hepatopancreas that has an

important role in metal dynamics due to the presence of metallothioneins (RAINBOW et al., 1979) However, we also have to take into account that metallic ions may be concentrated by adsorption on the exoskeleton and the gills (FOWLER et al., 1974 ; BENAYOUN et al., 1974 ; DAVIES et al., 1981). Mercury is particularly concentrated in the abdomen, which is mainly a muscular tissue. The difference in metal concentrations that have been found between the two populations at stations L and T2 may reflect real variations in environmental concentrations correlated with different hydrographic regimes and relative isolation of the basins : but they may also be due to differences in body size and sexual maturity, since C. macandreae is a proterandric hermaphrodite animal (the male gametes mature and are shed before female gametes mature). Cadmium could be present in higher concentrations in females, which are large size animals (> 30 mm), as was found in the case of the crab Cancer pagurus (DAVIES et al., 1981).

Conclusion

The values found for mercury and cadmium concentrations in brachiopods are lower than the values found for the two other species. The metal uptake for G. vitreus comes essentially from the water and the small suspended particles. The main part of mercury and cadmium accumulated by M. musculus and C. macandreae problably comes from the sediment. The sediment "sink" may ultimately act as a "source" of pollutants for the bottom dwelling animals because of the release of metals to the bottom water. Interstitial water probably plays an important role in the mechanism (RAY et al., 1980).

The biology of the species has a great influence on the uptake of metals by marine organisms. As one can see, at station T2 for March and April 1982, the uptake of mercury by C. macandreae decreased while the uptake by M. musculus increased. It is interesting to speculate on the actual mechanism of uptake, whether it occurs by cuticle adsorption or absorption by internal organs (BRYAN, 1971, in RAY et al., 1980). This point, as well as the physiological state of the organism (age, sex, maturation, etc.), are very important to know in order to understand to what degree these organisms can reflect the level of the environmental pollution in an area where oceanic basins have different hydrographic conditions.

References

BENAYOUN G., S.W. FOWLER and B. OREGIONI (1974). - Flux of cadmium through Euphausiids. Mar. Biol. 27, 205-212.

CARPINE C. (1970). - Ecologie de l'étage bathyal dans la Méditerranée occidentale. Mem. Inst. Oceanog. 2, 146 p.

DAVIES I.M., G. TOPPING et al., (1981). - Field and experimental studies on cadmium in the edible crab Cancer pagurus. Mar. Biol 64, 291-297.

EISLER Ronald (1981). - Trace Metal Concentrations in Marine Organisms. - New York : Pergamon Press. 685 p.

FOWLER S.W. and G. BENAYOUN (1974). - Experimental studies on cadmium flux through marine biota. In: Comparative studies of Food and Environmental Contamination, 159-178. Vienna : International Atomic Energy Agency.

FOWLER S.W., M. HEYRAUD and J. LA ROSA (1978). - Factors affecting methyl and inorganic mercury dynamics in mussels and shrimps. Mar. Biol. 46, 267-276.

GREEN F.A., J.W. ANDERSON et al., (1976). - Effects of mercury on the survival, respiration and growth of postlarval white shrimps, Penaeus setiferus. Mar. Biol. 37, 75-81.

HYMAN L.H. (1959). - The Intervertebrates : Smaller Coelomate Groups. vol 5 New York, London : Mc Graw-hill Book Co., Inc 783 p.

International Atomic Energy Agency (1978). - Intercalibration of analytical methods on marine environmental samples. Monaco : International Atomic Energy Agency 23 p. (Progress report : 19).

NIMMO Del Wayne, Donald V. LIGHTNER and Lowell H. BAHNER (1977). - Effects of cadmium on the shrimps Penaeus duorarum, Palaemonetes pugio and Palaemonetes vulgaris. In : Physiological Response of Marine Biota to Pollutants New York : Academic Press. pp 131-183.

RAINBOW P.S. and A.G. SCOTT (1979). - Two heavy metal-binding proteins in the midgut gland of the crab Carcinus maenas. Mar. Biol 55, 143-150.

RAY S., D. Mc LEESE and D. PEZZACK (1980). - Accumulation of cadmium by Nereis virens. Arch. Environm. Contam. Toxicol. 9, 1-8.

THURBERG F.P., A. CALABRESE, et al. (1977). - Response to the lobster Homarus americanus to sublethal levels of cadmium and mercury. In : Physiological Response of Marine Biota to Pollutants. New York : Academic press. pp. 185-197.

VAISSIERE R. and G. SEGUIN (1980). - Etude préliminaire de peuplements de Copépodes (juillet 1977) en relation avec l'hydrologie des mers Tyrrhénienne et Ionienne. Oceanologica Acta 3(1), 17-29

WHITE S.L. and RAINBOW P.S. (1982). - Regulation and accumulation of copper, zinc and cadmium by the shrimp Palaemon elegans. Mar. ecol. Prog. Ser. 8, 98-101.

BIOACCUMULATION DES METAUX Cr, Cd, Ni, ET Cu DANS LE GOLFE DE GERAS, ILE DE LESVOS, MER EGEE, GRECE (NOTE PRELIMINAIRE)

par

Angélique-Vassiliki CATSIKI et Hélène FLOROU
Institut de Recherches Océanographiques et Halieutiques
Agios Kosmas, Hellinikon, Athènes 16604 (Grèce)

Résumé

Au cours de cette étude préliminaire nous examinons, par la méthode d'absorption atomique, les concentrations des métaux Cr, Cd, Ni et Cu dans plusieurs espèces marines prélevées dans le golfe de Géras en novembre 1983, où est implantée une grande tannerie.

Abstract

In this preliminary study the concentrations of heavy metals Cr, Cd, Ni and Cu are determined by atomic absorption (flame atomization) in selected species collected from the gulf of Geras on November 1983, in the coast of which a large tannery is located.

*
* *

Introduction

Le golfe de Géras est un bassin allongé, semi-fermé, de faible profondeur et à lent rythme de renouvellement des masses aquatiques. Sur ses côtes est implantée depuis 1833, une des plus grandes tanneries de la péninsule balkanique. La production accrûe et le traitement chimique actuel des cuirs a provoqué, les dernières années, des effets défavorables (4).

Ce travail fait partie de l'étude entreprise par l'Institut de Recherches Océanographiques et Halieutiques (I.O.K.A.E.) dans la région (3), afin de juger son état écologique et de répondre à la question posée sur la comestibilité des produits halieutiques.

Méthodologie - Analyses chimiques

Le travail comporte deux phases. Dans la première, qui consiste en une première approche du milieu, des échantillons de plusieurs espèces comestibles (benthos et necton rencontrées dans le golfe de Géras, ainsi que quelques espèces caractéristiques, ont été prélevées en novembre 1983 dans un réseau de 11 stations (fig. 1) en vue de déterminer dans leur chair les niveaux de concentration des métaux et spécialement du chrome.

Dans la deuxième phase, six espèces benthiques (Ostrea edulis, Paracentrotus lividus, Pinna nobilis, Mytillus galloprovincialis, Posidonia oceanica et Cymodocea nodosa) ont été retenues en vue d'être étudiées à long terme dans six stations (fig. 1). L'étude comporte en outre une carthographie des biocénoses, monitoring, relations trophiques, bio-accumulation sélective des métaux par divers organes, rôle de l'âge, de la taille, de la localisation, du substrat étude de la teneur en métaux des sédiments superficiels.

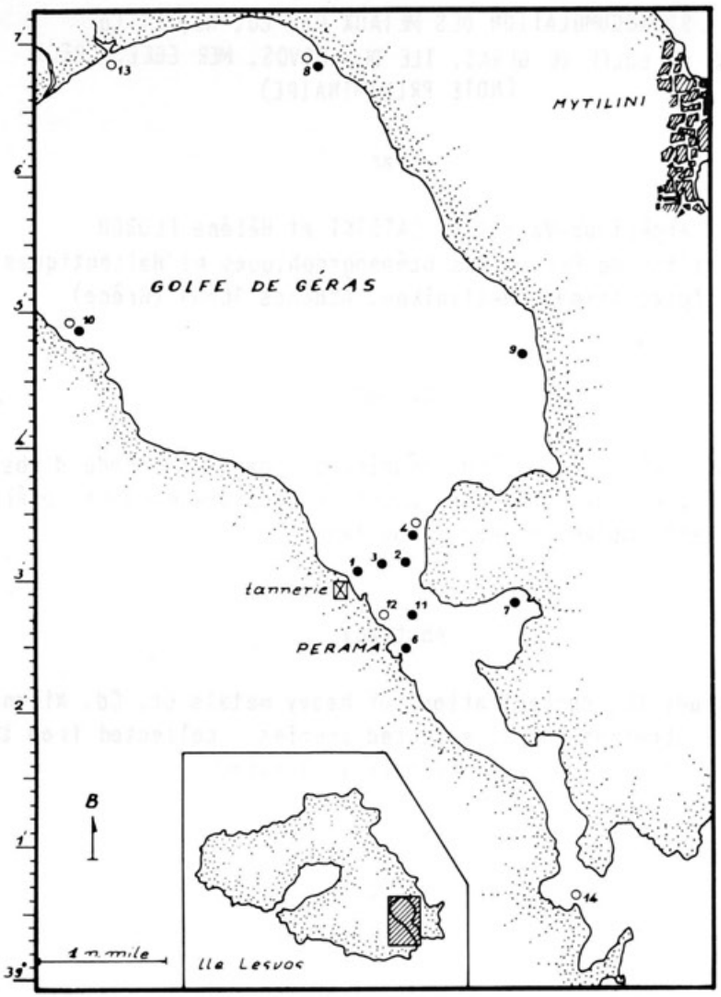


Figure 1. - Stations de prélèvements.
● Stations de l'étude à court terme.
○ Stations de l'étude à long terme.

L'échantillonnage du benthos en novembre 1983 a été effectué à l'aide d'un outil trainant utilisé par les pêcheurs locaux, tandis que dans la deuxième phase les échantillons sont recueillis en plongée autonome.

Les échantillons sont conservés au congélateur, ils sont ensuite lyophilisés et homogénéisés : tout traitement est conforme aux normes suggérées par la F.A.O. (2).

Nous avons travaillé sur des échantillons mixtes sauf dans le cas des poulpes où un individu d'une longueur de 30 cm environ, a été analysé. Les échantillons des gastéropodes comprenaient trois spécimens de 6 à 8 cm et celui des bivalves six individus de 3 à 5,5 cm, tandis que les échantillons de Paracentrotus lividus contenaient huit oursins de 4 à 6 cm de diamètre.

La détermination du Cr, Cd, Ni et Cu a été réalisée par spectrophotométrie d'absorption atomique avec flamme (air-acétylène) sur des solutions obtenues par la minéralisation d'environ 0,5 gr de chair par 5 ml de HNO_3 dans des bombes teflon pendant 6 heures (5). La précision de cette méthode est d'environ 5 % (exercice d'intercalibration de l'U.N.E.P. - 1984).

Résultats - Discussion

Dans ce travail sont présentés les résultats de la première phase de l'étude du golfe, basée sur le prélèvement de novembre 1983 et un nombre d'échantillons restreint.

TABLEAU 1

Espèces	$Cr_{(ppm)}$		$Cd_{(ppm)}$		$Ni_{(ppm)}$		$Cu_{(ppm)}$	
	A	B	A	B	A	B	A	B
Eledone moschata	1.60	*	5.80	0.80	3.00	2.80	26.4	23.2
Ostrea edulis	15.80 (2)		2.80 (2)		5.80 (2)		135.80 (2)	
Arca sp.	5.80 (5)		1.40 (5)		4.20 (5)		5.10 (5)	
Murex trunculus	3.70	3.50 (3)	6.10	13.90 (3)	3.50	6.00 (3)	532.00	110.50
Murex brandaris	5.20 (3)	13.40	2.90 (3)	3.00	7.10 (3)	3.80	71.40	53.40
Paracentrotus lividus	1.60 (5)	*	1.10 (5)	0.40	5.90 (5)	5.00	6.60 (5)	1.60
Dasycladus clavaeformis	25.50 (4)		1.50 (4)		21.80 (4)		5.00 (4)	

Concentrations en Cr, Cd, Ni et Cu (µg/g de poid sec) de quelques espèces prélevées dans deux régions distinctes du golfe de Géras en Nov. 1983.
(*) : concentration au-dessous du seuil de détection de l'A.A.

Les résultats des analyses chimiques, exprimés en µg par g de poids sec de chair (ppm), sont reportés sur les tableaux 1 et 2.

TABLEAU 2

Espèce	$Cr_{(ppm)}$	$Cd_{(ppm)}$	$Ni_{(ppm)}$	$Cu_{(ppm)}$
Mullus surmuletus	*	0.80	3.00	2.20
Mullus barbatus	*	0.80	(106.00)	2.60
Boops boops	*	0.80	3.00	3.00
Boops salpa	*	0.80	10.60	
Trachurus trachurus	15.20	1.00	13.60	2.60
Gobius sp.	16.20	1.00	10.60	3.60
Maena chryselis	6.20	1.20	6.40	2.40
Serranus scriba	*	1.30	22.00	2.40
Diplodus annularis	2.20	0.80	6.80	2.80
Pagellus erithrinus	*	0.80	9.80	2.20

Concentrations en ppm de Cr, Cd, Ni et Cu de quelques spécimens de poissons pêchés dans le golfe de Géras en Nov. 1983.
(*) : concentration au-dessous du seuil de sensibilité de l'appareil AA.

Les dragages à proximité immédiate de la sortie du conducteur des rejets de la tannerie n'ont pas fourni d'échantillons de macro-faune et macro-flore benthiques. On a constaté, en outre, que les rejets de l'usine contiennent une phase solide sur laquelle on a détecté des traces métalliques : le Cr y est extrêmement élevé (de l'ordre de 15,000.00 ppm), tandis que le Ni et le Cu se rencontrent en des concentrations assez basses (27.60 et 30.20 pppm respectivement) et le Cd en traces (0.70 ppm) :

les rejets de la tannerie ne semblent donc pas contribuer à la pollution du golfe par le Cd, le Cu et le Ni - par contre la tannerie semble être la source principale des ions de Cr.

Sur le tableau 1 sont données pour quelques espèces choisies comme caractéristiques deux valeurs de concentrations qui correspondent aux moyennes de contenance en traces métalliques des échantillons prélevés dans deux régions différentes la région A qui comprend les stations de prélèvements situées dans un rayon d'un kilomètre des sorties des conducteurs des rejets de la tannerie (st 1, 2, 3, 4, 5, 6, 7 et 11) et la région B qui comprend des stations plus éloignées (st 8, 9 et 10). De cette manière, compte tenu de la différence de la qualité de l'eau entre les deux régions (4) on a voulu rechercher une relation probable entre la contenance en traces métalliques des échantillons de certaines espèces et leur distance du conducteur de la tannerie.

Cette relation est positive pour les poulpes et les oursins - les échantillons d'oursins provenant des stations les plus proches du point de rejet du conducteur avaient assimilé le chrome d'une manière plus importante (Région A - st 1 : 36.00 ppm st 2 : 0.80-1.60 ppm, st 3 : 1.70 ppm, st 6 - 3.00 ppm - Région B - st 8 : 0.20 ppm). En effet, cette espèce présente l'avantage d'avoir une large répartition dans le golfe, d'etre abondante, comestible, facile comme matériel d'analyse et d'avoir un régime alimentaire bien déterminé : strictement herbivore Ceci nous a conduit à l'utiliser pour la deuxième phase de l'étude.

Pour cette étude à long terme, afin de déterminer l'accumulation des métaux par le premier échelon de la chaîne trophique, nous avons choisi les phanérogames Posidonia oceanica et Cymodocea nodosa qui sont abondants dans le golfe et sont connus dans la bibliographie comme matériel de recherche (1).

Mytillus galloprovincialis présente un grand intérêt comme organisme benthique étudié par de nombreux laboratoires. En outre, les bivalves Ostrea edulis et Pinna nobilis également sélectionnées, présentent une large répartition dans le golfe et ont un régime trophique similaire.

Parmi les poissons étudiés (tableau 2) nous avons choisi, pour une étude statistique de bioaccumulation des métaux par les poissons d'age et sexe différents, pêchés tous les mois dans le golfe de Géras, les espèces : Gobius sp. comme espèce benthique indicatrice de la pollution de la région. Trachurus trachurus, Maena chryselis, Diplodus annularis comme espèces accumulant le Cr (tableau 2) et Mullus barbatus comme espèce proposée par la F.A.O. (2).

Nous considérons que cette étude nous permettra d'une part de savoir si les produits piscicoles sont réellement comestibles et d'autre part d'étudier l'influence du chrome et d'autres traces métalliques sur des spécimens d'espèces caractéristiques appartenant à divers niveaux trophiques.

Références

1 - H. AUGIER, G. GILLE, G. RAMONDA (1976). - Recherche sur la pollution mercurielle en rade d'Hyères et dans l'archipel des Stoechades (Médit France) I - Teneur en mercure des eaux des sédiments et les phanérogames marines du milieu lagunaire dans l'Anse de Port Cros. - Travaux Scientifiques du Parc National de Port Cros. Tome II.

2. - M. BERNHARD (1976). - Manual of methods in aquatic environmental research. Per 3. Sampling and analysis of biological material. - FAO Fish. Techn. Pap. no 158.

3. - I.O.K.A.E. (1984). - Etude Océanographique du Chenal de Mytilini et étude Ecologique du golfe de Géras - Rapport Technique de I.O.K.A.E. (en grec).

4. - M. SCOULLOS, M. MIMIKOS, M DASSENAKIS, L. BACHAS and E STAURIANOUDAKIS (1982). - Trace metals and petroleum aromatic hydrocarbons in the gulf of Gera Lesvos Isl., Greece - VIes Journées Etud. Pollutions. Cannes, C.I.E.S.M.

5. UNEP (1982) - Determination of total Cd, Zn, Pb and Cu in selected marine organisms by atomic absorption spectrophotometry. - Reference methods for marine pollution studies no 11. July 1982.

MONITORING OF HEAVY METALS IN SUPERFICIAL SEDIMENT AND SOME MARINE ORGANISMS FROM THE WESTERN MEDITERRANEAN COAST

by

A.R. DE LEON, J. MAS,
J. GUERRERO and A. JORNET
Centro Oceanografico Mar Menor,
Instituto Espanol de Oceanografia
San Pedro del Pinatar, Murcia (Espana).

Abstract

During 1983, and with a periodicity of every three months, samples of organisms (Mullus barbatus, Mytilus galloprovincialis, Chamalea gallina, Donax trurculus and superficial sediment were analysed for heavy metals. Hg, Cd, Pb and Zn in the sediments and Hg, Cd and Pb in the organisms.

The survey area was from Valencia to Cartagena and the high levels from three sites, Valencia, Cartagena and Portman, showed a significant influence of industrial activity.

Introduction

Within the framework of MED POL from december 1982 to december 1983 we carried out a systematic monitoring of heavy metals in superficial sediment and organisms. The survey covered nine sites of the western Mediterranean coast from Castellon to Cartagena and the superficial sediment was analysed for Hg, Cd, Pb, Zn, Cu and the organisms for Hg, Cd and Pb.

Method

The location of the stations from each site are shown in figure 1. At each station, which were half a mile apart, superficial sediment was collected using a Shipek grab avoiding contamination. The fractions smaller than 63 µm were washed three times with distilled water and dried at 100-150° C for 24 h. Subsamples of 1 g were digested following the method of KRISHNAMURTY et al. (1976).

The organisms collected have been Mullus barbatus, Mytilus galloprovincialis, Chamelea (Chamelea) gallina and Donax trunculus.

Mytilus galloprovincialis was collected directly by us and were held in aerated sea-water for 96 hours to allow depuration. The rest of the organisms were collected through commercial fisheries and all the samples were deep frozen until used. The frequency of sampling was every three months; soft tissues were lyophilized and then digested following BERNARD (1976).

Analysis of samples and blanks were carried out with a Perkin-Elmer 603 Atomic Absorption spectrophotometer for Cd, Zn, Cu and Pb and with a 300 S Perkin-Elmer Atomic Absorption spectrophotometer for Hg, using background correction when necessary. Standard additions were used to check the recovery of metals.

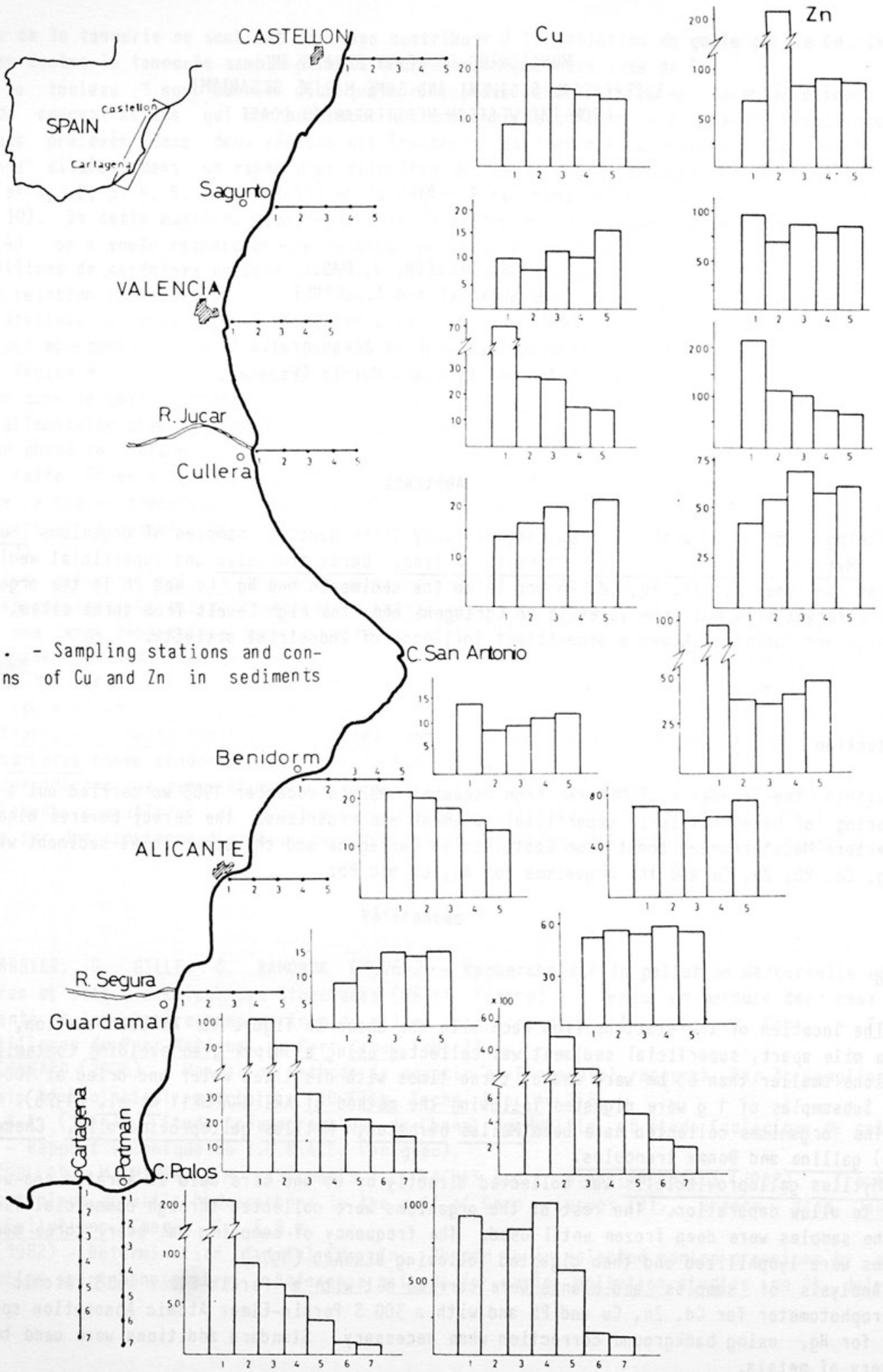


Figure 1. - Sampling stations and concentrations of Cu and Zn in sediments (ppm).

To check on the analytical method a sample of N.B.S. Standard Reference Material 1645 River sediment was analysed with the following results:

Element	Our results ug/g. dry wt.	N.B.S. values ug/g. dry wt.
Zn	1,720 ± 170	1,635 ± 34
Cu	109 ± 19	111 ± 4
Pb	714 ± 28	726 ± 16
Cd	10.2 ± 1.5	8.2 ± 0.3
Hg	1.1 ± 0.5	1.0 ± 0.1

Our analytical methodology was also consolidated in the following intercalibration exercise: 6th I.C.E.S. traces metal intercomparison exercise for cadmium and lead in biological tissue. Cooperative Research Report n° 111 February 1982.

Results and discussion

The concentrations of Hg, Cu, Zn, Pb and Cd in the superficial sediment of the areas sampled may be found in figures 1 and 2.

High concentrations of Hg are found in the stations closest to the coast in the sites near industrial effluents: Cartagena (20.7 ppm), Portman (13.1 ppm), Alicante (7.60 ppm), Valencia (6.80 ppm) and Sagunto (7.44 ppm). The Hg concentration in the Segura river mouth is also quite high 10.1 ppm. These concentration decrease with the distance from the coast.

High values of Cu are found in Cartagena (226 ppm), Portman (110 ppm) and Valencia (118 ppm). In the profiles opposite the river mouths, with low concentration at every station, the concentrations of Cu increase with the distance to the coast.

The concentration of Zn, Pb and Cd are very high in Cartagena (1145 ppm for Zn, 938 ppm for Pb and 1.69 ppm for Cd), Valencia (337 ppm for Zn, 151 ppm for Pb and 0.76 ppm for Cd) and Portman (6481 ppm for Zn, 3377 ppm for Pb and 10.40 ppm for Cd). In this last site the discharge from the exploitation of a lead-zinc mine has heavily polluted the sediment. (A.R. DE LEON et al., 1982).

Table I shows the concentrations of heavy metals for each organism in the different areas sampled. The accumulation of Cd and Pb in molluscs follow the same pattern as their concentrations in the sediment.

Molluscs exposed to the influence of industrial effluents show high levels. Mytilus galloprovincialis collected from Cartagena, Portman and Valencia showed high concentrations of Cd and Pb and striped venus (Chamelea gallina) collected near Portman had 1.90 ppm of Cd and 105.4 ppm of Pb.

The levels of Hg in M. barbatus were higher than in the molluscs with maximum concentrations in Valencia and near the river mouth.

We did not observe any significant seasonal variation or correlation between body burden of heavy metals and length or weight.

Conclusions

Industrial effluents have an impact on the levels of heavy metals in the sediment and in the organisms. The concentrations of Hg, Cu, Pb and Zn are high on the stations closest to the coast in

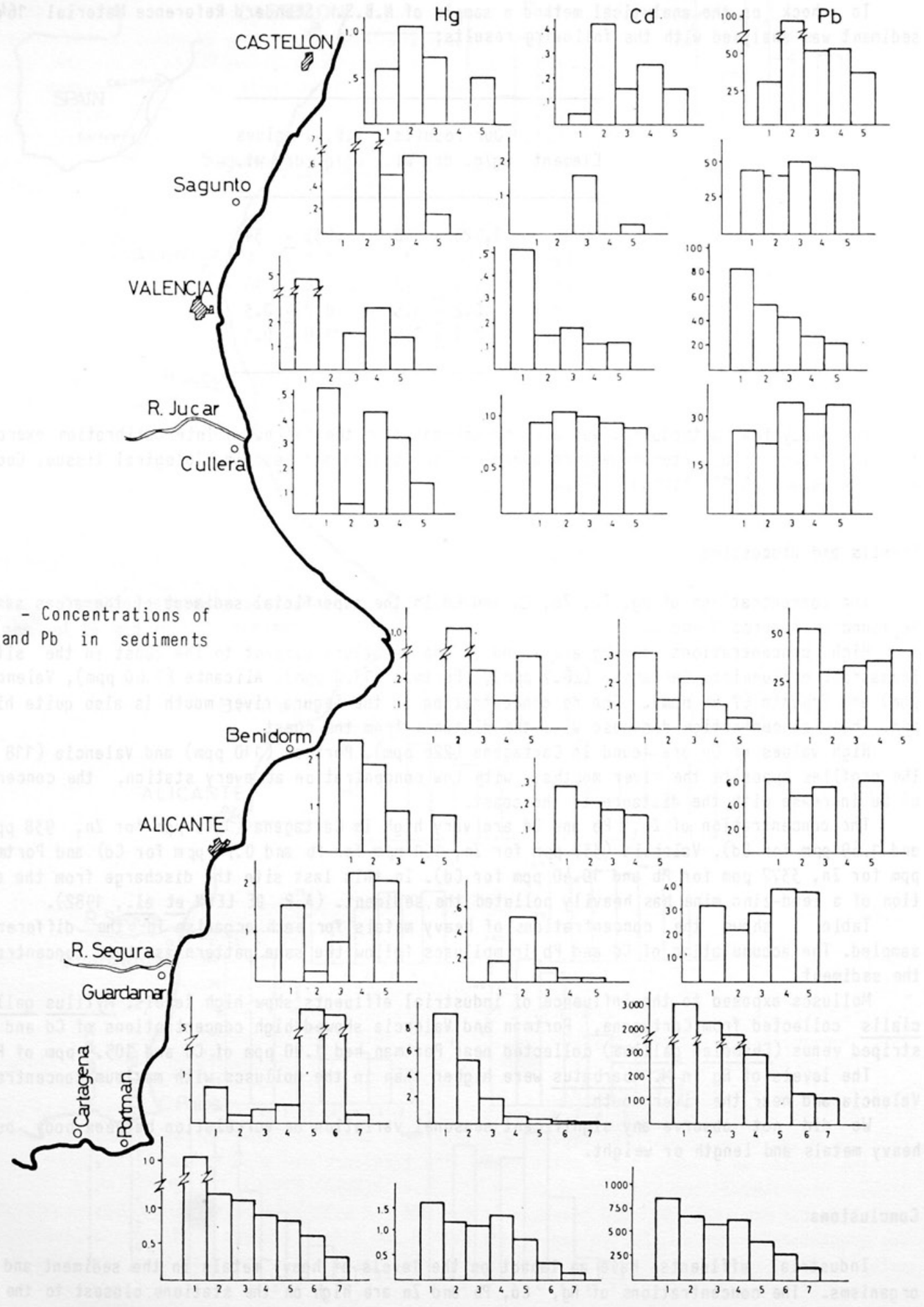


Figure 2. - Concentrations of Hg, Cd and Pb in sediments (ppm).

METAL	SPECIES	SAMPLING SITE & AREA		n	µg/Kg F W Mean	Range
Hg	MULLUS BARBATUS	Cartagena	I	24 H	93	3-200
		Guardamar	III	18 H	60	30-100
		Alicante	III	23 H	32	30-40
		Cullera	II	24 H	80	20-140
		Valencia	II	23 H	232	20-620
		Sagunto	II	18 H	80	70-100
		Castellon	II	28 H	154	60-310
	CHAMELEA GALLINA	Portman	I	19	10	
		Guardamar	III	60 H	<10	<10-<10
		Cullera	II	90 H	13	<10-20
		Valencia	II	60 H	290	10-570
		Sagunto	II	60 H	15	10-20
		Castellon	II	60 H	15	10-20
	MYTILLUS GALLOPRO-VINCIALIS	Cartagena	I	90 H	23	20-30
		Portman	I	60 H	20	20-20
		Guardamar	III	60 H	90	90-90
		Alicante	III	110 H	30	10-50
		Benidorm	III	60 H	<10	<10-<10
		Cullera	II	121 H	17	<10-30
		Valencia	II	60 H	35	20-50
		Castellon	II	90 H	10	10-10
	DONAX TRUNCULUS	Guardamar	III	30	10	
		Cullera	II	30	18	
		Valencia	II	90 H	13	10-20
		Sagunto	II	30	20	
		Castellon	II	60 H	15	10-20

METAL	SPECIES	SAMPLING SITE & AREA		n	µg/Kg F W Mean	Range
Cd	MULLUS BARBATUS	Cartagena	I	24 H	7	1-23
		Guardamar	III	18 H	6	2-11
		Alicante	III	23 H	2	1-4
		Cullera	II	24 H	2	1-4
		Valencia	II	23 H	5	3-8
		Sagunto	II	18 H	7	1-14
		Castellon	II	28 H	5	1-13
	CHAMELEA GALLINA	Portman	I	19	1900	
		Punta Espada	I	315 H	500	340-690
		Guardamar	III	60 H	72	46-99
		Cullera	II	90 H	24	9-34
		Valencia	II	60 H	37	24-50
		Sagunto	II	60 H	19	11-27
		Castellon	II	60 H	20	18-23
	MYTILLUS GALLOPRO-VINCIALIS	Cartagena	I	90 H	400	160-650
		Portman	I	60 H	940	930-950
		Guardamar	III	60 H	100	70-130
		Alicante	III	110 H	113	41-174
		Benidorm	III	60 H	123	110-136
		Cullera	II	121 H	61	35-90
		Valencia	II	60 H	86	53-120
		Castellon	II	90 H	67	38-100
	DONAX TRUNCULUS	Guardamar	III	30	6	
		Cullera	II	30	5	
		Valencia	II	90 H	22	6-41
		Sagunto	II	30	12	
		Castellon	II	60 H	8	5-11

METAL	SPECIES	SAMPLING SITE & AREA		n	µg/Kg F W Mean	Range
Pb	MULLUS BARBATUS	Cartagena	I	24 H	17	10 30
		Guardamar	III	18 H	17	10 23
		Alicante	III	23 H	13	10-20
		Cullera	II	24 H	146	10-540
		Valencia	II	23 H	10	10-12
		Sagunto	II	18 H	22	10-30
		Castellon	II	28 H	17	10-20
	CHAMALEA GALLINA	Portman	I	19	105400	
		Punta Espada	I	315 H	1650	590-4640
		Guardamar	III	60 H	40	20-60
		Cullera	II	90 H	56	20-110
		Valencia	II	60 H	190	130-250
		Sagunto	II	60 H	60	30-90
		Castellon	II	60 H	70	40-100
	MYTILLUS GALLOPRO-VINCIALIS	Cartagena	I	90 H	2013	310-4600
		Portman	I	60 H	94000	90600-97400
		Guardamar	III	60 H	495	290-700
		Alicante	III	110 H	520	330-700
		Benidorm	III	60 H	995	840-1150
		Cullera	II	121 H	262	130-530
		Valencia	II	60 H	1630	450-2810
		Castellon	II	90 H	480	160-840
	DONAX TRUNCULUS	Guardamar	III	30	10	
		Cullera	II	30	170	
		Valencia	II	90 H	33	20-50
		Sagunto	II	30	50	
		Castellon	II	60 H	75	40-110

Table 1. - Concentrations of heavy metals in organisms.

the industrial areas of Cartagena, Valencia and Portman, especially in the last one where the discharge from the exploitation of a lead-zinc mine has heavily polluted the sediment.

Molluscs collected from these areas also show high concentrations of Cd and Pb.

No significant seasonal variation in the levels of heavy metals in the organisms was observed nor correlation between body burden of heavy metals and length or weight of organisms.

References

BERNARD M. (1976). - Manuel of Methods in Aquatic Environment Research. F.A.O. Part. 3; 48-70.

KRISHNAMURTY, KOTRA V., E. SHPIRT and M. M. REDDY, (1976). - Trace metal extraction of soils and sediments by nitric acid-hydrogen peroxide. Atomic Absorption Newsletter. 15, 68-70.

DE LEON A.R., J. MAS and J. GUERRERO. - Impact of a lead-zinc mine waste disposal into the marine environment. Symposium on Contaminant Fluxes Through The Coastal Zone. I.C.E.S. in press.

HEAVY METAL ANALYSIS IN SEDIMENTS AND MARINE ORGANISMS OF THE BAY OF MALI STON AND THE ADJACENT SEA

by

I. VUKADIN, P. STEGNAR*, M. TUSEK* and T. ZVONARIC
Institute of Oceanography and Fisheries
Split (Yugoslavia)
* "J. Stefan", Institute "E. Kardelj" University
Ljubljana (Yugoslavia)

Abstract

Heavy metals from surface sediments and marine organisms were studied in the Bay of Mali Ston and their role in terms of pollution discussed. Results show that sediments of the Bay contain high levels of Hg, Zn, Fe and Sb, which are significantly higher than those in the open sea. These concentrations can be attributed to natural sedimentological processes, and drainage of the River Neretva and submarine springs carrying mafic constituents of igneous and limestone rocks.

*
* *

Introduction

The area of the Bay of Mali Ston and the adjacent sea are under very strong influence of the nearby land, particularly from the River Neretva and many submarine springs, called vrulje. This bay is a unique ecosystem in Adriatic, and even more so in Mediterranean terms. It is particularly well adapted for shellfish and fish culture. Therefore, the Bay of Mali Ston as a region of outstanding natural beauty and rarity requires special protection.

Materials and methods

Samples of sediments were collected by gravity corer from four stations in the Bay and one in the open sea, in the summer of 1980. Sediments were immediately sectioned into 5 cm lengths and stored frozen until analysis. Analysis of mercury and the other trace elements was performed by radiochemical neutron activation analysis. Accuracy and precision of method are ± 10 per cent. Samples of marine organisms (Mullus barbatus, Sardina pilchardus and Pagellus erythrinus) were collected by bottom trawl.

Stations	Temp. °C	S x 10^3	σ_t	O_2 (ml/l)	O_2%	pH	Transparency (m)
1	22.05	35.08	24.28	5.57	107.9	8.22	24
2	21.74	34.82	24.06	5.78	111.1	8.21	25
3	21.60	35.53	24.75	5.44	105.0	8.22	16
4	21.90	34.93	24.22	5.39	104.3	8.19	10
5*	23.40	37.48	25.71	5.30	106.9	8.17	23
1	16.47	37.81	27.81	5.81	104.1	8.19	
2	19.54	37.34	26.69	5.50	103.8	8.19	
3	18.68	37.49	27.03	5.75	106.9	8.20	
4	20.21	36.98	26.24	5.45	103.6	8.19	
5*	14.50	38.58	28.86	5.51	96.0	8.09	

Table 1. - Basic hydrographic parameters in the surface layer (0 m) and bottom layer of the sea.

Station	Hg	Cd	As	Sb	Cu	Zn	Co	Mn	Fe
1	1.53	0.15	18.7	0.63	18.5	48.0	6.8	665	1.69
2	1.38	0.22	21.0	0.68	22.0	100.0	14.9	660	1.35
3	3.78	0.19	12.1	0.20	16.7	68.0	14.0	960	3.35
4	1.00	0.18	16.0	0.45	15.4	78.0	3.8	-	-
5*	0.40	0.98	33.5	0.26	12.8	40.0	9.6	-	-

Table 2. - Trace element contents in upper sediment layer (0-5 cm) in mg/kg DW, (Fe in %).

Station	Hg	Cd	As	Sb	Cu	Zn	Co	Mn	Fe
1	0.21	0.10	19.4	0.54	22.5	80.0	12.6	650	1.37
2	0.82	0.17	21.1	0.56	17.0	78.0	5.5	705	1.75
3	1.94	0.20	12.7	0.19	19.1	82.5	14.5	875	3.04
4	1.94	0.17	18.0	0.50	15.6	75.0	6.7	-	-
5*	1.22	0.10	15.0	0.20	9.3	34.0	7.0	-	-

* Station in the open sea

Table 3. - Trace element contents in subsurface sediment layer (15-20 cm) in mg/kg DW, (Fe in %).

Element	Mafic igneous rocks	Limestone rocks	Marine sediments in the Adriatic
Hg	0.07-0.5	0.03	0.01-5.00
Cd	0.15	0.2	0.1 -0.3
As	5	5	10-20
Sb	1	-	0.1 -0.5
Cu	70	20	10-50
Zn	130	50	30-150
Co	23	-	1-20
Fe	0.5%	0.4%	1.0-2.0%
Cr	200	2	10-150
Mn	1000	385	300-3000

Table 4. - Average means of trace element contents in mafic constituent of igneous rocks, limestone rocks and marine sediments in the Adriatic (mg/kg) (RANKAMA, K. and SANHAMA, G. 1949).

Results and discussion

Spatial distribution of total heavy metals in surface and subsurface sediment layers are shown in tables 1 and 3.

The results show higher concentrations of mercury (> 3 mg/kg DW) at station 3, which is a closed station near the land, than at stations 1 and 2.

It is evident that the concentrations of mercury and some other trace elements (except arsenic and cobalt) are somewhat lower in sediments of the open sea (station 5).

These higher concentrations of all trace elements in the Bay of Mali Ston compared to other regions along the Adriatic coast (STEGNAR P. et al., 1981), could be attributed to the contribution of River Neretva, and submarine springs in this area. The drainage from land affects the quantity of materials and their transport as well as the type of weathering. Sediments are mainly muddy (30-50 cm) in the study area. This type of sediment has an extraordinary absorption capacity of trace elements from a water column. In fact, sediment itself is heavy metal reservoir.

This fact, together with the uniformity of the data, and the well documented trend towards higher concentrations in sediments of the south Adriatic coast (STEGNAR P. et al., 1981) points to the fact that general distribution of heavy metals in the Bay of Mali Ston is not due to pollution, but rather to natural supply by rivers and submarine springs as well as to other sedimentological processes (see table 4).

The results of the analysis of trace metals in marine organisms in Bay of Mali Ston are shown in table 5, and compared with the values for organisms from the open sea (Island of Vis - Central Adriatic).

Organisms	Weight (g)	Lenght (cm)	n	Hg	Se	Zn	Cu	As	Mn	Sb
Mullus barbatus	24-83	13-19	9	0.49--1.49	0.26--0.37	3.42--5.45	0.43--0.78	8.1--10.6	0.16--0.31	1.8--3.2
Sardina pilhardus	18-39	12-17	9	0.83	0.51	2.69	0.69	21.6	0.50	4.2
Pagellus erythrinus	31-98	15-18	9	0.58	0.38	3.45	0.26	5.66	0.16	0.8
Mullus * barbatus	20-79	15-19	9	0.15	0.34	4.46	0.47	3.27	0.31	1.1

* Data for organisms from the open sea station

Table 5. - Trace element content in flesh of living organisms from the Bay of Mali Ston (in µg/gFW, Sb in ng/g FW).

Almost all Hg values found are higher than the level permissitle by WHO (0.5 µg/kg FW). Values for some other trace metals are higher than the respective values inthe open sea. These higher values can be explained by the above-mentioned factors.

Conclusions

Basic hydrographic, chemical and biological parameters showed that the whole area of the Bay of Mali Ston and the adjacent sea are under very strong influence from the adjacent land (River Neretva and many submarine springs).

Our results show that sediments of the Bay contain higher levels of Hg, Zn, Fe and Sb than those in the open sea.

These concentrations can be attributed to natural sedimentological processes, drainage by the River Neretva and submarine springs of mafic constituents of igneous and limestone rocks.

References

BRYAN G.W., (1969). - Bioaccumulation of marine pollutants. Phil. Trans. R. Soc. Lond. B., 286; 483-505.

PAUL J. and D. MEISCHNER, (1976). - Heavy metal analysis from sediments of the Adriatic Sea. Senckenbergiana marit. 8, (1/3), 91-102.

PANKAMA K. and T.G. SANHAMA, (1949). - Geochemistry. The University of Chicago. 226.

STEGNAR P., I. VUKADIN, A. VAKSELJ, A. PROSENC, (1981). - Toxic trace elements in sediment and marine organisms from Adriatic sea. Thalassia Jugosl., in press.

VUKADIN I. (1981). - Hydrographic properties of Maloston Bay and the adjacent sea. JAZU, Malostonski Zaljev. 52-65.

TRACE METALS IN LAKE MANZALAH, EGYPT

by

Naim M. DOWIDAR*, Kurt J. IRGOLIC**
and Alaa R. ABDEL MOATI*
* Alexandria University, Faculty of Science
Oceanography Department (Egypt)
** Texas A and M University
Center for Energy and Mineral Resources (U.S.A.)

Introduction

Lake Manzalah is the largest and most productive delta lake in Egypt (surface area about 900 km2). It occupies the north eastern area between the Damietta branch of the Nile and the Suez Canal (long. 31°45' - 32°50' E· lat. 31°00' - 31° 35' N) (fig. 1). It is a shallow lake with an average depth about one metre. The lake receives about 6,680 x 106 m3 of drainage water annually most of which is discharged into the south-eastern part of the lake. This quantity includes about 3,500 x 106 m3 of agricultural drain water (Hadus and Ramsis drains) and about 1,700 x 106 m3 of highly polluted water (Bahr El-Bakar drain). El-Inanyia canal conveys about 156 x 106 m3 yr of fresh water from the Damietta branch of the Nile to the western region. To the north the lake is connected to the Mediterranean Sea through a narrow channel known as Boughaz El-Gamil which is a pathway for the marine fishes entering the lake. The aim of the present work is to study the role and effect of drain water reaching the lake on the concentrations of Al, Cd, Co, Cu, Fe, Mn, Ni, Zn and Pb both in the dissolved and particulate forms in the lake water.

Materials and methods

Throughout the period from January to December 1982, monthly samples for trace metal analysis were collected from eight stations I-VIII (fig 1). At each station 2-5 litres were collected from 30 cm below surface by a Van Dorn sampler. Samples were filtered on Millipore filters (0.45 u) for determination of T.S.M. The filtrates were used for determinations of dissolved elements using the Chelax-100 concentration technique (RILEY and TAYLOR, 1968· ABDULLAH et al., 1976). The metals associated with suspended matter were determined using the method of TESSLER et al. (1979). Concentrations were measured using Model 34,000 Inductively Coupled Plasma (I.C.P.) Emission Spectrometer.

Blanks were found to be below the detection limit of the instrument. The precision and accuracy for the analytical techniques were checked and found statisfactory.

Results and discussion

The average salinity of the lake varies between 1.38 ‰ and 2.88 ‰. The highest average (i.e. 5.54 ‰) was recorded at station VIII lying opposite the lake-sea connection which is intermittently exposed to marine water invasion (maximum 18.81 ‰ in July).

	STATIONS							
	I	II	III	IV	V	VI	VII	VIII
Al	184 ± 80	171 ± 132	91.5 ± 78	97 ± 83	149.5 ± 97	118 ± 51	177.5 ± 137	86 ± 46
Cd	0.41 ± 0.25	0.16 ± 0.07	0.19 ± 0.10	0.19 ± 0.09	0.37 ± 0.35	0.20 ± 0.09	0.13 ± 0.04	0.2 ± 0.11
Co	0.049 ± 0.008	0.060 ± 0.02	0.058 ± 0.018	0.059 ± 0.03	0.06 ± 0.04	0.062 ± 0.03	0.059 ± 0.04	0.069 ± 0.04
Cu	3.52 ± 1.29	4.48 ± 1.22	3.88 ± 1.14	2.87 ± 1.69	4.64 ± 2.51	4.14 ± 1.74	3.97 ± 2.67	3.72 ± 2.73
Fe	16.44 ± 5.7	15.32 ± 6.3	10.06 ± 5.1	10.27 ± 8.01	15.04 ± 8.1	11.17 ± 3.61	15.58 ± 8.91	11.21 ± 6.91
Mn	22.1 ± 13.3	1.63 ± 1.19	1.81 ± 1.45	1.84 ± 1.79	5.88 ± 6.92	3.29 ± 4.31	4.82 ± 5.51	2.44 ± 1.58
Ni	2.16 ± 1.11	1.09 ± 0.22	1.03 ± 0.41	0.70 ± 0.45	1.12 ± 0.49	1.13 ± 0.54	0.89 ± 0.39	0.82 ± 0.41
Zn	15.55 ± 6.47	10.10 ± 3.07	10.69 ± 4.92	8.08 ± 2.74	15.06 ± 9.77	11.53 ± 3.47	13.40 ± 9.39	8.45 ± 5.43
Pb	2.21 ± 0.99	1.03 ± 0.51	1.66 ± 0.78	1.34 ± 0.89	1.63 ± 1.27	1.52 ± 1.22	1.53 ± 0.90	1.51 ± 1.54

Table 1. - Mean and standard deviation (±) of concentrations of dissolved metals (µg/l) at the stations sampled in Lake Manzalah.

	STATIONS							
	I	II	III	IV	V	VI	VII	VIII
Al	1743 ± 969	2185 ± 1711	1356 ± 1496	65 ± 25	2113 ± 2195	1274 ± 761	1104 ± 801	1127 ± 626
Cd	0.46 ± 0.37	0.58 ± 0.53	0.24 ± 0.31	0.02 ± 0.30	0.38 ± 0.41	0.24 ± 0.14	0.22 ± 0.17	0.20 ± 0.13
Co	1.37 ± 0.80	1.95 ± 1.73	0.77 ± 0.85	0.07 ± 0.04	1.20 ± 1.16	0.75 ± 0.40	0.69 ± 0.52	0.67 ± 0.38
Cu	8.88 ± 6.45	2.77 ± 2.26	1.75 ± 1.97	1.02 ± 0.57	3.48 ± 4.23	1.91 ± 1.27	2.18 ± 1.66	1.19 ± 0.53
Fe	1340 ± 532	1423 ± 891	826 ± 600	69 ± 22.8	1275 ± 935	1078 ± 517	876 ± 551	939 ± 480
Mn	62.32 ± 23.4	82.16 ± 64.7	60.07 ± 62.5	5.79 ± 2.67	71.77 ± 61.6	58.60 ± 47.7	49.14 ± 37.1	18.56 ± 9.20
Ni	3.91 ± 1.81	3.91 ± 3.71	2.90 ± 2.66	0.30 ± 0.09	3.35 ± 2.70	2.45 ± 1.42	2.20 ± 1.37	1.66 ± 0.77
Zn	46.79 ± 39.6	11.78 ± 10.5	12.06 ± 13.9	3.90 ± 2.2	14.22 ± 10.0	7.62 ± 7.1	12.90 ± 9.5	6.27 ± 3.09
Pb	8.67 ± 6.16	3.87 ± 2.66	4.60 ± 5.78	2.16 ± 1.37	5.54 ± 4.48	5.03 ± 6.83	5.59 ± 7.69	2.88 ± 1.65
TSM	72.23 ± 81.7	53.95 ± 57.6	30.69 ± 30.4	15.35 ± 11.4	39.70 ± 25.6	35.97 ± 42.7	46.14 ± 49.4	34.62 ± 41.2

Table 2. - Mean and standard deviation (±) of concentrations of particulate metals µg/l) as well as total suspended matter (mg/l) at stations sampled in lake Manzalah.

The pH of the lake is mostly on the alkaline side varying between 7 53 and 8.71 The lowest

values occurred in the polluted area affected by El-Baqar drain (station I) varying between 7.15 and 8.14.

Except for station I the lake water is always oxygenated. The average D O. values ranged between 2.39 ml l at station V and 9.33 ml/l at station VII. It is interesting to note that the water at station I is always anoxic with high concentrations of H2S ranging from 8.10 ml/l to 20.64 ml/l (average 15.3 ml/l).

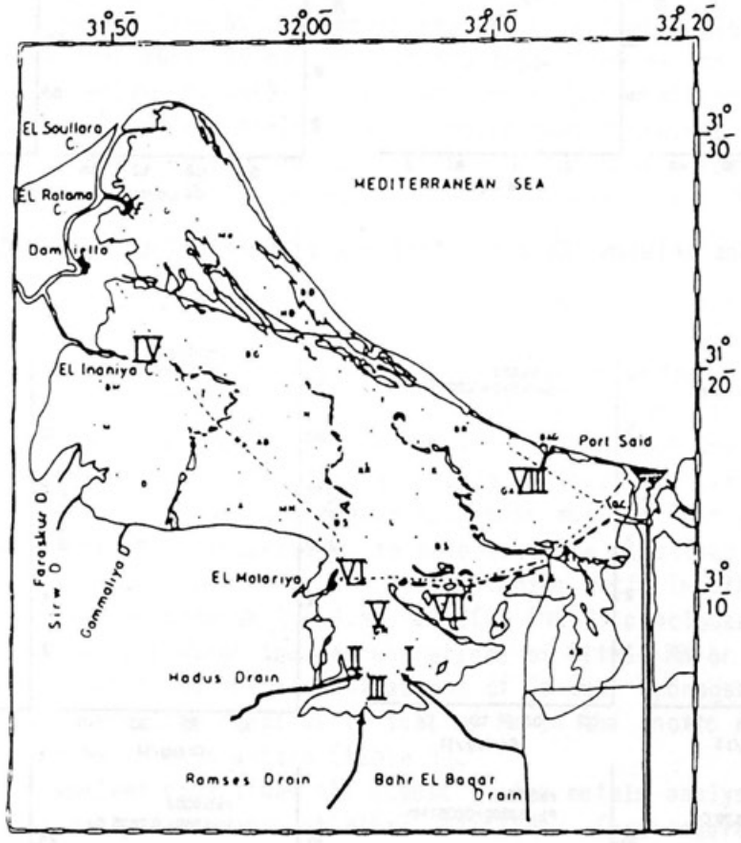


Figure 1. - Map of Lake Manzalah showing the main drains and the stations sampled.

Trace metal studies

The mean concentrations of trace metals studied in Lake Manzalah both in the dissolved and particulate forms are shown in tables 1 and 2.

The high coefficient of variation shown for most metals reflects the wide variations in the concentrations of these metals in different months and or the patchy distribution of suspended matter. Despite this fact significant positive correlations were found between several couples of elements in particulate form. particularly at stations I and II (fig 2) On the other hand no significant correlations were observed between the concentrations of most elements in dissolved form. Zinc cadmium and nickel do not change their oxidation state under reducing conditions (KREMLING 1983). The concentrations of these metals in the dissolved form were higher in anoxic waters (station I) than at any other oxic stations (table 1) This condition may indicate the release of these metal ions from the bottom sediments and/or the suspended particles in anoxic waters and their adsorption in oxygenated waters. However the balance between adsorption and desorption in the anoxic area of the lake is mostly a dynamic one because the water is continuously removed from the anoxic to oxic areas by the continuous flow.

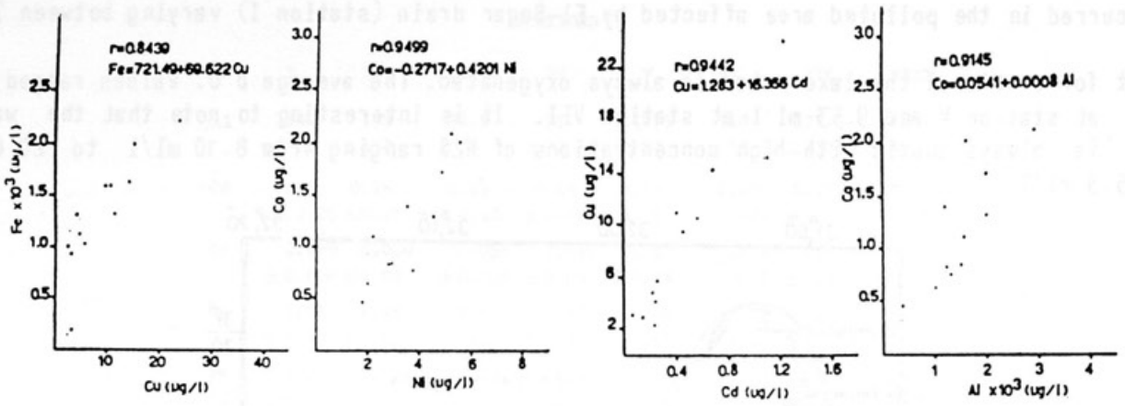


Figure 2a. - Correlations between the concentrations of particulate Fe-Cu. Co-Ni. Cu-Cd and Co-Al at station I.

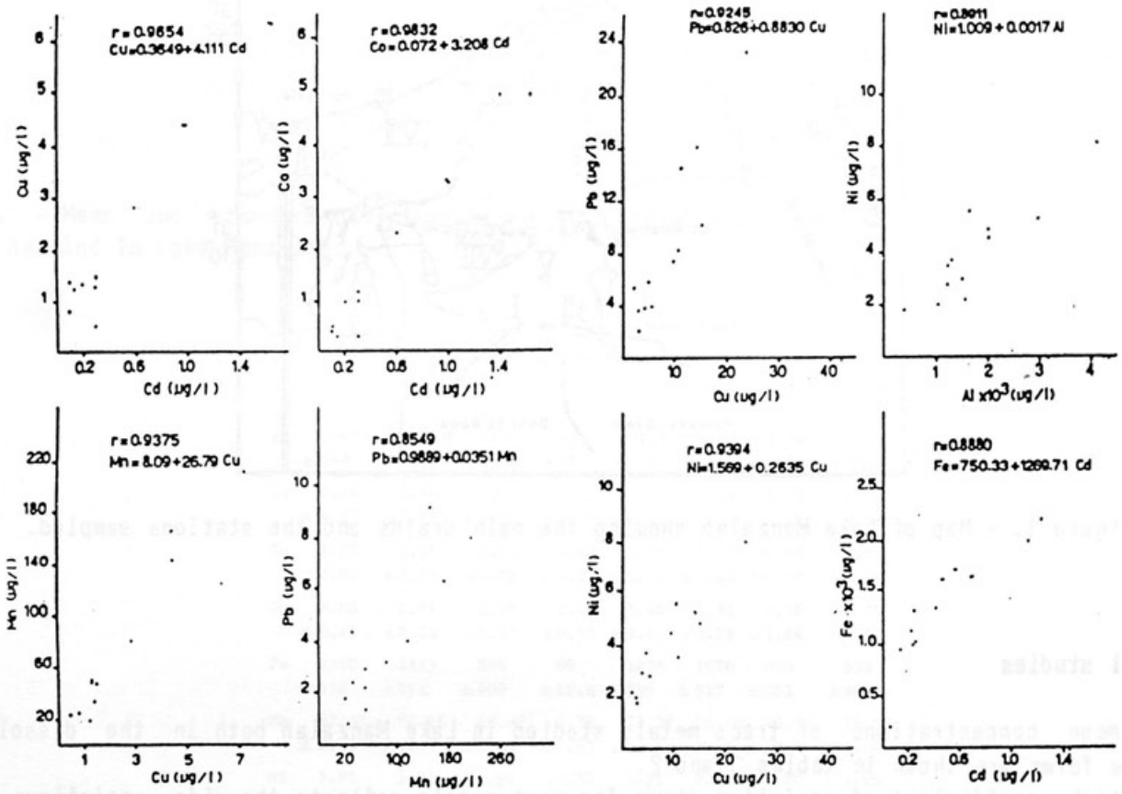


Figure 2b. - Correlations between the concentrations of particulate Cu-Cd. Co-Cd. Mn Cu. Mn Pb. Pb-Cu. Ni-Al. Ni-Cu and Fe-Cd at station II.

	STATIONS							
	I	II	III	IV	V	VI	VII	VIII
Al	9.6	7.3	6.3	59.9	6.6	8.5	13.9	7.1
Cd	47.1	21.6	44.4	90.9	49.6	45.4	37.8	49.9
Co	3.5	3.0	7.0	44.7	4.8	7.7	7.9	9.3
Cu	26.2	61.8	68.9	73.9	57.1	68.4	64.5	75.7
Fe	1.2	1.1	1.2	13.0	1.2	1.0	1.7	1.2
Mn	26.1	1.9	2.9	24.1	7.6	5.3	8.9	11.6
Ni	35.5	21.9	26.1	70.1	25.1	31.6	28.8	33.0
Zn	24.9	46.2	47.0	67.4	51.4	60.2	51.0	57.4
Pb	20.3	21.1	26.5	38.3	22.8	23.2	21.5	34.4

Table 3. - Percentage abundance of dissolved species relative to the total metal concentration of different stations sampled in Lake Manzalah.

The concentration of dissolved copper ions at the anoxic station (about 26 per cent of the total) was relatively low compared to that at oxic stations (57 to 75 7 per cent of total) (except station IV). In pure anoxic waters dissolved Cu is most probably present as Cu species (KREMLING 1983). The polluted water of El-Baqar drain contains high concentrations of various organic and inorganic pollutants As mentioned by HUTCHINSON (1975) water rich in brown humic matter in contact with mud particles of lake water takes up Cu more easily than does lake water poor in such matter.

The concentration of dissolved iron at the anoxic station is slightly higher than that at other oxic stations. Table 3 shows that about 99 per cent of the total iron in the lake is in the particulate form and the ratio of dissolved to particulate fractions at all stations (except station IV) is almost constant. Mc MAHON (1969) and HUTCHINSON (1975) showed that dissolved iron concentrations in lake water increase following the dissolved oxygen depletion. but that prolonged anoxic conditions result in hydrogen sulphide formation and iron sulphide precipitation thus decreasing dissolved Fe concentrations. This may explain the unexpected relatively low dissolved iron at the anoxic station

The concentration of dissolved manganese species in the hydrogen sulphide region of the lake is very much higher than that at oxic stations (table 1) This high concentration may be explained by the release of Mn-2 from the sediments through a steady diffusion process as well as from the continuous supply of the reducing water of El-Baqar drain. Analysis of the bottom sediments in both anoxic and oxic areas of the lake showed that the adsorbed Mn on the bottom sediments in the oxic area is six times higher than the corresponding values in the sediments of anoxic regions (unpublis hed data). Table 3 indicates that the ratio of dissolved to particulate species of Mn is higher in anoxic than in oxic waters of the lake and this ratio decreases more or less gradually by increasing distance from the anoxic region.It is interesting to note that the dissolved Fe· Mn ratio in the anoxic region i.e 0.75 was much lower than the corresponding ratio in other regions of the lake where the value of this ratio varied between 2.6-9.4 EINSELE (1937) concluded that while the precipitation of MnS was never likely to control the concentrations of either MN or hydrogen sulphide the precipitation of FeS often controlled the concentrations of Fe and hydrogen sulphide. This may explain the low values of dissolved iron relative to that of Mn in the anoxic region and the prepon derance of dissolved iron over Mn in oxic waters (table 1).

The concentration of dissolved cobalt was the lowest of the metals analysed. Its concentration in the anoxic region was slightly lower than at other stations. Furthermore. variations in the dissolved forms of Co in oxic regions of the lake were almost insignificant Except at station IV dissolved Co constitutes about 3-9 per cent of the total Co and the ratio increases more or less gradually away from the anoxic region (table 3).

The solubility of aluminium as an amphoteric element varies greatly with the hydrogen ion concentration and is minimal at about neutrality (HUTCHINSON. 1975) As stated by HUTCHINSON (1975) above pH 8 appreciable amounts of the aluminate ion HAl2O4- will be present. The concentrations of dissolved Al (table 1) showed some irregularities which may be related to experimental errors rather than chemical affinities. Except at station IV. most of the Al present in the lake water occurred in particulate form (table 2).

It is important to note that station IV which is mainly affected by the fresh water discharge from the Damietta branch of the Nile contained the lowest concentrations of T.S.M The particulate metal content at station III is several orders of magnitude greater than that of station IV (table 2) Further the percentage of the dissolved metal ions at station IV (table 3) was significantly higher than at any other station. This may be due to the fact that the water flow through El-Inaniya Canal is slow and that bacterial disintegration of suspended matter may be more efficient.

The average annual inflow of fresh and brackish water into the lake is estimated as 6.680 x 106 m3. After making allowances for precipitation and evaporation the net outflow into the Mediterranean Sea would be about 5.300 x 106 m3 yr. The balance sheet of the trace metals studied shown in Table 4 indicates a net gain of all these elements in the lake which is exceedingly high in terms of weight in case of Al. Fe and Mn. However the percentage of the amount retained in the lake from various metals varied between a minimum of 25 5 per cent for iron and maximum of 77 0 per cent for Mn. Apart from these two extremes the percentage gain varied from 34 4 to 57 7 per cent for other

Metal	Total inflow	Total outflow	Net gain Tons	%
Al	10414.27	6426.23	+ 3988.04	38.0
Cd	3.71	2.13	+ 1.58	42.6
Co	8.22	3.92	+ 4.30	52.3
Cu	44.77	26.00	+ 18.77	42.0
Fe	6761.35	5035.87	+ 1725.48	25.5
Mn	483.56	111.27	+ 372.29	77.0
Ni	26.31	13.12	+ 13.19	50.1
Zn	184.62	78.04	+ 106.58	57.7
Pb	35.46	23.26	+ 12.20	34.4
TSM	304×10^3	183.5×10^3	$+120.5 \times 10^3$	39.6

Table 4. - Balance sheet of total trace metals in Lake Manzalah (in tons).

metals. Most of these amounts are precipitated or adsorbed on to the bottom sediments. A small fraction may however be consumed by the biota of the lake.

On the other hand, the large amounts of water flowing out from the lake to the Mediterranean with its high metal and nutrient contents increase the levels of these elements in the coastal Mediterranean waters affected by this outflow. DOWIDAR and MOUSTAFA (1983) found that the biological productivity of the coastal Mediterranean waters east of the Damietta branch was consistantly higher than any other region of the Nile Delta.

Acknowledgement

This paper was carried out in the framework of a co-operative research project on'Fishery Management of Lake Manzalah'supported by grant A/D/NE-CA 1,706 from the US Agency of International Development. Most of the trace metal analyses were carried out at Texas A & M University. The technical help provided by Dr Wendy K. KENNICUTT in these analyses is gratefully acknowledged.

References

ABDULLAH M.I., O. EL-RAYIS and J.P. RILEY (1976). - Reassessement of chelating ion exchange resins for trace metals analysis of sea water. Analytica Chemica Acta, 84: 363-368.

COPELAND R.A. and J.C. AYERS (1975). - Trace elements distribution in water, sediment, phytoplankton, zooplankton and benthos of Lake Michigan. Environ. Res. Group, Inc., Ann Arbor, Mich.

DOWIDAR N.M. and H.M. MOUSTAFA (1983). - Phytoplankton biomass and primary production in the South Eastern Mediterranean off the Egyptian Coast. Rapp. Comm. int. Mer Médit., 28, 9.

EDINGTON D.H. and J.A. ROBBINS (1976). - Records of lead deposition in Lake Michigan sediments since 1800. Envirom. Sci. Technol. 10, 266-273.

EINSELE W. (1973). - Physikalisch-chemische Betrachtung einiger Probleme des limnischen Mangan-und Eisenkreislaufs. Verh. int. Ver. Limnol., 8: 69-84.

EL-WAKEEL S.K. and S. WAHBY (1970). - Hydrography and chemistry of Lake Manzalah, Egypt. Arch. Hydrobiol. 67, 2: 170-200.

FAHMY M.A. (1981). - Seasonal distribution of heavy metals in Damietta branch of the Nile. M.Sc. Thesis, Alexandria University, 130 p.

GUERGUESS S.K. (1978). - Ecological study of zooplankton and distribution of macrofauna in Lake Manzalah. Ph.D. Thesis, Alexandria University, 361 p.

HAFEZ H.M. (1982). - Distribution of heavy metals in Lake Mariut and Nozha Hydrodrome and their accumulation in fish. M.Sc. Thesis Alexandria Univerersity 177 p.

HUTCHINSON C.T. (1975). - A treatise of Limnology. Wiley, New York, N.Y., 1015 p.

KREMLING K. (1983). - The behaviour of Zn, Cd, Cu, Ni, Co, Fe and Mn in anoxic Baltic waters. Marine chemistry, 13, 87-108.

LOPEZ J.M. and G.F. LEE (1977). - Environmental chemistry of copper in Torch Lake, Michigan. Water, Air and Soil Pollution, 8, 373-385.

McMAHON J.W. (1969). - The annual and diurnal variation in the vertical distribution of acid-soluble ferrous and total iron in small dinictic lake. Limnol. Oceanogr., 14: 357-367.

NISSENBAUM A. (1977). - Minor and trace elements in Dead Sea water. Chemical Geology, 19: 99-111.

RILEY J.P. and D. TAYLOR (1968). - Chelating resins for the concentration of trace elements from sea water and their analytical use in conjunction with atomic absorption spectrophotometry. Anal. Chem. Acta, 40: 479-485.

SHAHEEN A.H. and S. YOSEF (1978). - The effect of the cessation of Nile flood on the hydrographic features of Lake Manzalah, Egypt. Arch. Hydrobiol. 84, 3: 339-367.

TANAKA M. (1953). - Etude chimique sur le métabolisme minéral des lacs. J. Earth Sci., Nagoya Univ., 1: 119-134.

TESSLER A., P.G. CAMPBEIL and M. BISSON (1979). - Sequential extraction procedure for the speciation of particulate trace metal. Analytical chemistry, 51, 7: 844-851.

WAHBY S., S. YOSEF and N. BISHARA (1972). Furthur studies on the hydrography and chemistry of Lake Manzalah. Bull. Inst. Oceanogr. & Fish., A.R.E. 2: 421-422.

POLLUTANT LEVELS IN SEDIMENTS OF CATALONIAN SEA. DATA ANALYSIS

by

José OBIOLS SALVAT, Xavier TOMAS MORER
and Lluis A. PEIRO MIR
Instituto Quimico de Sarria
Barcelona - 17 (Espana)

Abstract

From 1981 to 1982, heavy metal analysis (Hg, Cd, Cu, Mn, Ni, Zn, V) of water and sediments of 91 sampling stations, located in the southern area of the Catalan Sea, was carried out. Special characteristics (industrial, agricultural, demographic, etc.) of the coastal area covered - extending from the Ebro Delta to the river Besos - enhanced the study of actual pollutant levels in such a zone.

This paper reports experimental data analysis, showing the correlation between pollutants, the characterization of particular polluted zones, and (for certain stations) the trends of principal pollutants are estimated.

*
* *

Introduction

As a second stage in the study of the pollutant levels in the Catalan Sea, we have expanded the coastal area previously covered (1), in order to reach the singular zone of the Barcelona metropolitan environment.

Therefore the limits of the total area are now located at the river Besos (41°25' N) and the Ebro Delta (40°40' N), with 91 designated sampling stations (figures 1 and 2).

The study of actual pollutant levels and trends of this South Catalan sea zone is enhanced bythe presence of local sources of pollution summarized as follows :

- The Ebro River Delta. Third Mediterranean river (average annual outflow 17,270 HM3) ; its basin is eminently agricultural (specially its delta) with some industrial and urban effluents upstream (chlor-alkali, manufactures, automobiles. nuclear power plant, etc.) :
- Nuclear power plant at Vandellos, with effluents interacting directly with the coastal zone :
- Petrochemical area in Cap Salou-Tarragaon environment. One of the main Spanish refineries, with related chemical industries and base chemicals production :
-Llobregat and Besos rivers. Natural effluents of the two industrial valleys of Catalonia ;
- Urban areas located in Tarragona (109,112 inhabitants) and Barcelona Metropolitan (3,029,904). Total population of the area (3,512,944) is equivalent to 9.3 per cent of the Spanish population :

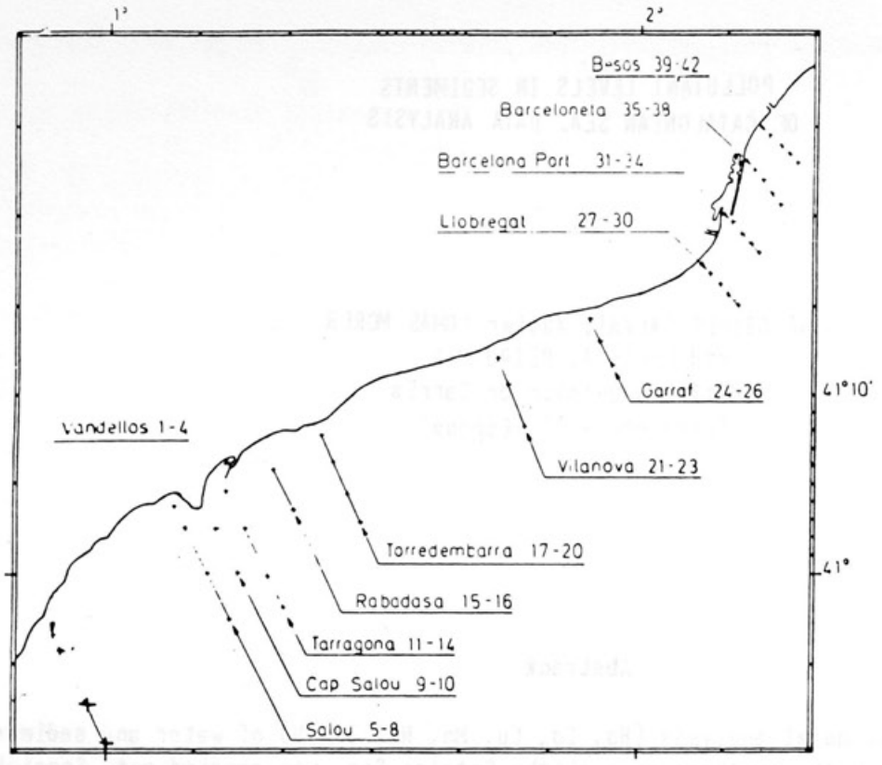


Figure 1. - 1982 stations.

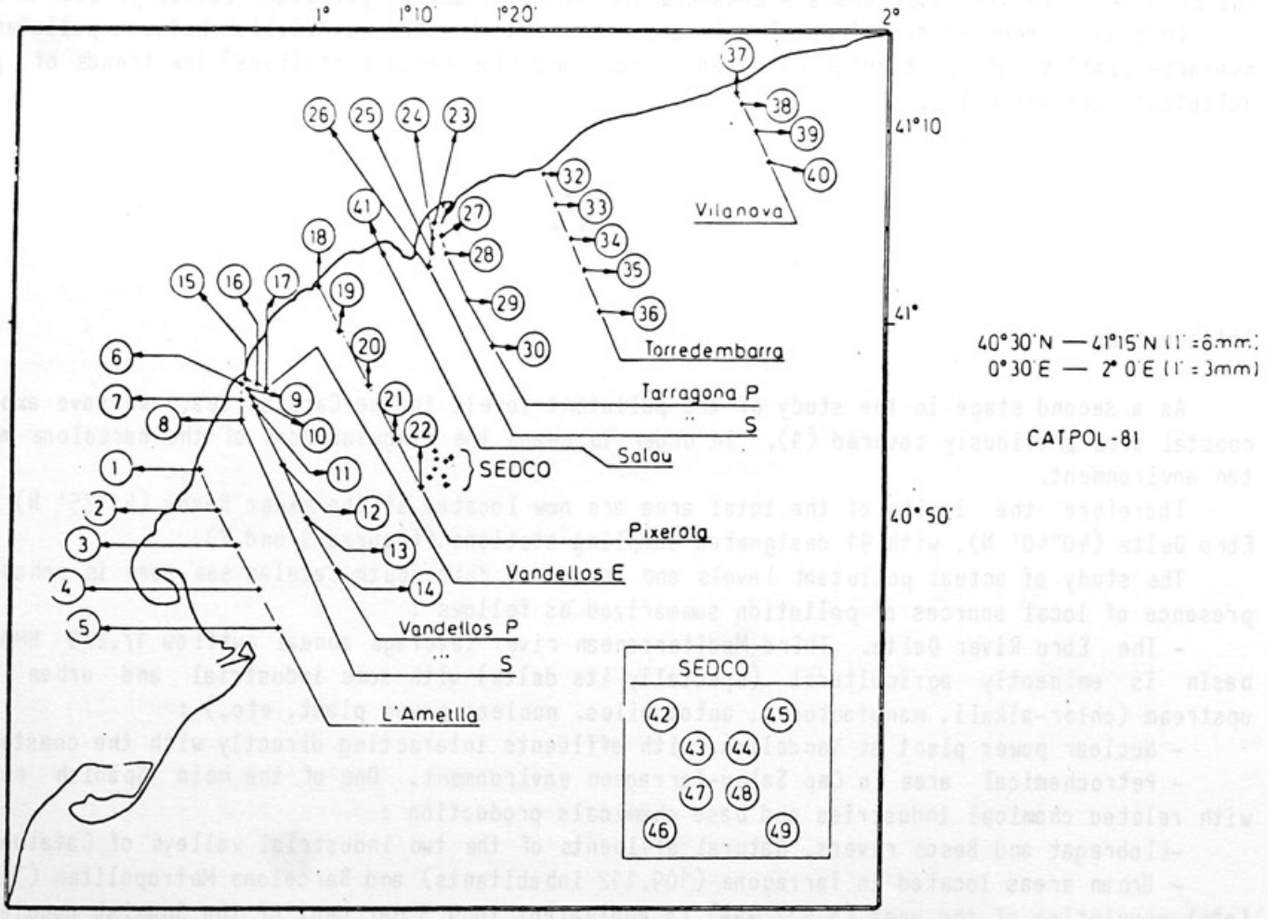


Figure 2. - 1981 stations.

- Harbour areas. Besides minor ports, Barcelona and Tarragona are the first and second ones in the Spanish Mediterranean ranking, with approximately 38 million tonnes in transit (18 per cent of all Spanish ports).

At this stage, new data from the river Besos (Barcelona) to the previous northern limit (Vilanova) are reported, and previous values of the overlapped Vilanova-Vandellos area are verified. The SEDCO oil project is not included.

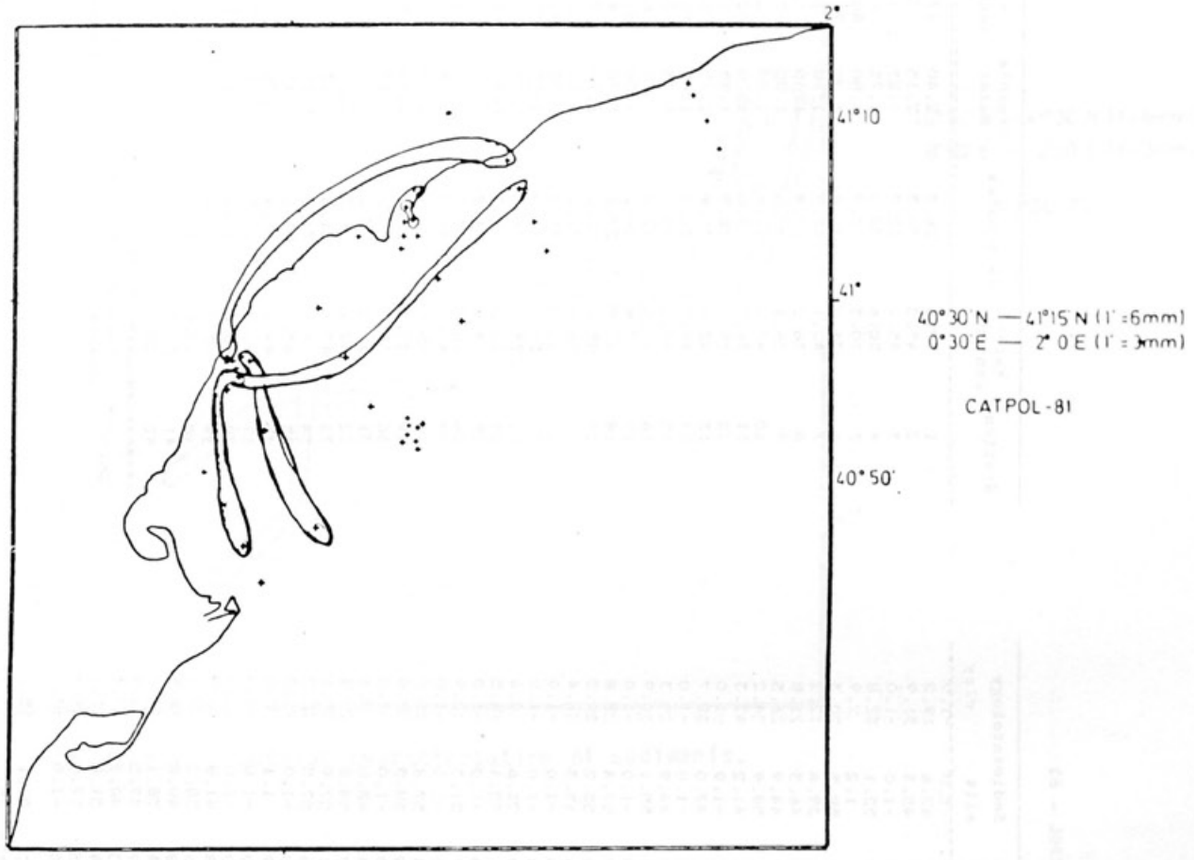


Figure 3. - Heavy Metals in Sediments.

Material and methods

Samples of surface water were taken with a Niskin type bottle. All samples were filtered through a glass fibre filter, stored in 25 ml. polyethylene flasks (previously cleaned with acid) containing 2 ml of 5M hydrochloric acid, and frozen until their analysis. Every ten samples a bottle was filled with distilled water for test analysis.

In the laboratory, the water was kept at room temperature, and cadmium, zinc, lead and copper were analysed by Potentio metric Stripping Analysis (P.S.A.) according to the method developed by D. JAGNER and K. AREN (2), using standard additions, which ensures accuracy in all the samples (Ion Scanning System, Radiometer ISS-820). The precision of the method is between 10 and 20 per cent of the results obtained.

Mercury was analysed by cold vapour Atomic Absorption Spectrophotometry, using $NaBH_4$ as a reducing agent, at 253.7 nm - with an Electrodeless Discharge Lamp.

CATPOL - 81

Station	Water content	Carbonates	Loss of weigh at500°	Loss of weigh at1000°	Sedimentology sand	Sedimentology slit	Sedimentology clay
1	-1.0	-1.0	-1.00	-1.00	-1.0	-1.0	-1.0
2	34.1	9.8	16.02	34.20	3.3	62.6	19.2
3	42.2	13.9	11.82	32.10	7.7	45.3	31.9
4	42.5	11.2	15.27	30.90	-1.0	-1.0	-1.0
5	37.3	8.9	17.74	34.20	0.9	52.1	32.8
6	17.5	8.6	32.78	64.90	89.7	1.2	7.9
7	36.6	13.7	13.58	25.80	6.0	58.5	20.3
8	41.7	9.5	15.93	30.00	12.8	39.6	32.2
9	38.3	10.0	11.40	22.40	20.2	46.3	21.8
10	43.4	11.7	13.00	28.50	17.2	44.9	26.5
11	36.3	22.7	8.74	29.40	1.3	59.9	24.2
12	47.4	13.0	12.20	30.80	0.5	41.2	43.3
13	-1.0	-1.0	-1.00	-1.00	-1.0	-1.0	-1.0
14	46.1	13.2	10.47	29.30	2.0	47.0	38.7
15	-1.0	-1.0	-1.00	-1.00	-1.0	-1.0	-1.0
16	44.3	13.2	11.30	24.20	9.8	44.9	29.3
17	44.6	14.7	9.49	26.90	4.0	55.1	30.9
18	-1.0	-1.0	-1.00	-1.00	-1.0	-1.0	-1.0
19	38.7	10.5	12.52	32.90	1.9	57.4	25.8
20	41.8	13.4	14.37	32.60	2.0	55.2	29.2
21	41.1	14.7	9.82	30.40	3.4	45.3	36.4
22	-1.0	-1.0	-1.00	-1.00	-1.0	-1.0	-1.0
23	-1.0	7.6	16.32	35.70	-1.0	-1.0	-1.0
24	28.1	7.3	16.01	36.70	45.8	21.6	8.6
25	33.9	10.7	19.76	37.70	40.1	33.4	16.5
26	-1.0	12.1	11.23	30.50	-1.0	-1.0	-1.0
27	28.9	10.0	20.15	39.80	23.7	51.3	12.9
28	-1.0	-1.0	-1.00	-1.00	-1.0	-1.0	-1.0
29	39.4	11.6	10.59	30.60	1.4	57.4	25.0
30	41.7	15.1	10.98	30.20	1.8	58.5	25.0
31	-1.0	-1.0	-1.00	-1.00	-1.0	-1.0	-1.0
32	21.1	4.3	19.19	29.90	42.4	48.0	2.1
33	37.4	10.8	16.42	32.20	2.2	58.8	20.8
34	37.8	10.5	15.09	30.40	2.1	57.9	25.1
35	41.7	9.4	17.02	28.80	2.6	57.5	25.2
36	-1.0	-1.0	-1.00	-1.00	-1.0	-1.0	-1.0
37	24.3	4.0	14.88	29.60	93.9	1.1	3.0
38	-1.0	3.7	14.80	31.70	-1.0	-1.0	-1.0
39	-1.0	-1.0	-1.00	-1.00	-1.0	-1.0	-1.0
40	27.9	6.1	17.78	31.90	39.8	41.8	10.9
41	31.1	7.2	13.93	29.20	43.6	38.3	9.6
42	35.8	9.1	14.59	29.50	29.8	40.8	18.1
43	35.7	9.1	15.78	31.00	32.4	38.1	17.4
44	37.6	7.3	16.99	28.20	28.9	37.5	21.4
45	34.9	7.8	14.14	27.70	32.3	40.9	17.1
46	42.3	12.2	11.36	35.50	8.4	51.8	25.3
47	37.6	8.5	16.64	29.00	29.5	37.9	20.6
48	29.7	6.7	17.85	32.90	-1.0	-1.0	-1.0
49	27.7	5.6	16.43	31.10	59.3	20.1	15.0
Unity	<----------		% on dry sediment				---------->

Table 1. - General characteristics of sediments.

CATPOL - 82

Station	Water content	Carbonates	Organic mater	Sedimentology sand	Sedimentology slime	Sedimentology clay	Sedimentology grave
1	39.6	26.3	2.40	17.40	61.6	21.0	0.0
2	45.5	30.5	2.15	2.10	56.8	41.1	0.0
3	45.1	32.7	1.85	6.10	52.8	41.1	0.0
4	50.6	32.0	1.75	4.00	52.9	43.1	0.0
5	30.5	32.7	0.80	80.40	15.2	4.4	0.0
6	31.0	40.4	1.20	71.20	8.7	20.1	0.0
7	40.1	35.7	1.60	1.60	67.5	30.9	0.0
8	36.2	32.4	1.30	1.80	65.1	33.1	1.4
9	34.1	37.8	1.80	10.30	65.3	24.4	0.0
10	43.3	32.6	1.80	12.10	62.9	15.0	0.0
11	36.3	39.0	1.70	42.80	41.1	16.1	0.0
12	39.1	32.3	1.85	1.10	67.9	31.0	0.0
13	42.0	33.4	1.70	1.10	67.6	31.3	0.0
14	44.2	31.3	1.45	12.20	61.5	26.3	0.0
15	40.0	38.0	1.75	18.60	61.6	19.8	0.0
16	40.6	32.7	1.75	6.60	69.5	23.9	0.0
17	17.8	24.2	0.50	86.00	7.5	6.5	6.1
18	42.9	27.1	1.70	4.80	71.1	24.4	0.0
19	45.8	32.2	1.70	2.90	69.3	27.8	0.3
20	36.5	32.0	1.30	4.60	64.0	31.4	0.2
21	23.4	35.2	0.50	94.50	2.2	3.3	0.7
22	27.2	50.0	0.55	75.90	23.5	0.6	0.0
23	33.5	34.3	1.75	27.10	51.7	21.2	0.0
24	22.2	21.3	0.40	96.30	1.7	2.0	0.0
25	24.6	34.2	1.70	29.20	50.9	19.9	20.0
26	26.4	35.7	1.25	61.60	24.2	14.2	0.0
27	37.5	30.0	2.95	30.90	50.7	18.4	0.6
28	45.6	27.6	2.05	11.80	61.6	26.5	4.7
29	38.7	28.1	1.70	9.50	64.1	26.4	0.2
30	33.0	40.7	1.10	34.50	39.5	26.0	3.1
31	37.3	23.2	2.65	33.70	45.7	20.6	3.9
32	30.1	16.2	1.85	44.60	40.1	15.3	14.9
33	42.6	21.6	2.20	16.90	55.0	28.1	3.1
34	36.7	31.8	1.35	25.80	47.5	26.7	0.8
35	49.6	20.6	4.40	11.50	69.2	19.2	0.0
36	49.4	17.9	3.70	14.80	63.4	21.8	0.0
37	40.7	19.0	1.90	10.80	62.3	26.9	0.5
38	40.0	23.1	1.60	24.30	41.1	34.7	0.9
39	47.7	15.0	4.70	15.20	58.9	25.9	0.5
40	43.2	17.9	2.60	15.90	60.1	24.0	4.6
41	31.4	21.4	1.40	26.40	49.4	24.3	1.5
42	25.7	27.1	0.90	76.00	13.3	10.7	3.1
Unity	<----------		% on dry sediment				---------->

Table 2. - General characteristics of sediments.

Sampling, manipulation and analytical methods were applied to sediments in order to discover the content in Hg, Cd, Cu, Mn, Ni Pb, Cr, Zn, carbonates, moisture, organic matter, and particle-size distribution (> 50 um sand : between 50 and 2 um silt : > 2 um, clay) were described previously (1).

The results of the analyses were stored in a computer data base, MARPOL, also described previously (3).

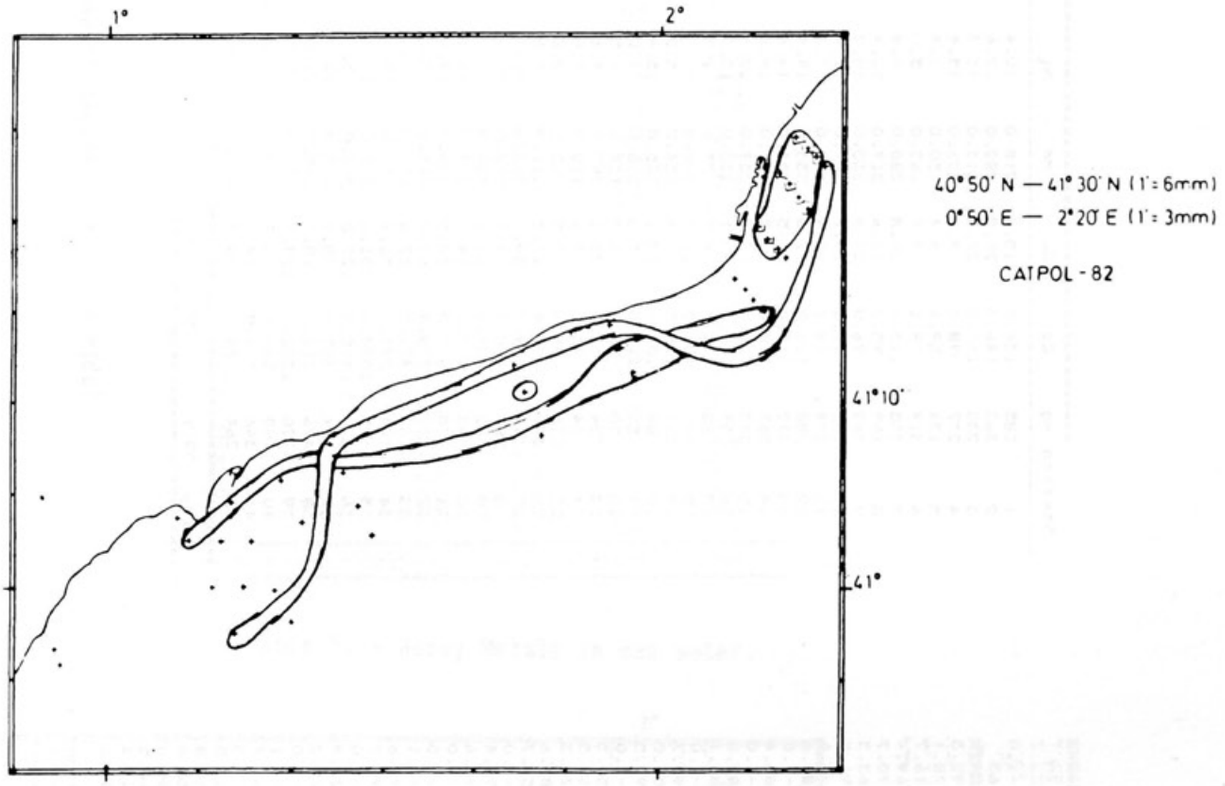


Figure 4. - General characteristics of sediments.

Results and discussion

Tables 1 to 5 contain the values obtained to which are applied the statistical treatment of Principal Component Analysis and Clustering techniques (3, 4, 5).

Figures 3 to 5 represent the cluster of stations according to the values they recorded, which were :

Heavy metals level (Hg, Cu, Cd, Pb, Cr, Zn, Mn, Ni) in sedimentological parameters (sand, slime, clay, moisture and carbonate).

Satisfactory results from the Principal Component Analysis of data for 1981 and 1982 point towards a special influence of carbonate and sand percentage and one of the remaining variables. For heavy metals, it seems that Hg influence is clearly significant, Ni and Mn have no specific value as pollution-sensitive parameters, and Cd, Cu, Pb, Cr, Zn have a similar distribution pattern, specially Zn and Pb.

Although data interpretation when considering geographical and population factors is not simple, a significant difference between the industrial and urban Barcelona area and the rest of the coast studied is apparent (figures 4 and 5). Reference to the remaining 81 stations (figure 3) reveals an homogeneous and unpolluted coastal zone.

CATPOL - 81

Station	Hg	Cd	Cu	Mn	Ni	Pb	Cr	Zn
1	-1	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0
2	404	231.0	13.4	260.0	14.5	21.5	16.3	63
3	640	236.0	16.9	320.0	18.7	55.9	21.6	[illegible]
4	1512	251.0	17.9	304.0	20.4	28.9	22.3	92.3
5	960	228.0	16.2	318.0	18.1	23.6	20.9	78.1
6	134	74.6	3.3	98.0	3.8	10.2	8.8	16.7
7	114	359.0	14.3	232.0	12.4	35.3	29.2	69.4
8	149	149.0	14.4	278.0	15.7	25.4	24.0	70.7
9	365	106.0	12.8	241.0	11.8	35.6	23.6	63.3
10	577	147.0	31.8	262.0	17.6	33.3	18.5	96.9
11	1300	170.0	15.2	281.0	16.5	63.4	20.9	81.6
12	75	46.5	17.1	344.0	21.9	46.5	29.3	80.6
13	-1	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0
14	71	66.0	16.8	354.0	22.4	34.6	27.6	80.4
15	-1	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0
16	129	88.2	17.5	254.0	15.1	45.7	32.1	82.8
17	121	100.0	18.0	317.0	15.5	40.1	32.2	83.1
18	-1	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0
19	761	179.0	31.7	299.0	19.7	34.9	21.5	98.7
20	263	323.0	32.6	322.0	20.4	31.1	19.1	106.0
21	533	187.0	33.1	336.0	21.1	29.2	17.4	99.5
22	-1	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0
23	2240	174.0	12.6	154.0	6.4	21.7	13.0	50.5
24	1076	167.0	27.7	177.0	11.2	27.4	15.8	58.5
25	914	212.0	18.0	220.0	11.3	22.5	20.6	70.3
26	758	100.0	33.3	272.0	15.6	26.8	22.1	87.6
27	2060	215.0	13.4	206.0	9.6	46.6	16.9	58.7
28	-1	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0
29	755	191.0	30.1	340.0	18.4	30.3	20.4	96.4
30	507	122.0	27.2	320.0	19.1	31.9	17.0	92.6
31	-1	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0
32	60	67.5	10.5	181.0	3.9	5.9	4.5	22.5
33	796	214.0	34.5	316.0	16.9	46.5	25.0	98.1
34	582	104.0	30.4	335.0	17.4	34.4	18.8	93.6
35	861	108.0	33.9	323.0	19.4	30.2	18.6	99.7
36	-1	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0
37	630	165.0	4.1	231.0	4.3	9.8	7.3	27.9
38	580	135.0	3.7	206.0	3.8	15.9	8.2	24.2
39	-1	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0
40	490	117.0	24.5	176.0	8.9	29.1	14.5	64.1
41	759	130.0	23.4	261.0	8.0	25.5	14.1	61.7
42	429	87.9	26.5	333.0	15.3	20.3	14.4	77.3
43	149	137.0	13.0	368.0	15.0	21.7	25.8	63.5
44	186	157.0	12.0	308.0	14.5	24.1	22.7	58.8
45	94	94.1	12.9	301.0	12.5	29.5	22.4	58.8
46	371	198.0	16.0	327.0	19.4	23.5	22.9	80.0
47	119	121.0	12.3	295.0	14.1	24.2	24.9	63.5
48	320	117.0	7.7	213.0	11.2	26.0	14.1	149.4
49	126	116.0	7.9	239.0	9.1	21.6	19.0	42.6
Unity	ng/g	ng/g	<----------µg/g---------->					

Table 3. - Heavy Metals in sediments.

CATPOL - 82

Station	Hg	Cd	Cu	Mn	Ni	Pb	Cr	Zn
1	285	134.0	15.7	218.0	10.4	33.4	9.7	67.5
2	363	146.0	20.9	282.0	12.6	45.0	13.4	83.7
3	230	117.0	20.4	313.0	15.3	41.1	13.8	80.0
4	237	98.0	19.8	326.0	15.4	40.5	12.4	80.8
5	134	59.6	6.4	150.0	3.9	63.0	6.2	33.7
6	319	78.1	9.9	163.0	5.0	22.5	6.7	41.8
7	355	113.0	21.8	288.0	8.8	41.7	13.0	76.0
8	189	95.0	18.6	317.0	10.4	32.2	10.0	71.5
9	362	96.0	20.8	233.0	10.2	38.1	16.1	71.5
10	282	111.0	20.7	307.0	12.4	37.8	14.8	77.3
11	379	89.7	17.4	195.0	7.2	31.2	13.2	57.1
12	288	126.0	22.3	335.0	14.4	38.4	19.7	91.9
13	272	113.0	21.5	384.0	14.4	44.2	16.3	87.7
14	267	118.0	20.2	373.0	14.6	47.7	14.1	84.2
15	343	112.0	18.7	259.0	10.4	53.6	18.5	74.4
16	354	122.0	23.1	335.0	13.3	62.0	20.7	90.5
17	65	45.7	4.7	77.0	2.3	25.2	5.9	20.4
18	269	126.0	22.3	310.0	13.0	53.0	17.9	86.8
19	211	116.0	22.6	334.0	15.2	48.2	21.9	91.5
20	88	103.0	18.2	335.0	15.4	33.0	20.0	83.0
21	55	119.0	5.6	216.0	4.0	17.2	7.8	29.3
22	50	17.2	3.0	134.0	1.5	39.6	6.2	23.0
23	324	115.0	25.3	292.0	9.6	57.4	32.2	97.4
24	34	35.1	3.6	118.0	1.8	14.6	6.3	23.2
25	524	149.0	30.5	222.0	8.8	65.3	45.9	109.0
26	349	87.4	21.5	211.0	7.6	60.0	30.3	87.7
27	1111	879.0	59.3	310.0	12.7	91.5	71.7	188.0
28	987	460.0	49.7	312.0	13.8	102.0	72.2	156.0
29	462	281.0	31.1	324.0	14.0	46.1	30.5	94.4
30	383	207.0	24.3	269.0	10.3	34.6	17.8	79.2
31	778	677.0	39.5	286.0	13.5	57.8	44.3	206.0
32	1157	538.0	62.0	213.0	9.4	113.0	46.3	190.0
33	1162	431.0	57.8	270.0	13.7	96.2	75.8	186.0
34	484	271.0	30.9	272.0	13.5	48.8	28.5	102.0
35	4125	2090.0	151.0	319.0	17.0	353.0	292.0	666.0
36	4022	1490.0	120.0	295.0	15.8	222.0	271.0	373.0
37	654	352.0	38.7	278.0	12.2	63.8	57.7	119.0
38	476	210.0	25.1	249.0	10.7	50.6	44.1	99.0
39	3720	1330.0	123.0	276.0	15.3	212.0	358.0	350.0
40	1100	568.0	56.4	280.0	12.1	103.0	111.0	180.0
41	346	194.0	19.9	209.0	9.2	37.2	33.7	83.6
42	264	78.0	8.8	128.0	4.6	26.6	15.5	48.0
Unity	ng/g	ng/g	<----------µg/g---------->					

Table 4. - Heavy Metals in sediments.

CATPOL - 82

Station	Hg	Cd	Pb	Cu	Zn
1	0.7	6.1	35.7	6.3	28.8
2	0.2	2.8	10.2	5.8	15.0
3	0.2	3.3	5.8	3.5	8.6
4	2.0	2.8	8.8	2.1	7.5
5	0.2	2.7	39.0	3.8	34.0
6	0.2	3.7	6.2	6.4	12.1
7	0.3	4.7	14.1	11.1	17.0
8	0.3	5.1	5.5	3.6	9.0
9	0.3	3.4	5.5	3.4	8.7
10	0.8	7.0	17.0	8.5	21.4
11	0.3	2.6	5.7	14.8	31.1
12	0.4	4.2	6.7	25.0	36.5
13	0.3	3.4	9.0	2.3	10.2
14	0.3	3.5	12.0	3.1	57.6
15	0.2	2.5	2.1	2.8	6.8
16	0.2	4.2	2.5	2.4	29.6
17	0.2	1.3	1.8	1.6	5.9
18	0.2	2.5	2.8	3.0	7.8
19	0.2	1.6	4.0	2.8	9.8
20	0.2	3.1	9.0	2.8	10.5
21	0.2	1.7	3.7	2.8	14.1
22	0.2	4.0	7.5	4.2	13.9
23	0.2	1.7	4.2	2.5	10.0
24	0.2	1.5	4.5	3.6	18.7
25	0.2	3.7	6.8	3.7	39.1
26	0.2	4.3	24.0	6.2	107.0
27	0.2	3.0	6.4	4.3	20.5
28	0.2	2.1	2.6	2.5	6.7
29	0.2	2.2	4.9	11.0	21.3
30	0.2	1.8	2.2	2.2	4.4
31	3.4	7.3	8.3	10.8	53.0
32	3.1	10.1	19.0	8.6	33.5
33	1.2	3.5	3.8	3.8	7.6
34	1.3	2.5	2.5	2.9	13.0
35	1.0	4.8	7.4	2.8	17.0
36	1.0	3.4	6.5	2.2	11.0
37	0.8	2.0	1.7	3.1	9.8
38	0.2	1.9	3.2	4.8	14.3
39	0.2	4.3	7.6	3.4	13.0
40	0.2	2.4	2.1	3.2	9.1
41	0.2	2.8	3.9	2.2	5.1
42	0.2	3.4	4.6	4.6	11.4
Unity	<----------		ng/g		---------->

Table 5. - Heavy Metals in sea water.

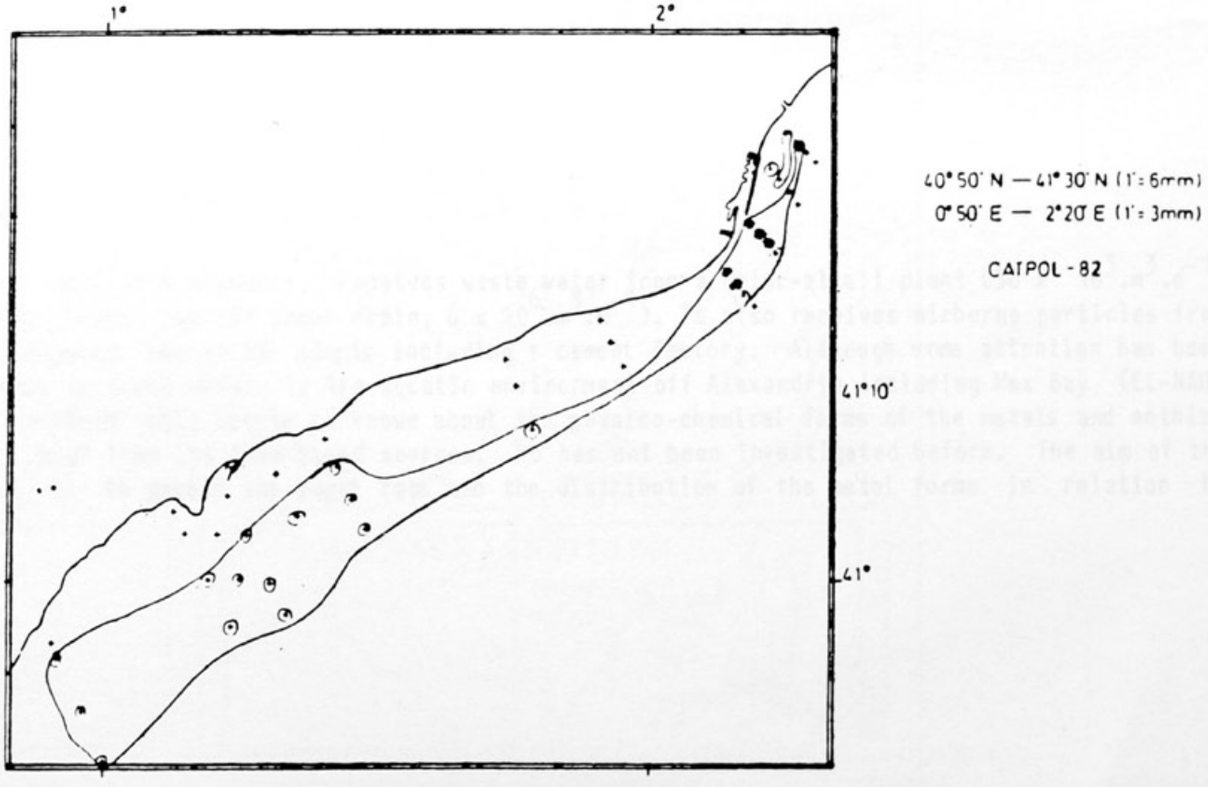


Figure 5. - Heavy metals in sediments.

In such a way, differences between 1981 and 1982 parameters and inter-metal correlations point to these two different areas (figures 3 and 5).

Manganese and nickel levels in sediments call for another comment. Both 1981 and 1982 data, including the Barcelona zone, show a different distribution from the other metals, and poor pollution control could be responsible for the levels of both metals. The same phenomenon was found in the analysis of Huelva Bay sediments (4) carried out in 1981.

References

1. PEIRO L., X. TOMAS, J. OBIOLS, J. ROS (1982). - Contenu en métaux lourds des sédiments de la Côte de la Catalogne : Méthodologie et résultats. VIes Journées d'études sur la pollution de la Méditerranée. Cannes C.I.E.S.M. 431-434.
2. JAGNER D., K. AREN (1979). - Potentiometric stripping analysis for zinc, cadmium, lead, and copper in sea-water. Analytica Chemica Acta 107 29-35.
3. TOMAS X., J. OBIOLS et al. - Méthodologie traitement et présentation des données accumulées sur la contamination dans une large zone de la mer Catalane. VIes Journées d'Etudes sur la pollution de la Méditerranée. Cannes C.I.E.S.M. 461 to 464.
4. TOMAS X., J. OBIOLS, L. PEIRO (1983). - La contaminacion por metales pesados en sedimentos de la ria de Huelva. Analisis de resultados. Afinidad (1983) 40. 413 to 420.
5. J.L. RINGOT. These 3e cycle. Université Paris-sud (1980).

ENVIRONMENTAL CONDITIONS IN MEX BAY WEST OF ALEXANDRIA 1.-PHYSICAL SPECIATION OF FOUR TRACE METALS IN THE BAY WATER*

by

O. ABOUL DAHAB, O. EL-RAYIS and Y. HALIM
Oceanography Department, Faculty of Science
Alexandria University, Alexandria (Egypt)

Abstract

Dissolved, colloidal and particulate Cu, Pb, Cd and Zn were separated by Nuclepore filters and measured in 74 water samples collected from Mex Bay and from two effluents by solvent extraction-graphite furnace AAS technique. Most of the Pb was in the particulate form (75 % of the total). Particulate Cu, Zn and Cd represent only 43 %, 20 % and 17 % of their respective total concentrations. The agricultural drain input largely exceeds that of the chlor-alkali waste water. The impact of the two sources on the coastal waters is discussed.

*
* *

Introduction

Mex Bay, west of Alexandria, receives waste water from a chlor-alkali plant (30 x $10^3.m^3.d^{-1}$) and mixed agricultural run-off (Umum drain, 6 x $10^6.m^3.d^{-1}$). It also receives airborne particles from the fumes of adjacent industrial plants including a cement factory. Although some attention has been given recently to trace metals in the aquatic environment off Alexandria including Mex Bay (EL-NADY 1981, SAAD, in HALIM 1983) little is known about the physico-chemical forms of the metals and nothing about their input from the land-based sources. Pb has not been investigated before. The aim of the present work is to assess the input rate and the distribution of the metal forms in relation to particle size.

---

* Aquatic Environment Pollution Project EGY/73/058.

Material and methods

The chlor-alkali effluent and Umum drain were sampled six times (August 1983 to May 1984). Two cruises were carried out (August 1983, FEBRUARY 1984) involving 11 stations in Mex Bay (fig. I). Samples were collected with a peristaltic pump from standard depths down to 20 m and kept in acid-washed polyethylene containers. All precautions recommended by KREMLING (1983) were followed during collection, treatment and filtration of the samples. Acid precleaned Nuclepore filters of 0.4 µm (particulate form) and 0.2 µm (particulate plus colloidal forms) were used. The filterate from the latter represents the dissolved phase which includes ionic, chelated and sorbed metals on finer colloids. The filters were dried and subsequently treated with suprapure nitric acid in PTFE pressure decomposition vessels. A double beam Perkin-Elmer AAS was used applying flameless technique. All manipulations were carried cut in a laminer flow clean bench in a dust-free room.

Table 1
Average daily discharge from the agricultural drain
and the chlor-alkali effluent. (g/day, six monthly samples)

Metal	Chlor-Alkali	Drain
Pb	82.2	34722
Cu	354.0	121200
Cd	6.8	2496
Zn	1620.0	594000

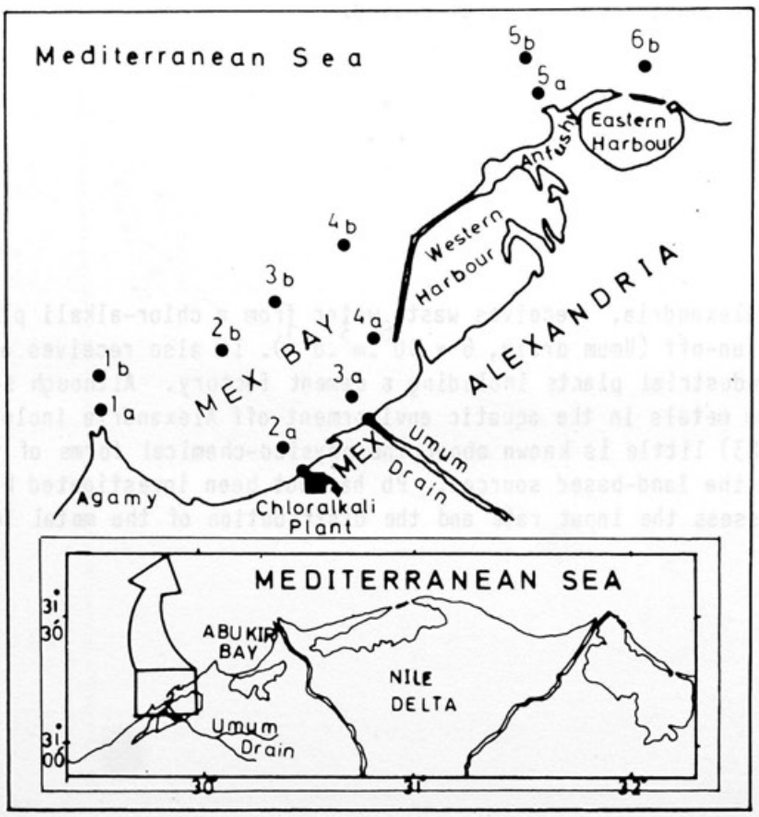


Figure 1. - Area of study and sampling stations Positions.

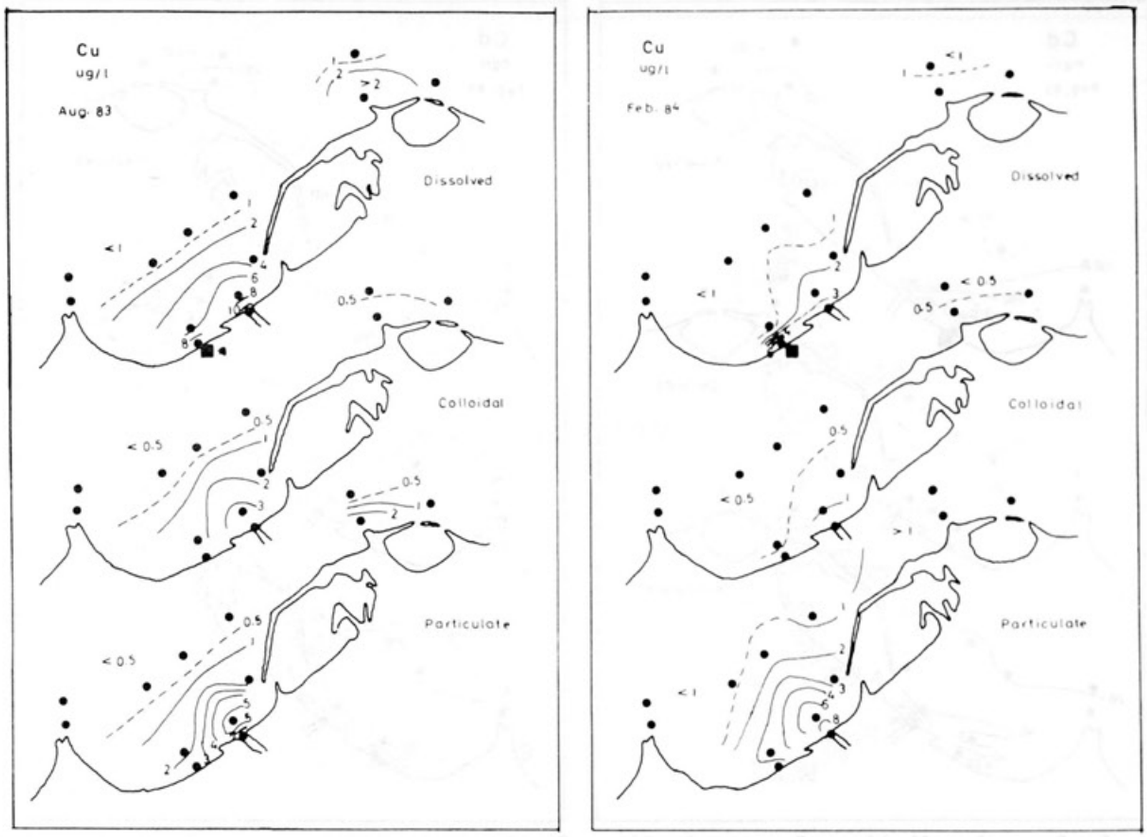


Figure 2. - Surface distribution of concentration of different Copper phases in the coastal water, west of Alexandria.

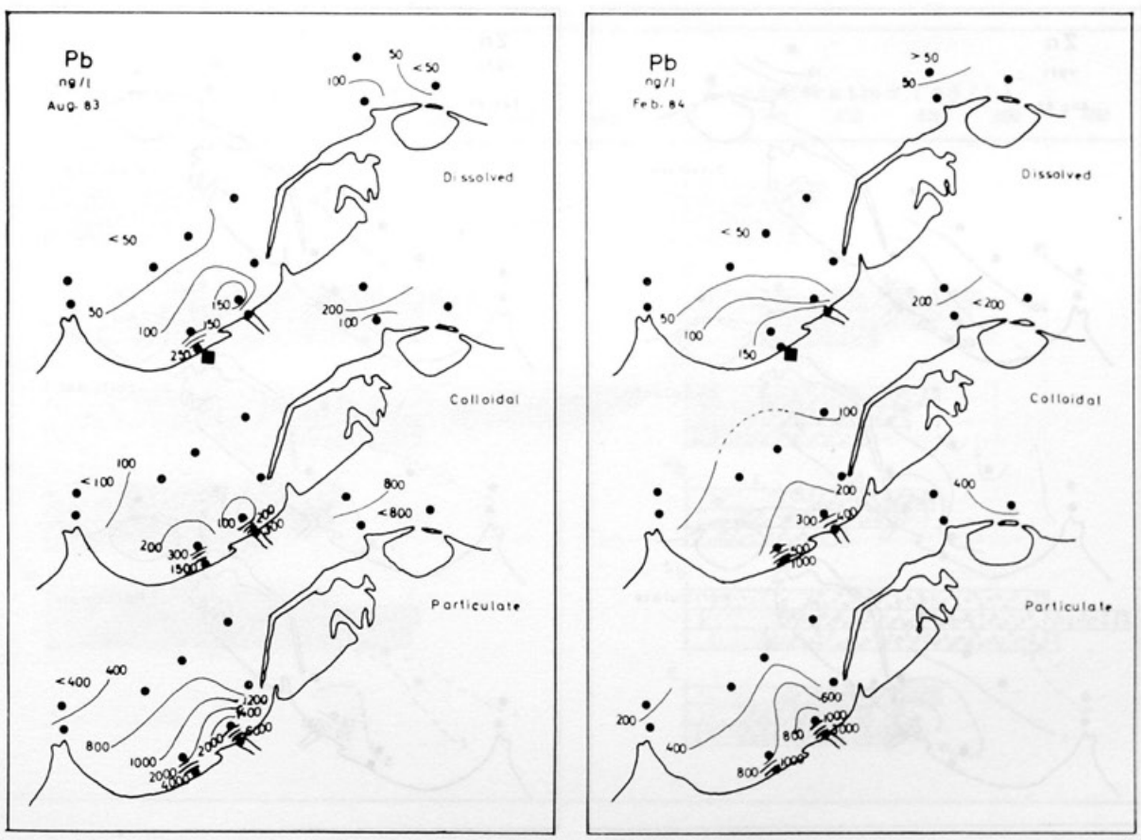


Figure 3. - Surface distribution of concentration of different Lead phases in the coastal water, west of Alexandria.

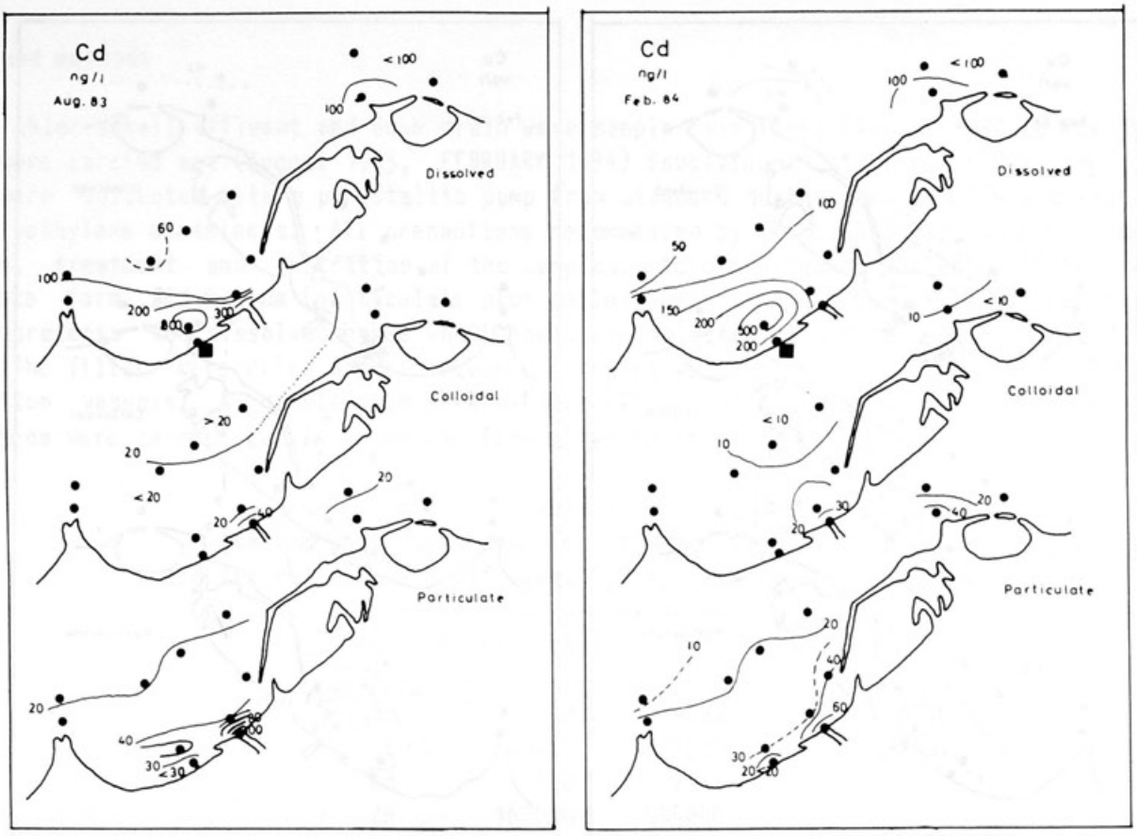


Figure 4. Surface distribution of concentration of different Cadmium phases in the coastal water, west of Alexandria.

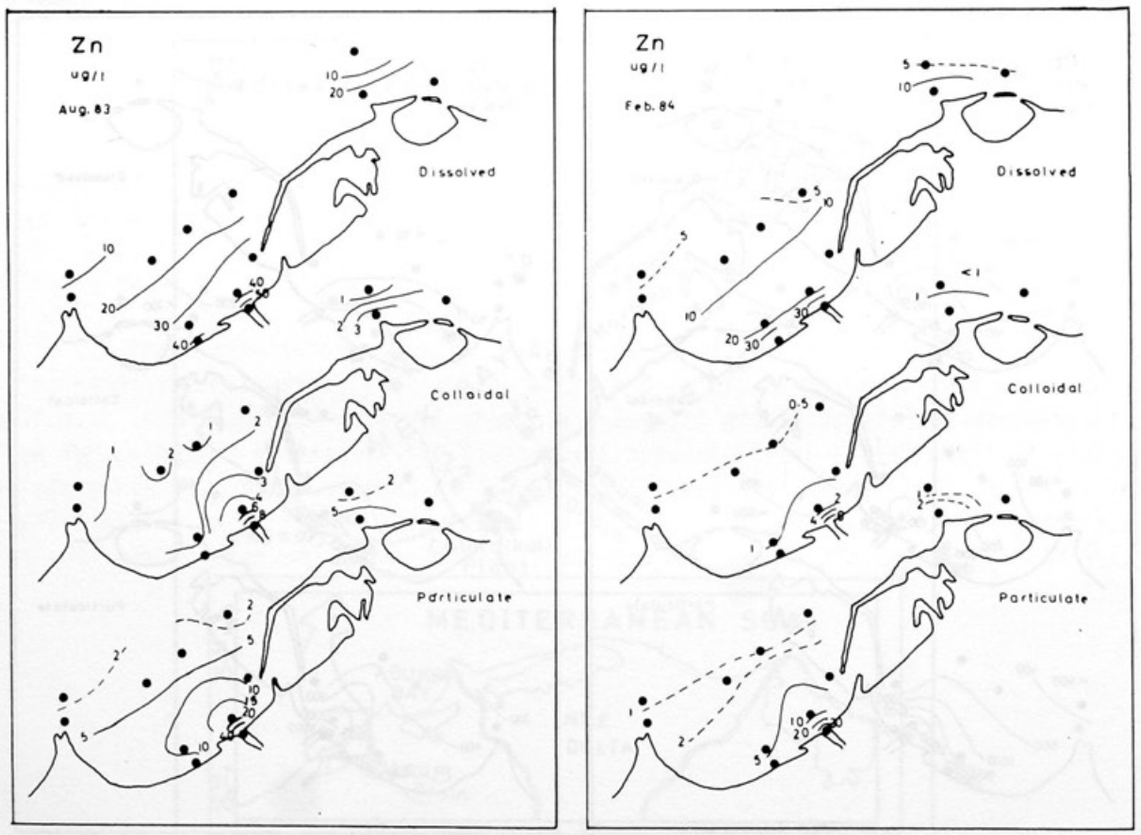
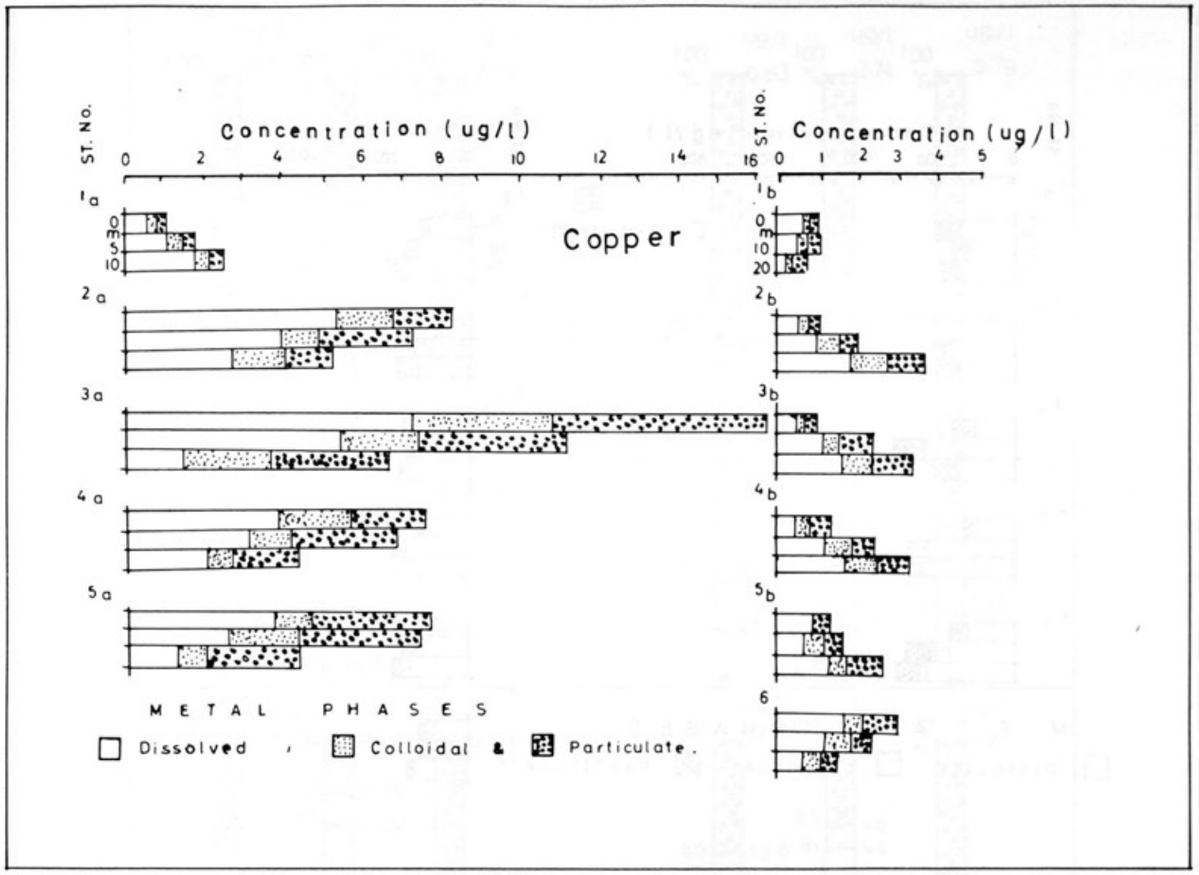


Figure 5. - Surface distribution of concentration of different Zinc phases in the coastal water, west of Alexandria.



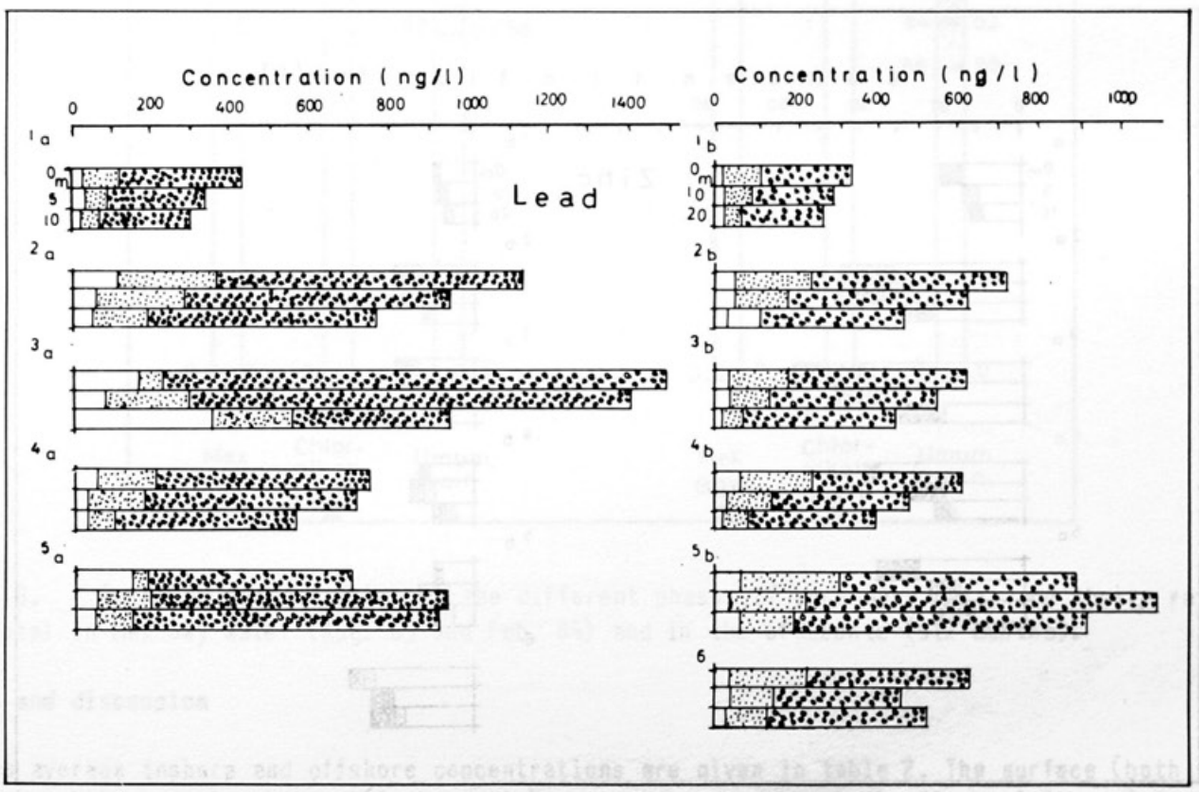


Figure 6. - Histograms of the concentrations of the three phases of Copper and Lead in each water sample collected from the shore area west of Alexandria, Aug. 1983.

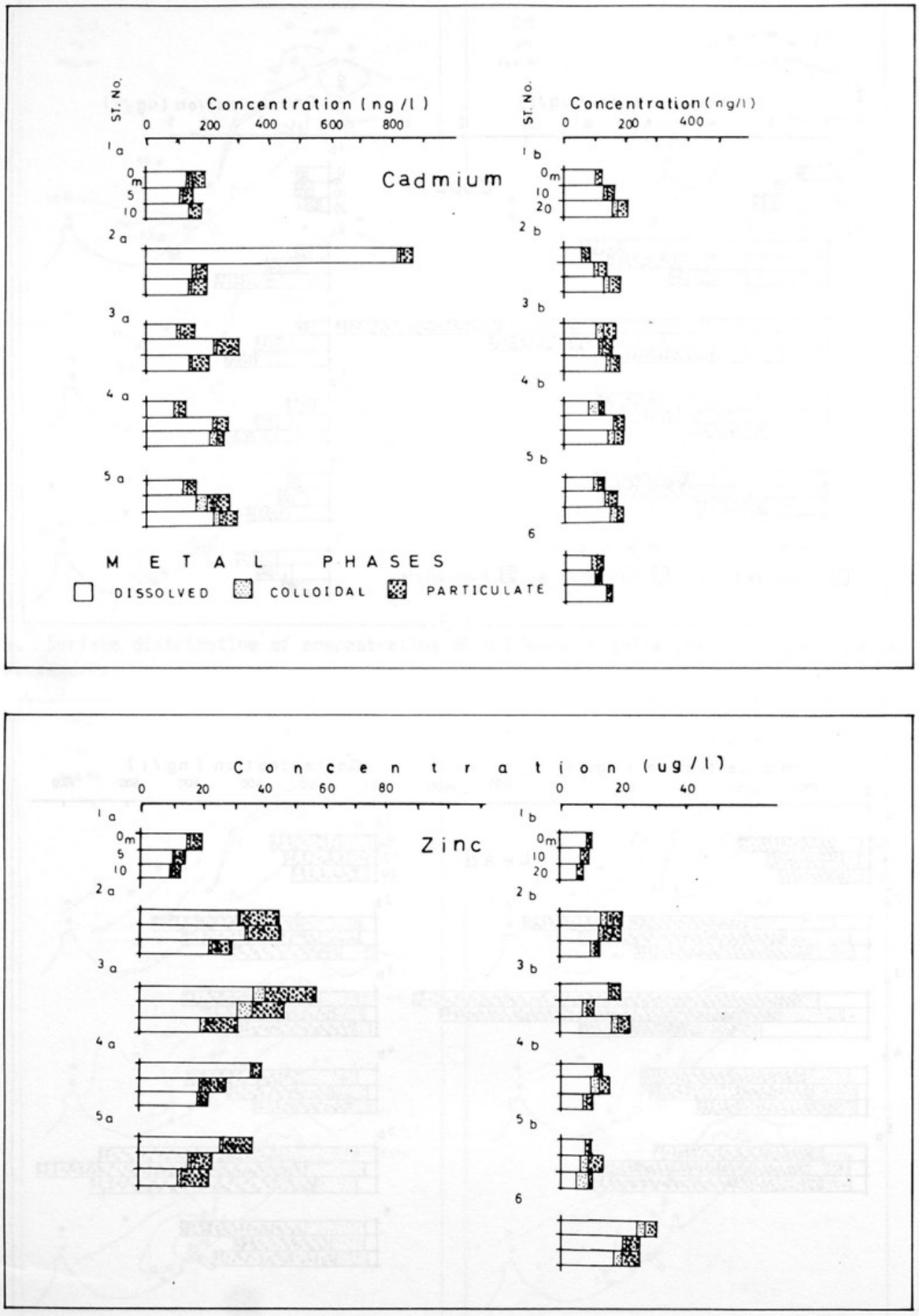


Figure 7. - Histograms of concentrations of the three phases of Cadmium and Zinc in each water sample collected from the shore area, west of Alexandria, August 1983.

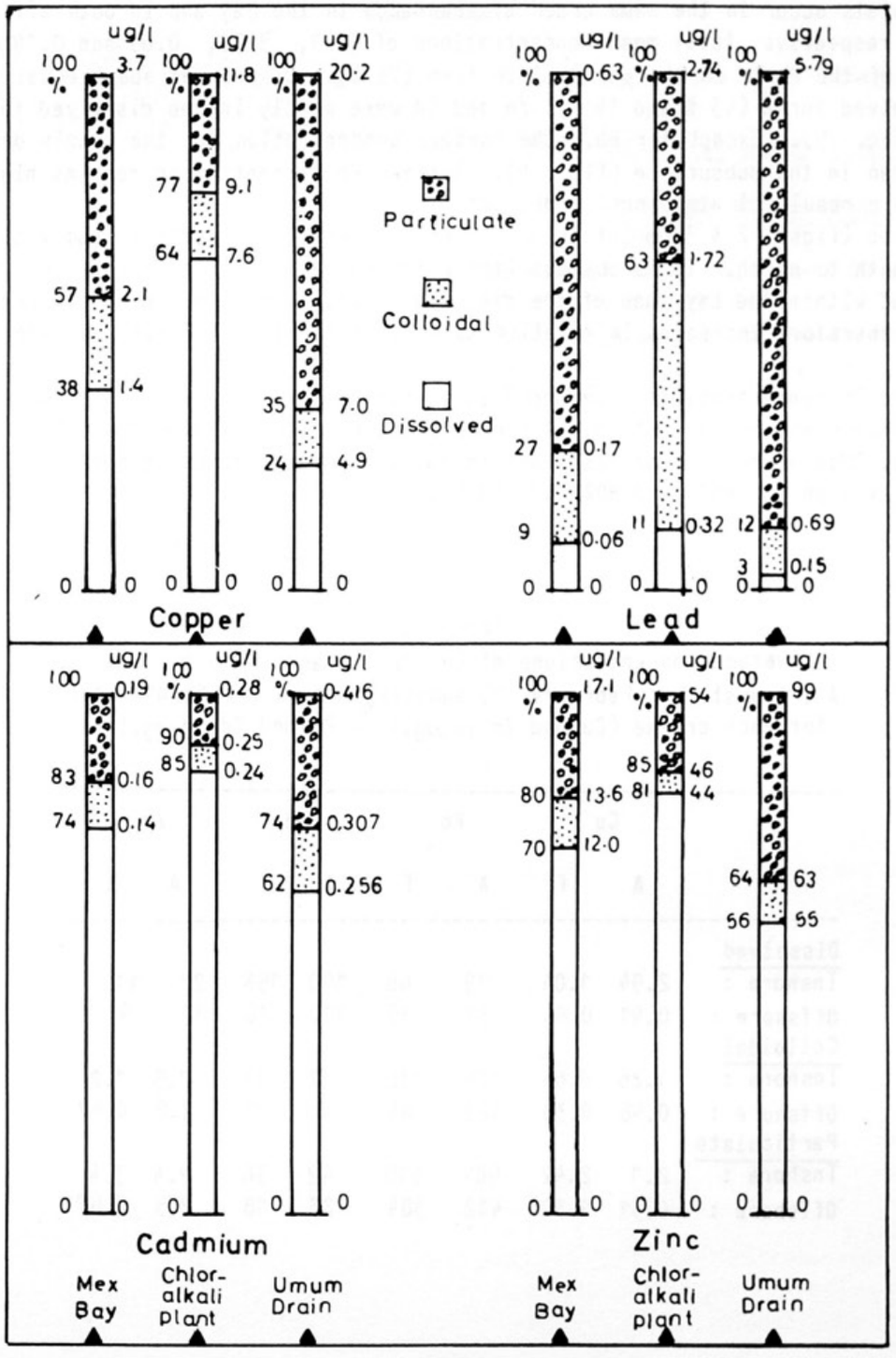


Figure 8. - Average concentration of the different phases of each hevy metal and their ratios to their total in Mex Bay water (Aug. 83 and Feb. 84) and in the effluents (six months).

Results and discussiom

The average inshore and offshore concentrations are given in table 2. The surface (both cruises) and the vertical distributions (August only) are shown respectively in figs. 2-5 and 6-7 and the average phase concentrations on fig. 8. The metal concentration and the daily input rate of the drain largely exceed those of the chlor-alkali effluent (table 1).

The four metals occur in the same order of abundance in the bay and in both effluents, Zn, Cu, Pb, Cd, with respective total mean concentrations of 17.1, 3.72, 0.63 and 0.19 $\mu g l^{-1}$ for both cruises. Most of the Pb is in the particulate form (73 %). Cu occured about equally in the particulate and dissolved forms (43 % and 39 %). Zn and Cd were mostly in the dissolved form, respectively 70 % and 76 % (fig. 5). Except for Pb, the surface concentration for the metals decreases offshore more rapidly than in the subsurface (fig. 4). Surface Pb concentration remains higher everywhere, most probably as a result of atmospheric fall-out.

The isolines (figs. 2 & 3) point to a smaller concentration gradient from east to west within the bay than south to north. It is obvious (table 3) that a greater proportion of the particulate forms is retained within the bay than of the dissolved form. The fraction of the metal input trapped within the bay therefore increases in relation to their respective affinity to associate with particulate matter.

Cu, Cd and Pb concentrations obtained fall within the ranges given by NURNBERG (1977) for the Ligurian and Tyrrhenian coastal waters. All earlier results from Alexandria waters are higher than the present ones. Measurements from Rosh Haniqra to Hifa yielded twice as much ZN, 4 times as much Cd and 8 times as much Pb (ROTH and HORNUNG, 1975).

Table 2.

Average concentrations of Cu, Pb, Cd and Zn in Mex bay.
A : August, F : February. 15 samples inshore and 18 offshore for each cruise (Cu and Zn in $\mu g.l^{-1}$, Pb and Cd in $ng.l^{-1}$)

	Cu		Pb		Cd		Zn	
	A	F	A	F	A	F	A	F
Dissolved								
Inshore :	2.94	1.04	79	66	190	158	22	11
Offshore :	0.91	0.84	33	35	110	70	11	4
Colloidal								
Inshore :	1.26	0.64	124	132	17	17	2.5	1.2
Offshore :	0.46	0.33	123	84	16	16	2.0	0.47
Particulate								
Inshore :	2.1	2.42	607	510	42	36	7.4	3.4
Offshore :	0.51	1.37	412	304	23	18	2.5	0.67

Table 3

Offshore to drain concentration (%) of the metal phases

	Cu	Pb	Cd	Zn
dissolved :	8	17.7	32.1	19.9
colloidal :	11.8	37.2	23.2	21.1
particulate :	6.5	7.0	21.3	5.4

References

EL-NADY F. (1981). - Survey of some heavy metals in Alexandria waters and their effect on some marine animals. (unpublished) Ph. D. Thesis, Alexandria University, 7 pp.

HALIM Y. (1983). - Mid-Term Report, 1983, Aquatic Environmental Pollution Project EGY/73/058. Alexandria University.

KREMLING K. (1983). - Determination of trace metals. In : "Methods of sea-water Analysis", K. Grassoff, Edt., Verlag Chemie. Weinh.

NURNBERG H.W., L. MART and P. VALENTA (1977). - Concentrations of Cd, Pb and Cu in Ligurian and Tyrrhenian coastal waters. Rapp. Comm. Int. Mer. Médit., 24(8) : 25-29.

ROTH I. and HORNUNG H. (1975). - Heavy metal concentration in water, sediments and fish from the Mediterranean coastal area, Israel. Final Report, Israel Oceanogr. and Limn Ltd., Haifa pp 1-12.

ENVIRONMENTAL CONDITIONS IN MEX BAY, WEST OF ALEXANDRIA
2. - MERCURY SPECIES IN THE BAY WATERS

by

Osman A. EL-RAYIS, Y. HALIM and O. ABOUL DAHAB
Oceanography Department, Faculty of Science, Alexandria University, Alexandria (Egypt)

Abstract

Dissolved reactive and organic mercury as well as the particulate form were measured in 66 surface and subsurface water samples collected from 11 stations in Mex Bay, between Agamy and Anfushy, in addition to 12 water samples collected from two outfalls, a chloralkali effluent and an agricultural drain. A flameless AAS-Cold vapour technique and the U.V. photo-oxidation method for releasing Hg bound to organic matter were used. Mercury shows a strong tendency to be associated with particulate matter, particulate mercury contributing 80 per cent to the total Hg. Dissolved reactive form represents 75 per cent, on average, of the total dissolved mercury. The most toxic form, organic mercury, represents only 5 per cent of the total. There is a general tendency for all mercury species to decrease seawards. High mercury contamination in the vicinity of both the industrial and the drain outfalls is observed, the level downstream form the chloralkali plant outfall being of the same order as that reported for a similar area on the Mediterranean coast of Tuscany, Italy.

The rate of Hg input to the bay through agricultural run-off, however, largely exceeds the chloralkali input, despite the higher concentration of the latter.

*
* *

Introduction

The present work is part of a more comprehensive investigation of environmental conditions in Mex Bay, and in particular, the level and fate of heavy metals in their different forms downstream from industrial and agricultural effluents (ABOUL DAHAB et al., this volume) both in water and in biota (HALIM et al., ABOUL DAHAB et al., EL-RAYIS et al. in press).

Considerable efforts have been directed in recent years at assessing the mercury problem in the Mediterranean basin (U.N.E.P., 1983). In the area of Alexandria, investigation of total Hg in recent sediments on the inner shelf (EL-SAYED and HALIM, 1979) and its level in several fish species (EL-SOKKARY, 1980) have revealed the existence of a mercury problem west of Alexandria. Ther anomalous concentrations found in sediments and fish were naturally related to the presence of a chloralkali plant in Mex Bay. The present work, carried out in the frame of the Aquatic Pollution Project of the University of Alexandria, aims at the determination of the physico-chemical forms of Hg, their level and fate in coastal waters. No information is available, at present, on either total Hg or its different forms in Egyptian waters.

	Station&Depth (m)	Total dissolved Hg T		Reactive Hg R		Organic Hg T-R		%R/T Hg		Particulate Hg	
		Aug.	Feb.	Aug.	Feb.	Aug.	Feb.	Aug.	Feb.	Aug.	Feb.
Inshore water	1-a 0	7.6	8.3	5.5	6.0	2.2	2.3	71.3	72.3	21.1	23.6
	5	3.3	6.4	2.3	4.3	1.0	2.1	70.9	67.2	21.8	30.3
	10	9.4	9.2	6.1	6.1	3.3	3.1	65.1	66.3	38.1	37.9
	2-a 0	11.7	15.7	8.9	11.4	2.8	4.3	76.3	72.6	69.5	73.9
	5	22.3	15.9	17.9	12.1	4.5	3.8	79.9	76.1	81.8	76.2
	10	33.1	22.0	23.0	15.4	10.1	6.6	69.5	70.0	86.7	90.6
	3-a 0	12.3	8.2	8.4	5.4	4.0	2.8	67.9	65.9	50.2	46.7
	5	11.4	9.1	9.4	6.8	2.0	2.3	82.4	74.7	49.1	40.1
	10	13.5	10.4	13.0	9.7	0.5	0.7	96.2	93.3	37.2	27.3
	4-a 0	10.2	10.3	8.1	8.1	2.1	2.2	79.3	78.6	44.8	36.3
	5	7.6	11.1	5.9	8.2	1.7	2.9	77.4	73.9	37.5	50.1
	10	9.9	7.8	8.1	6.0	1.8	1.8	81.4	76.9	63.0	51.3
	5-a 0	5.6	6.2	4.3	4.2	1.5	2.0	73.7	67.7	33.7	43.2
	5	12.5	10.1	8.7	6.1	3.9	3.9	69.2	61.0	34.6	34.9
	10	13.4	9.6	8.1	6.0	5.3	3.6	60.5	62.5	40.8	47.7
	Cruise average	12.21	10.70	9.17	7.80	3.04	2.90	75.1	71.9	47.3	47.3
	Total average	11.46		8.49		2.97		73.50		47.31	
Offshore water	1-b 0	7.4	8.7	5.9	7.1	1.5	1.6	79.3	81.2	13.9	17.2
	10	7.9	7.6	5.9	5.1	2.0	2.5	75.1	66.6	16.2	20.5
	20	7.4	9.3	4.7	5.8	2.7	3.5	63.8	62.7	24.4	23.3
	2-b 0	6.5	9.2	5.3	7.3	1.2	1.9	81.9	79.3	36.9	24.2
	10	9.7	10.4	7.8	7.5	1.9	2.9	80.1	72.1	40.5	38.3
	20	5.4	14.0	3.9	9.6	1.5	4.4	72.4	68.7	56.9	57.9
	3-b 0	4.9	6.0	3.2	4.2	1.6	1.8	66.4	69.7	35.4	26.1
	10	8.0	9.1	5.7	5.8	2.4	3.2	70.6	64.3	34.8	34.6
	20	15.5	12.3	9.3	7.8	6.1	4.5	60.3	63.4	42.7	37.5
	4-b 0	8.9	6.2	6.4	4.6	2.5	1.6	71.9	73.5	36.4	22.1
	10	8.2	8.7	5.7	5.7	2.5	3.0	69.4	65.7	32.9	40.2
	20	9.9	13.3	6.0	8.1	3.8	5.2	61.3	60.9	29.5	38.3
	5-b 0	11.6	12.1	7.6	8.2	3.9	3.9	65.9	68.1	28.6	23.1
	10	11.8	20.1	7.5	13.1	4.3	7.0	63.4	65.0	33.5	36.2
	20	10.0	7.8	5.6	4.8	4.4	3.0	56.3	61.2	34.1	33.9
	6-b 0	10.0	4.1	7.1	2.9	2.9	1.2	71.4	70.3	18.4	21.2
	10	7.3	4.8	5.1	3.0	2.2	1.8	70.1	62.7	28.7	17.9
	20	6.4	11.5	4.1	6.9	2.3	4.6	63.6	59.9	23.0	26.1
	Cruise average	8.7	9.73	6.29	6.53	2.4	3.2	72.4	67.5	31.5	29.9
	Total average	9.20		6.41		2.80		69.94		30.71	
	General mean	10.34		7.45		2.89		71.72		39.01	

Table 1. - Mercury species in coastal and offshore waters, west of Alexandria (ng l^{-1}). (Aug. 1983 and Feb. 1984).

Month	Total dissolved Hg T (ng l^{-1})		Reactive Hg R (ng l^{-1})		Organic Hg T-R (ng l^{-1})		%R/T		Particulate P (ng l^{-1})	
	Ind.	Agr.	Ind.	Agr.	Ind.	Agr.	Ind.	Agr.	Ind.	Agr
Aug.1983	1979	46	1943	16	36	30	98.2	35.3	927	548
Jan.1984	2474	75	2422	24	52	51	97.9	31.6	1035	386
Feb.1984	1757	53	1732	16	25	37	98.6	29.9	971	345
Mar.1984	2228	62	2148	19	80	43	96.4	30.1	754	580
Apr.1984	2151	47	2056	17	95	30	95.6	36.2	975	742
May 1984	1792	53	1740	19	52	34	97.1	35.5	878	670
Average	2064	56	2007	19	57	38	97.3	33.1	923	546
SD	± 275	± 10.9	± 263	± 3.6	± 26.4	± 8.2	± 1.1	± 2.9	± 98	± 155

Table 2. - Mercury in the Chlor-alkali effluent and the agricultural drain (Aug. 1983 to May 1984).

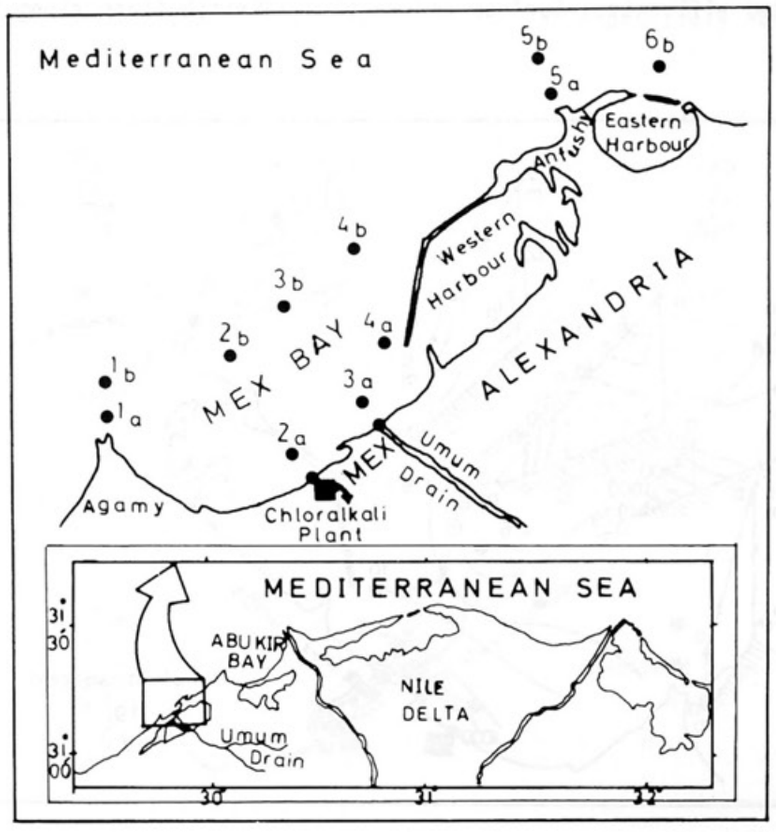


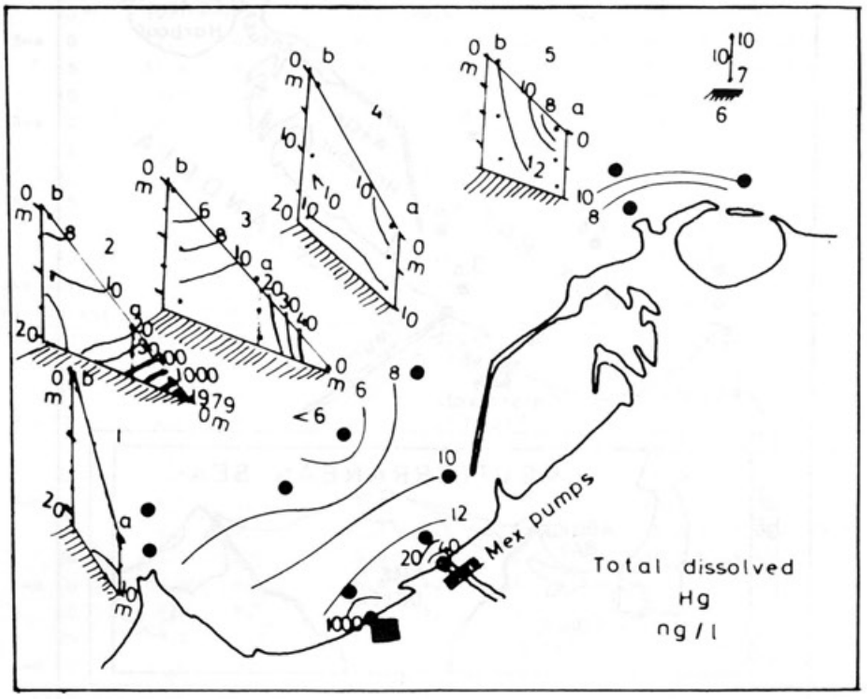
Figure I. - Area of study and sampling stations positions.

Material and methods

Water samples were collected by means of a peristaltic pump from depths of 0, 5, 10 and 20 m at 11 stations along 5 traverses (figure I) in August 1983 and February 1984. Six monthly samples were also collected from the two effluents, "Umum" drain and the chloralkali plant in the same period. The samples were collected in pre-cleaned pyrex glass bottles following the precautions described by P. ERICKSON (KREMLING, 1983). The samples were filtered over 0.45 µm Sartorius membrane filters previously cleaned with suprapure HCl. Analysis was performed following FREIMANN and SCHMIDT (1982). A filtered sub-sample was UV irradiated for two hours and the total dissolved Hg determined. This includes both reactive and organically bound Hg. Reactive Hg was measured in a separate filtered subsample without irradiation. The difference between total dissolved and reactive represents the organically bound Hg. The filter with the retained suspended matter was decomposed with suprapure nitric acid in a teflon pressure digestion kit. Cold vapour AAS was used with Argon aeration, $SnCl_2$ reduction, pre-enrichment and purification by amalgamation on finely dispersed gold. The gold was deposited on pure silica wool under high vacuum. The detection limit of this procedure was of 0.5 $ng.l^{-1}$ and the coefficient of variation 4 per cent for concentrations of 1 to 5 $ng.l^{-1}$. All manipulations were carried out in a laminar flow clean-bench in a dust free room.

Results and discussion

Results from both cruises for Hg species are given in table 1. Table 2 includes six months measurements for the two effluents. Surface and vertical distributions along the five traverses of



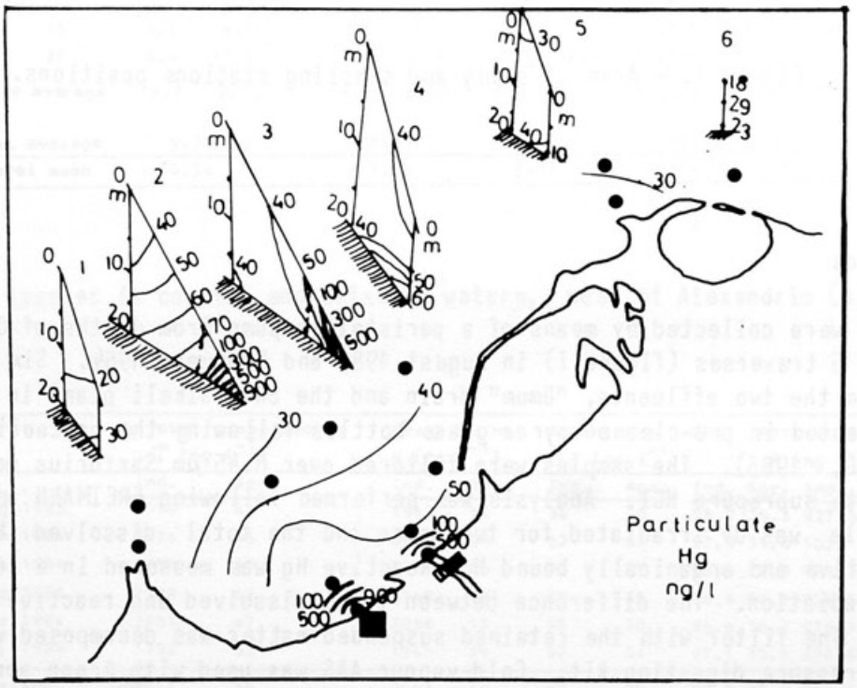
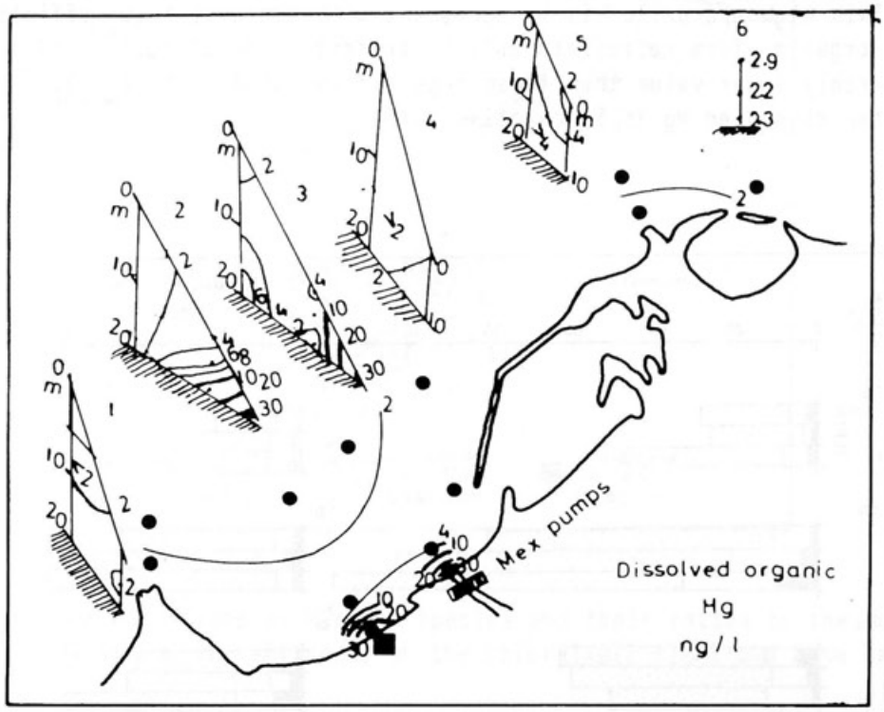


Figure II. - Surface and vertical distribution of total dissolved and particulate Mercury in the water west of Alexandria (August 1983).

August are represented in figure II for total dissolved and particulate Hg, and figure III for reactive and organic forms. Concentrations of the three species at three levels for each of the August stations are shown by the histogram on figure IV. The three histograms in figure V show the total average concentrations and their relative proportions respectively for the two effluents and for both cruises.



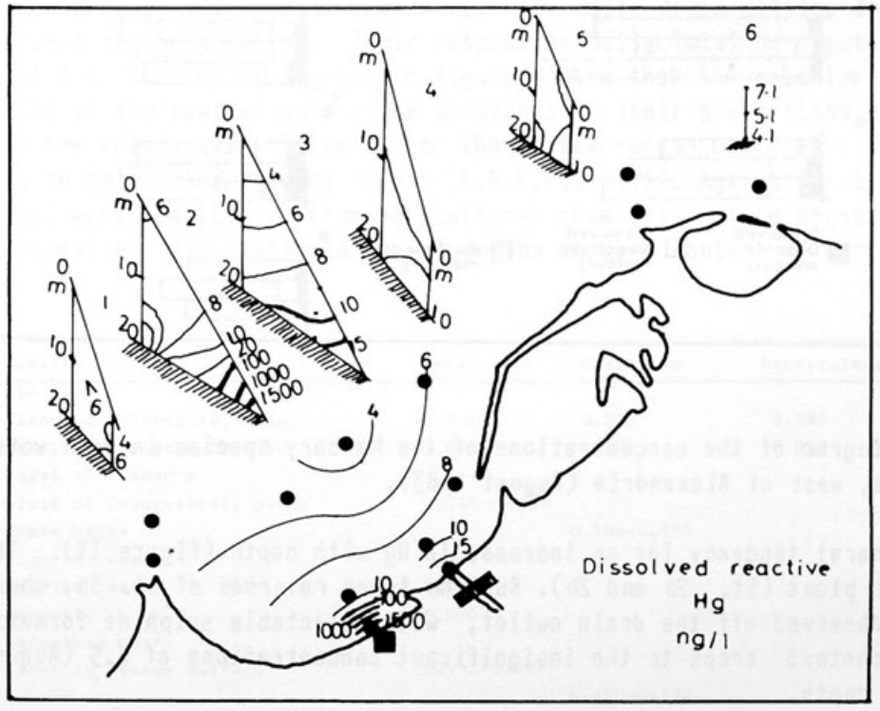


Figure III. - Surface and vertical distribution of dissolved organic and reactive Mercury in the water west of Alexandria. (August 1983).

Both cruises are in agreement with regard tc the levels of concentration, the relative proportions of the Hg species and the distributions trends. The cruise averages for inshore and offshore water showed remarkably small differences from August to February (table 1). All forms of Hg decrease slightly seawards, but the decrease is more drastic for the particulate form, namely 34 per cent from inshore to offshore.

About 80 per cent of the total Hg (79-80 per cent inshore, 77 per cent offshore) is in the particulate form. This high proportion is in agreement with the well known affinity of Hg for suspended matter. The organic form represents only 5 per cent (2.8-2.9 ng.l^{-1}) of the total for both cruises, a considerably lower value than those reported by BARGHIGIANI et al. (1980). About 70-73.5 per cent of the total dissolved Hg is in reactive form.

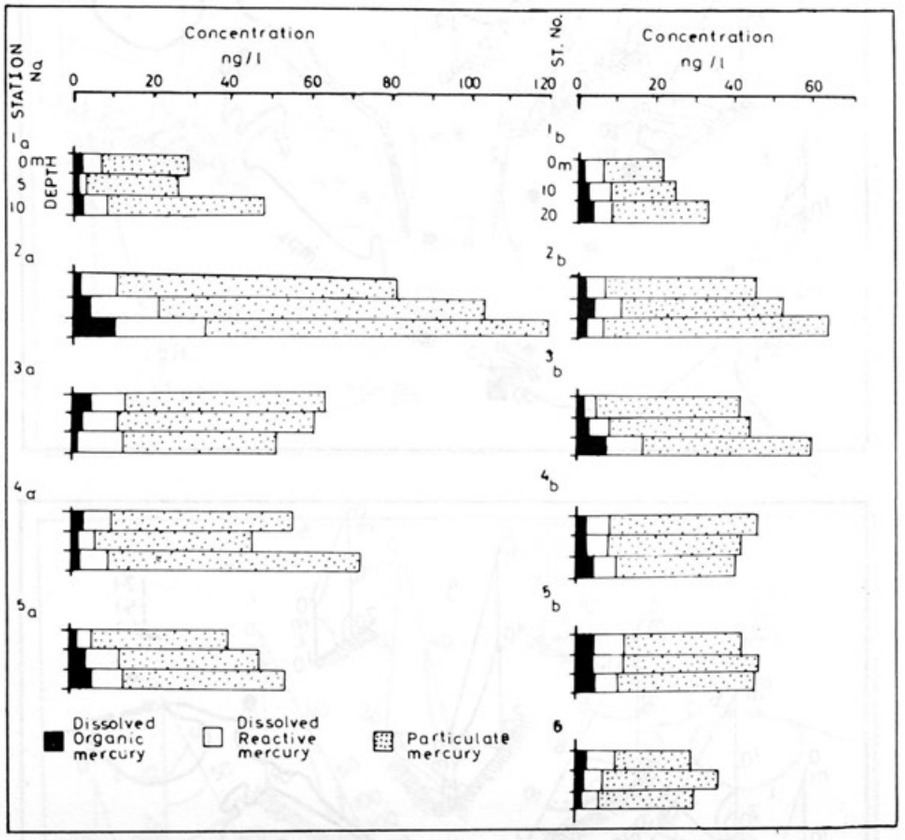


Figure IV. - Histogram of the concentrations of the Mercury species in each water sample collected from the shore area, west of Alexandria (August 1983).

There is a general tendency for an increase in Hg with depth (figure IV). This is most obvious off the chloralkali plant (St. 2a and 2b). But the trend reverses of st. 3a, where a strong salinity stratification is observed off the drain outlet, with detectable sulphide formation over the bottom. Its organic Hg content crops tc the insignificant concentrations of 0.5 (August) to 0.7 ng.l^{-1} (February) at 10 m depth.

The surface spreading cf the drain run-off is also shown by the shape of the isolines for particulate Hg in figure II. Off the outlet (traverse 2), the lines are inclined towards the offshore surface, while off the chloralkali effluent, their inclination is towards the offshore bottom. Despite the higher Hg concentration in the latter effluent, the Hg input to Mex Bay through agricul-

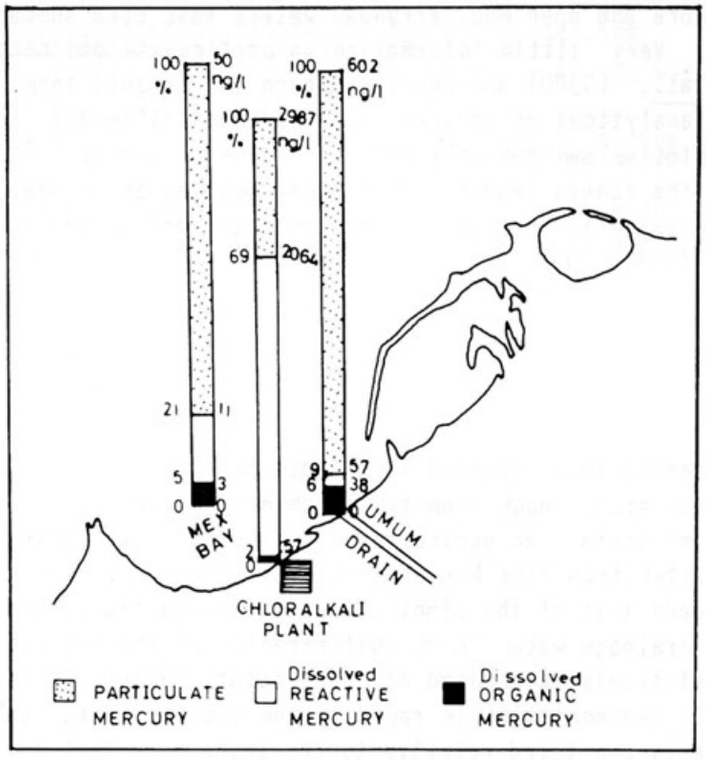


Figure V. - Average concentrations of Mercury species and their ratios to the total Mercury in Mex Bay (Aug. 1983 & Feb. 1984) and the effluents of the Chloralkali Plant and Umum Drain (six months).

tural run-off is much greater and spreads much more. The daily drain outflow amounts to 6×10^6 m^3 and that of the effluent to 150×10^3 m^3. Their respective daily total Hg inputs, 3612 g and 450 g, are in the ratio of 8 : 1. The histograms in figure V show that the relative proportions of the different Hg species in the coastal waters are analogous to their proportions in the agricultural run-off, and not to the chloralkali effluent. For the Mediterranean basin as a whole, 94 per cent of the Hg input appears to be carried down by rivers (U.N.E.P., 1979). Agricultural run-off is likely to drain Hg from inland municipalities, from agricultural chemicals, from degassing and weathering activities. No information was available as yet on the mercury content of agricultural drains in Egypt.

Locality	Total	Dissolved	Particulate
Mex Bay			
-Close to Chlor-alkali plant	0.130	0.020	0.080
-Mean(66 samples,0-20 m)	0.049	0.010	0.039
Coast of Tuscany			
-Close to Chlor-alkali plant	0.020-0.180		
-Rhone Delta		0.100-0.190	
-Ligurian coast	0.012-0.260		
-Istrian coast	0.040		
-Adriatic	0.070		
Saronikos Gulf			
-Close to sewage outfall	0.150-0.600		
-Israel		0.160-0.180	
-East Mediterranean open waters		0.120-0.020	

Table 3. - Mercury levels ($\mu g\ l^{-1}$) in Mex Bay compared to other Meditarrean localities (references in UNEP, 1983, tables 2 & 3).

Values for Hg in inshore and open Mediterranean waters have been summarized in a recent U.N.E.P. Report (U.N.E.P., 1983). Very little information on particulate and reactive Hg is available and except for BARGHIGIANI et al., (1980) apparently none on the organic form. The various authors have often followed different analytical procedures and determined different forms. Intercomparison on this basis can only be tentative and can only deal with general trends. The mean dissolved form from the present area is below the ranges reported from other Mediterranean areas, the open sea included. The mean total Hg is comparable to that on the Istrian coast and in the Adriatic and much lower than the ranges from elsewhere (table 3).

Conclusions

The anomalous Hg concentrations reported from sediments and fish from Mex Bay, west of Alexandria, result from the cumulative input from two neighbouring sources of Hg contamination, a chloralkali plant and an "Umum" drain, an agricultural effluent. The latter effluent is known to drain also highly contaminated water from lake Maryut, south of Alexandria. The rate of input from agricultural run-off largely exceeds that of the plant effluent, though its concentration is much lower. The role of this agricultural drainage water in Hg contamination of the bay was completely overlooked in earlier surveys. The relatively high load of particulate matter in drainage water is the main vehicle for Hg in the bay. Sedimentation is rapid in the coastal belt, but the offshore particulate mercury is decreased by only one third relative to the inshore concentration. Sedimentation is more rapid off the chloralkali effluent than off the drain outlet, as a result of salinity stratification beyond the outlet. It is likely that much of the particulate mercury (higher than 0.45 µm diameter) is taken up by various filter-feeding organisms, thus becoming incorporated in the food-chain.

The results of the two cruises are remarkably consistent regarding the levels of concentration and the relative proportions of the three Hg species determined. The organic form is much lower, namely 5 per cent, than reported from elsewhere in the Mediterranean. The reactive and particulate forms account respectively for 15 and 80 per cent of the total. The level of total Hg contamination of sea-water in this area is comparable to, if not lower than that reported from analogous Mediterranean localities. Comparisons, however, should only be made with great caution, because of differences in analytical techniques in the various Mediterranean laboratories, and of the probability of contamination at all stages.

References

ABOUL DAHAB, O., O. EL-RAYIS and Y. HALIM (1984). - Environmental conditions in Mex Bay. 1 - Physical speciation of four trace metals in the Bay waters. VIIes Journées Etud. Pollutions, Lucerne, C.I.E.S.M. (this volume).

ABOUL DAHAB, O., O. EL-RAYIS and Y. HALIM (1984). - Mercury species in coastal marine organisms from different trophic levels west of Alexandria. Submitted to Biogeochemical cycle of mercury in the Mediterranean meeting in Siena, Italy.

BARGHIGIANI, C., R. FERRARA, A. SARITTI, A. PETROSINO, A. MASONI and E. MORELLI, (1980). Determination of reactive, total and particulate mercury in the coastal waters of Tuscany (Italy) by atomic fluorescence spectrometry. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. 127-130.

EL-RAYIS O., Y. HALIM and O. ABOUL DAHAB, (1984). - Total mercury in the coastal marine ecosystem west of Alexandria. Submitted to Biogeochemical cycle of mercury in the Mediterranean meeting in Siena, Italy.

EL-SAYED M. and Y. HALIM, (1978). - Survey of the trace metals pollution in the sediments from the Alexandria region, Egypt. 1-Mercury. IVes Journées Etud. Pollutions, Antalya, C.I.E.S.M. 187-191.

EL-SOKKARY I.H., (1980). Mercury accumulation in fish from Mediterranean coastal area of Alexandria, Egypt. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. 493-498.

FREIMANN P. and SCHMIDT, (1982). - Determination of mercury in sea water by cold vapour atomic absorption spectrophotometry. Analy. Chem. 313, 200-202.

KREMLING K. (1983). - Determination of trace metals. In "Methods of sea water analysis". K. Grasshoff, 2nd Adt. Verlag Chemie Weinh.

U.N.E.P., (1983). - Assessment of the present state of pollution by mercury in the Mediterranean sea and proposed control measures. Long-term programme for pollution monitoring and research in the Mediterranean sea (MED POL-Phase II). U.N.E.P./WG.91/5.49 pp.

U.N.E.P., (1984). - Pollutants from land-based sources in the Mediterranean. Regional Seas Programme Activity and Studies n° 32.

ASSESSMENT OF MERCURY POLLUTION IN THE COASTAL REGION OF EL MAX AT ALEXANDRIA, EGYPT

by

I. H. ELSOKKARY
University of Alexandria Research Centre
UNARC-Horreya Avenue
P. O. Box 832, Alexandria (Egypt)

Abstract

Samples of water, sediment and fish were collected from El Max region in order to evaluate the magnitude of their contamination by Hg. The concentration of total Hg (TOTHG) and organic Hg (ORGHG) were measured in these materials.

The results show that sea-water contained, on the average, appreciable amounts of TOTHG especially in Summer relative to those in winter. However, TOTHG and ORGHG in sediments did not vary with seasons of sampling. Also, it is found that about 0.10 per cent of TOTHG in sediments are present as ORGHG.

The concentrations of TOTHG and ORGHG in fish fillet were mostly lower than those in fish liver. Samples of fishes collected in summer contained higher amounts of Hg than those collected in winter. Generally, ORGHG constituted, on the average, about 55 per cent and 70 per cent of TOTHG in fish fillet and liver respectively. The results also show that tissue organs of Solea vulgaris contained higher amounts of Hg than those of Mullus surmuletus.

* * *

Introduction

The widespread pollution of El Max region by mercury has been well established in the literature (EL SAYED and HALIM 1978 ; ELSOKKARY, 1980 and ELSOKKARY 1982). The source of mercury in that area is the industrial effluents discharged from the chlorine alkali plant of El Max which is considered the most important contributor to mercury contamination. Since El Max is considered an important fishing ground, determination of mercury concentration in fish as well as water and sediment samples will contribute seriously to an evaluation of the rate of mercury pollution. Moreover, measurement of the concentration of organic mercury (methylmercury), which is regarded as the most potential health hazard, will give valuable information about the dramatic effect of the contamination of that region by mercury.

This work was partly carried out under the framework of the "Aquatic Environ. Poll. Project" (U.N.E.P./ U.N.E.S.C.O. / U.N.A.R.C., EGY / 73 / 058) and U.N.EP. / F.A.O. co-Programme MED POL PHASE II, in order to monitor mercury levels in waters, sediments and fishes in the coastal area of Alexandria.



El-Max area

Materials and methods

El Max region is located beyond Alexandria city. It is subjected to high pollution by mercury from the effluents discharged from Chlorine alkali plant in the area (see figure).

Samples of water, sediment and fish were collected in two seasons : winter (Jan., Feb., and March, 1982) and summer (June, July. and Aug. 1982).

Water : Surface sea-water was sampled by a plastic bucket, filtered immediately into a glass bottle and stored in a deep freezer for mercury analysis.

Sediments : Nearshore sediment samples were collected by a grab. The clay fraction (< 2 u) was separated by sedimentation and then collected, dried at 35 °C and stored for mercury analysis.

Fishes : Two fish species were used in this study : Mullus surmuletus and Solea vulgaris. The fish were collected by fishermen's hand nets, transported to the laboratory then stored in the deep freezer for mercury analysis.

Mercury analysis : Wet digestion technique described by F.A.O. fish Tech. Per her No. 158 (F.A.O., 1976) was used after some modification for water and sediments. Total mercury (TOTHG) and organic mercury (ORGHG) were measured in the fillet and liver of the fish, and also in the sediment samples (F.A.O. Tech. N° 158, 1976). Mercury was measured in the extract by flameless atomic absorption spectrophotometer, Varian model 175. The analytical technique used in our laboratory for mercury measurement in fish was intercalibrated. The results of Hg analysis, for samples obtained from I.A.E.A., Monaco, having code names : MA-A-1, MA-A-2 and orchards leaves NBS standards, were highly accurate.

Organic carbon was measured in the clay fraction according to the method described by EL WAKEEL and RILEY (1957).

Results and discussion

Sea-water

The results given in table 1 show that samples of water collected in winter contained on the average lower amounts of Hg (0.063 ug/l) than those collected in summer (0.128 ug/l). Generally, the present values are greater than other data reported by FUKAI and HUYNH-NGOC (1976) who found that values varied from 0.017 to 0.32 ug Hg/l in the filterate of surface sea-water samples collected from the western area of the Mediterranean sea.

Table 1
The range and average values of TOTHG (ug/l) in the filtrate of surface sea-water samples collected in winter and summer 1982 from El Max.

Season of Sampling	Number of Samples	Range	Average	S.D.
Winter	6	0.020 - 0.140	0.063	0.049
Summer	8	0.040 - 0.250	0.128	0.086

Sediments

The results in table 2 show very low variations in the average levels of TOTHG and ORGHG in sediment samples collected in winter and summer. It is also clear that the ORGHG fraction constituted a very small portion of the TOTHG in all sediment samples (winter and summer) which varied from 0.05 to 0.15 per cent with an average 0.10 per cent of TOTHG.

Comparing our results with others reported in the literature (BERNARD and RENZONI, 1977), it is clear that the amounts of TOTHG in El Max sediments are extremely high. This can be attributed to the property of the analysed portion of sediment which was only the clay fraction. This part is the most active in sediment and can accumulate high amounts of Hg. In agreement with our results are the data reported by EL SAYED and HALIM, 1978. They also assumed that 0.100 ppm Hg is the background level for uncontaminated sediment in the area. Accordingly high enrichment values of El Max sediments by Hg caused by the surrounding industries have been found and reached on an average more than 200.

The results also show the presence of a highly significant relation between the O.C. in sediment samples and both TOTHG ($r = 0.87$) and ORGHG ($r = 0.78$).

Table 2
The range and average values of TOTHG and ORGHG (D.W. basis) in the clay fraction (< 2 u) of sediment samples collected in winter and summer 1982 from El Max

season of sampling	number of samples		O.C. %	TOTHG ppm	ORGHG ppb
winter	9	range	5.0 - 11.0	9.55 - 21.50	7 - 24
		average	8.5	16.27	18
		S.D.	± 2.8	± 5.15	± 6
summer	12	range	4.9 - 12.0	8.90 - 21.95	8 - 22
		average	6.8	14.31	15
		S.D.	± 2.1	± 6.0	± 6

Fish

The concentration of TOTHG and ORGHG in the fillet and liver of the two fish species ; M. Surmullus and Solea vulgaris are summarized in tables 3 and 4. It is clear that the amounts of TOTHG in fish varied widely with the fishing season since it was higher in fish collected in summer than in those collected in winter. This relation was also found by UYSAL (1978), ELSOKKARY (1980) and AYDOGDU et al. (1982). It is also clear that liver accumulated higher amounts of Hg than fillet. This trend can easily be found with changes in fishing seasons and can also be found in the two fish species.

The concentrations of ORGHG in fish fillet were generally lower than those present in the liver and clearly follow the same trend of seasonal variations.

Table 3

The range and average values of TOTHG and ORGHG (ug/kg FW) in fillet and liver of M. Surmuletus fishes collected in winter and summer 1982 from El Max.

number of fishes		standard length mm	fillet		liver	
			TOTHG	ORGHG	TOTHG	ORGHG
			winter 1982			
	range	65 - 200	420 - 1,400	250 - 700	1,200 - 2,815	900 - 2,400
27	average	122	830	470	1,940	1,468
	S.D.	± 58	± 455	± 210	± 665	± 515
			summer 1982			
	range	65 - 192	650 - 2,600	300 - 1,350	1,650 - 3,650	1,040 - 2,600
29	average	125	1,425	732	2,435	1,754
	S.D.	± 60	± 650	± 490	± 715	± 650

Table 4

The range and average values of TOTHG and ORGHG (ug/kg FW) in fillet and liver of Solea vulgaris fishes collected in winter and summer 1982 from El Max

number of fish		standard length mm	fillet		liver	
			TOTHG	ORGHG	TOTHG	ORGHG
			winter 1982			
	range	96 - 180	680 - 1,110	350 - 700	1,600 - 3,150	980 - 2,650
19	average	134	931	482	2,333	1,790
	S.D.	± 48	± 253	± 185	± 650	± 750
			summer 1982			
	range	89 - 180	1,000 - 1,900	485 - 960	2,000 - 4,150	1,500 - 3,100
26	average	128	1,412	692	3,230	2,494
	S.D.	± 39	± 368	± 265	± 960	± 865

The calculated ORGHG as percentages of the TOTHG were found to vary considerably between liver and fillet organs. On the average for the two fish species, this percentage was about 55 per cent in fish fillet, and was much higher in fish liver reaching 70 per cent. The data in the literature report a value of 66.6 per cent of TOTHG as methylmercury (WHO, 1976).

Comparing the concentration levels of Hg in the organs of the two fish (tables 3 and 4), it is clear that, on the average, fillet and liver of Solea vulgaris contained higher amounts of both TOTHG and ORGHG than those of M. surmuletus.

It is clear from the data presented below in table 5 that there is no significant correlation between fish length and concentration of Hg in the fillet of the two fish species. However, positive significant correlations have been found between fish length and concentration of Hg in the liver of the two fish species.

Table 5
The correlation coefficient values calculated between fish lengths and their mercury conctnets

fish species	season of sampling	fillet		liver	
		TOTHG	ORGHG	TOTHG	ORGHG
Mullus surmuletus	winter	0.11	0.28	0.69	0.75
	summer	0.32	0.12	0.82	0.72
Solea vulgaris	winter	0.20	0.30	0.87	0.75
	summer	0.09	0.16	0.76	0.79

Conclusion

From the data presented in this study, we can infer that the concentrations of Hg in the water, sediment and fish samples investigated are much higher than those found in the literature. This is due to contamination of the area by Hg discharged in the effluents from the chlorine alkali plant of El Max.

It is obvious that the Hg introduced into the marine environment is concentrated in marine organisms by a factor varying from 1,000 to 10,000 (VINOGRADOV, 1953). It is also well established that there is a positive relation between Hg content in fish and its concentration in the water recipient (FRIBERG and VOSTAL, 1972 ; BERNARD and RENZONI, 1977). Thus the fate of Hg introduced into El Max ecosystem can be arranged according to its concentration as follows : water < fish < sediment.

The high Hg concentration observed in fish of El Max raises the question of possible health risks. According to WHO (1976); a provisional tolerable weekly intake of 200 ug Hg as methylmercury or 300 ug Hg as total Hg per 70 kg man has been recommended as a safety limit. Following our results, a value of TOTHG 1,000 ug/kg FW fillet, as an average in El Max fish, and according to the WHO (1976) recommendation. the amounts of fish fillet which could be consumed by 70 kg man will not exceed 300 g per week. By consuming more than this permissible diet, poisoning by Hg is highly probable.

References

AYDOGDU, T., T.I. BALKAS, F. BINGEL, I. SALIHOGLU and S. TUGRUL (1982). - Mercury in some benthic fish of the north Levantine (East Mediterranean). VIth Workshop on Marine Poll. of the Mediterranean, Cannes.

BERNARD, M. and A. RENZONI (1977). Mercury concentration in Mediterranean marine organisms and their environment : natural or anthropogenic origin. 2nd Inter. Marine Pool. Symposium, Dubrovnik, Croatia, Yugoslavia, October 10 - 13 .

BERNARD M., (1976) F.A.O. Fish Techn. Pap N° 158. Manual of methods in aquatic environmental research Part 3. Sampling and analysis of biological materials.

EL SAYED M.Kh. and Y. HALIM (1978). - Survey of the trace metals pollution in the sediments from Alexandria region, Egypt. I-Mercury. IVes Journées Etud. Poll. Antalya. C.I.E.S.M. pp. 187 - 191.

ELSOKKARY I.H. (1980). - Mercury accumulation in fish from Mediterranean coastal area of Alexandria, Egypt, Ves Journées Etud. Poll., Cagliari, C.I.E.S.M. pp. 493 - 496.

ELSOKKARY, I.H. (1982). - Comparative study on the levels of total mercury in sediments and fishes collected from three fishing sites in Alexandria, Egypt, VIth workshop on Marine Poll. of the Mediterranean Cannes.

EL WAKEEL S.K. and J.P. RILEY (1957): - The determination of organic carbon in marine muds. J. Cons. Int. Explor. Mer. 22 : 180 - 183.

FRUBERG L. and J. VOSTAL (1972). - Mercury in the Environment. Chemical Rubber Co. Ohio. pp. 23 - 26.

FAUKAI R. and L. HUYNH-NGOC (1976). - Trace metals in Mediterranean sea-water. Activities of the Inter. Lab Of Marine Radio activity, Monaco. Report, I.A.E.A. Vienna. Tech. Doc. I.A.E.A. - 137. pp. 122 - 132.

UYSAL H. (1978). - Accumulation and distribution of heavy metals in some marine organisms in the bay of Izmir and Aegean coasts. IVes Journées Etud. Poll. Antalya C.I.E.S.M. pp. 213 - 217.

VINOGRADOV A.P. (1953). - The elementry chemical composition of marine organisms. Sears Fed. Mar. Res - Nex Haven., Conn.

WHO (1976). - Environmental health criteria I, mercury. WHO, Geneva.

ESTIMATION OF METAL POLLUTANT LEVELS IN SEDIMENTS FROM LAKE BRULLUS (MEDITERRANEAN COAST, EGYPT)

by

Atef A. MOUSSA
Institute of Oceanography and Fisheries
Alexandria (Egypt)

Abstract

Lake Brullus is one of the large Nile Delta brackish water lakes having a direct connection with the Mediterranean Sea. Land run-off including solid materials from topsoil erosion is carried to the lake through the drainage system.

Samples were collected from the surface layer of sediments in the lake and analysed for their grain size, total carbonates, organic carbon, iron, phosphorus, zinc, copper, and lead.

The results indicate that the composition of sediments is homogeneous over the area in spite of some local variations. The sediments are composed of an admixture of sand, silt and clay. Calcareous shells form the greater part of the sands and they act as a diluent to the lithogenic materials.

The heavy metals are found in low concentrations comparable to those in the lithogenic derived sediments.

On comparison of the present results with others that have been reported for different Egyptian aquatic environments it is revealed that the sediments in lake Brullus are not polluted and therefore have no influence on the nearly Mediterranean waters.

*
* *

Introduction

Natural reservoirs such as lagoons and coastal lakes act as containment basins for pollutants. During containment, metal pollutants may to a varying degree become incorporated in the basin sediments. Anthropogenic contamination can thus be traced from the level of excess metal concentration in sediments whose initial natural composition is known.

The magnitude of subsequent pollutant transfer from lakes and lagoons to the coastal marine environment is dependent on both the degree of contamination of the reservoir and the nature and size of output to the sea.

The main purpose of this paper is to follow the changes in element concentrations going from the source area (Nile Delta) to the marine environment through a brackish water lake (lake Brullus). In order to obtain valuable information on this matter, the present work is based on :

1. Determination of the concentration levels of the trace metals : zinc, copper and lead in the surface sediments of the lake and the study of their distribution in conjunction with the grain size of sediments and the distribution of some essential components such as total carbonates, organic matter, iron and phosphorus.

2. Comparison of the results obtained with those previously reported for other polluted and unpolluted Egyptian aquatic environments in an attempt to find out how great the capacity of the lake could be to assimilate pollutants or regulate their release.

Table 1. - Results of sediment analyses.

component	range	mean	variation coefficient (V)
sand	4 - 100 %	36 ± 9.9 %	0.66
silt	0 - 50.5 %	34 ± 4.8 %	0.35
clay	0 - 57.8 %	31 ± 5.9 %	0.48
median	2.3 - 8.6 Ø	5.7 ±0.8 Ø	0.34
sorting	2.0 - 3.9 Ø	2.9 ±0.3 Ø	0.25
carbonate	1.7 - 73 %	26 ± 7.9 %	0.74
organic C	0.8 - 8.3 %	2.5 ± 0.6 %	0.61
Fe	1 - 4.5 %	3.5 ± 0.2 %	0.17
P	197 - 568 ppm	353 ± 34.5ppm	0.24
Zn	21 - 55 ppm	40 ± 3.6 ppm *	0.21
Cu	10 - 29 ppm	18 ± 2.2 ppm	0.31
Pb	18 - 34 ppm	24 ± 2.0 ppm	0.20

* The precision is ± 5% for trace elements

Table 2. - Correlation matrix.

	median	sand	silt	clay	CO_3^{--}	C	Fe	P	Zn	Cu	Pb
median	1										
sand	-0.82	1									
silt	0.68	-0.84	1								
clay	0.90	-0.76	0.68	1							
CO_3^{--}	-0.36	0.36	-0.13	-0.23	1						
C	0.13	-0.27	0.37	0.08	0.03	1					
Fe	0.27	-0.80	0.58	0.41	0.23	0.23	1				
P	-0.29	0.23	-0.41	-0.20	0.01	0.40	-0.39	1			
Zn	0.49	-0.61	0.31	0.57	-0.15	-0.09	0.32	-0.23	1		
Cu	0.06	-0.31	0.25	0.46	-0.40	0.23	0.26	0.02	0.3	1	
Pb	0.11	-0.14	0.06	0.17	0.03	-0.11	0.30	0.14	0.3	0.25	1

Area of study

Lake Brullus is a shallow basin connected to the Mediterranean through a narrow outlet of 250cm depth (figure 1). Its area covers 550 km2 and the water depths in the lake range from 50 to 200cm. The northern shores of the lake are bordered by high land covered with sand dunes, while the southern shore is low land having marsh-like characteristics caused by the discharge of drainage waters and dense plant vegetation.

The salinity range of the water is 1.56 - 8.46 with a mean value of 3.51, the highest salinity values being found in the vicinity of the lake-sea connection and near the northern shore (EL-SHERIF, 1983). The pH of the water ranges from 7.5 to 8.3 attaining its highest values in the northern parts and the lowest near the outlet of the drains. With a tidal range of 20 cm the average outflow to the Mediterranean is estimated to be roughly 500 x 106m3/year.

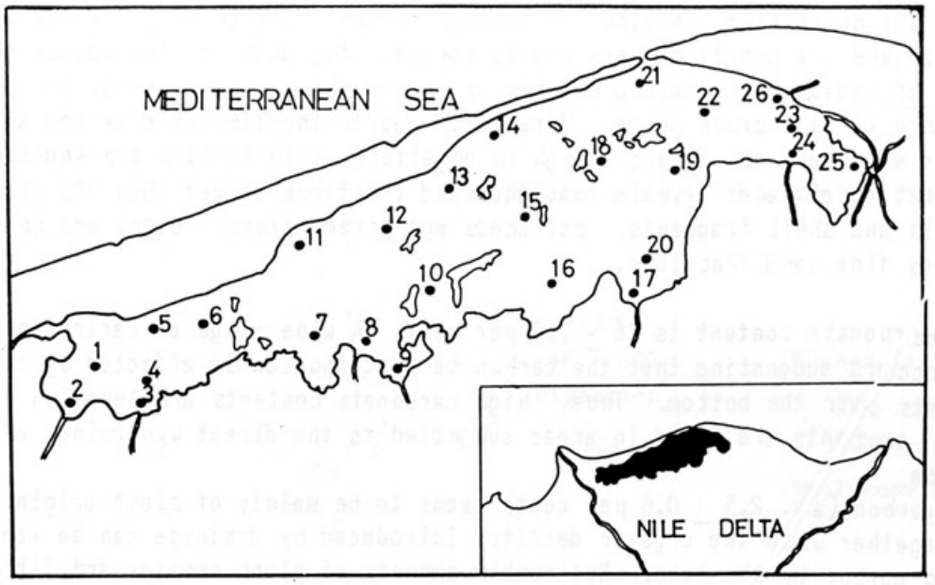


Figure 1. - Lake Brullus and sampling locations.

Material and methods

Twenty-six grab samples were taken from the surface layer of sediments and dried in air prior to storage and analyses.

Grain-size analysis was carried out by dry sieving for sand and by pipette technique for silt and clay fractions (KRUMBEIN and PETTIJOHN, 1938). The data were treated following FOLK (1974).

The total carbonate contents were estimated from gasometric measurement of the carbon dioxide released from the sample upon treatment with hydrochloric acid using a calcimeter technique.

The organic carbon was determined according to the method of EL-WAKEEL and RILEY (1957) which is based on wet oxidation with chromic acid.

Iron and phosphorus were determined in solutions prepared from sediment digestion with hydrofluoric and perchloric acids, while the trace metal concentrations were measured later on in other solutions prepared by treatment of the same sediments with nitric and sulphuric acids.

The standard colorimetric methods were used for the determination of iron and phosphorus. Iron is reduced to the ferrous state by hydroxylammonium chloride and a red colour is developed using 2.2 dipyridine. The phosphorus is determined using ammonium molybdate reagent and the resulting phosphomolybdate complex is reduced by ascorbic acid to a blue colour. The intensity of colours is measured in a Shimazu double-beam spectrophotometer UV-150-02. Zinc, copper and lead concentrations were measured by AAS.

Results

The ranges and mean values of the different parameters are shown in table (1) and the interrelations between them are shown in the correlation matrix (table 2).

Grain size and sediment composition

The grain size data reveal that the sediments are composed of an admixture of sand, silt and clay in almost equal quantities, having an average median diameter of 5.7 ± 0.8 Ø. The sediments everywhere in the lake are poorly or very poorly sorted, the mean sorting values being 2.9 ± 0.3 Ø. This poor sorting of sediments is caused maintly by the crushing of calcareous shells into fragments having a wide range of size-grade scale. Moreover, due to the limited size and shallowness of the lake, currents and waves are not strong enough to be effective in sorting the sediments.
Microscopic examination of sands reveals that the sand fractions larger than 125 microns are entirely of mollusc shells and shell fragments, ostracods and foraminifers. Light and heavy minerals are predominant in very fine sand fractions.

Carbonates

The average carbonate content is 26 ± 7.9 per cent. A wide range of variation (V = 0.74) around the mean value occurs suggesting that the carbonate distribution is affected by the flow of shells and their movements over the bottom. Thus, high carbonate contents are found in sheltered areas, while the lower contents are found in areas subjected to the direct winnowing effects of water.

Organic carbon

The organic carbon (av. 2.5 ± 0.6 per cent) seems to be mainly of plant origin. The high rate of plankton growth together with the organic detritus introduced by drainage can be considered the main sources of organic carbon in the lake. Noticeable amounts of plant remains and fibres were also seen incorporated in sediments.

Iron

All the samples have iron contents close to the mean value which is 3.5 ± 0.2 per cent . Iron is positively correlated with silt (r = 0.58) and to a less extent with clay (r = 0.41), meanwhile, it is negatively correlated with sand (r -0.8). These data suggest its abundance in fine sediments specially in minerals.

Phosphorus

Phosphorus is found in moderate concentrations (353 ± 34 ppm) lower than the estimated values for muds which ranges between 0.069 and 0.12 per cent according to RITTENBERG et al. (1955). The main factors probably responsible for the relative decrease in phosphorus are first, the dilution of phosphorus-rich organic matter by in organic particles and calcareous materials, and second, the continuous return of phosphorus from sediments as a result of the high rate of its uptake by phytoplankton. Morever, regeneration of phosphate bound to soft organic material may occur in oxidized conditions (OLAUSSON, 1972).

Trace Metals

The mean concentrations of zinc, copper and lead are 40 ± 3.4 ; 18 ± 2.2 and 24 ± 2.0 ppm respectively. These data indicate their presence in uniform low concentrations. Zinc has the highest affinity to clay (r = 0.57) followed by copper (r = 0.46), while, lead did not show this tendency. Moreover, lead is not correlated with any of the sediment components studied suggesting the probability of its atmospheric source. REGIONI and FUKAI (1980) estimated that the atmospheric input of lead

on the Mediterranean Sea is 8 x 103 tonne/year, while the river input is only 3 x 103 tonne/year. On the other hand the chief source of zinc and copper seems to be the lithogenic minerals.

Discussion and conclusions

The sediments in lake Brullus have a particular composition which is characteristic of the coastal Egyptian lakes in general . Significant amounts of mollusc shells are incorporated in the sediments affecting their composition and grain size distribution. Moreover, these shells act as a diluent to the terrigenous sediments and consequently the metal concentrations.

It appears that the main source of elements is the lithogenic derived minerals which are biologically non-degradable and only undergo transformations during the various stages of the biogeochemical cycle (BREDER et al., 1980).

area	element	concentration	method of analysis	auther
lake Maryut (industrial pollution)	Zn	70 - 2960	spectrographic	Wahby (1979)
	Cu	16 - 500		
	Pb	38 - 1650		
Nile Delta Shelf (unpolluted)	Zn	2 - 119 89.4	AAS HF + HNO_3 digestion	Moussa (1982)
	Cu	5 - 77 33.4		
Nile River (unpolluted)	Zn	62 - 216 108	AAS	Emelyanov et al (1978)
	Cu	31 - 70 52		
Eastern Harbour (sewage pollution)	Zn	8 - 174 53	AAS HCL + HNO_3 digestion	El-Sayed et al (1980)
	Cu	5 - 104 27		
Abu Qir Bay (restricted pollution)	Zn	9 - 758 102	AAS	Saad et al (1980)
	Cu	tr.- 91 12		
Eastern Mediterranean	Zn	tr.- 200	spectrographic	Moussa (1977)
	Cu	5 - 50		

Table 3. - Trace element concentrations (ppm) in sediments from different aquatic environments.

On comparison of the present results with others previously obtained for different Egyptian aquatic environments as well as with those of the Eastern Mediterranean basin in general (table 3) it is found that the sediments in lake Brullus have the lowest trace element concentrations. Moreover, the table shows that significantly high values of zinc, copper and lead are limited to polluted basins. Generally, a high level of metal concentrations in sediments reflects industrial activity. In Egypt the number of industries is not sufficiently great to have a noticeable effect and its influence is found only restricted areas.

The present work can thus be used as evidence that lake Brullus is neither polluted by trace metals nor has it a polluting effect on the nearly Mediterranean waters.

Acknowledgment

I am indebted to Dr. Gustaf ARRHENIUS for reading the paper and making many helpful comments.

References

BREDER R. H.W. NURNBERG and M. STOEOOLER (1980). - Toxic trace metal levels in water and sediments from the estuaries of the southern Ligurian and northern Tyrrhenian coasts : a comparative study, Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. 285 - 292.

EL-SAYED M.Kh.,M.A. EL-SAYED and A.A. MOUSSA (1980). - Anthropogenic material in sediments from the Eastern Harbour of Alexandria, Egypt, Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. 215 - 222.

EL-SHERIF Z.M. (1983). - Limnological investigations on the aquatic plant life in lake Brullus in relation to the dominant environmental conditions. Ph.D. Thesis, Faculty of Science, Alexandria University, 385 p.

EL-WAKEEL S.K. and J.P. RILEY (1957). - The determination of organic carbon in marine muds, J. Cons., 22 : 180 - 183.

EMELYANOV E.M., A.A. MOUSSA and A.Ju. MITROPOLSKY (1978). Mineralogical and chemical composition of the Nile alluvial deposits, Lithol. and Miner. Resour., 4 : 134 - 139 (in Russian).

FOLK R.L. (1974). - Petrology of sedimentary rocks, Hemphill Publ. Co., Austin, Texas, 170 p.

KRUMBEIN W.C. and F.T. PETTIJOHN (1938). - Manual of sedimentary petrography, Appleton Century Crofts Inc., New York, 252 P.

MOUSSA A.A. (1977). - Distribution of trace elements : Zr, Cd, V, Cu, Ni, Co and Mo in the Eastern Mediterranean sediments in relevance to sedimentary processes, Ph.D. Thesis, Odessa State University, 140 p. (in Russian).

MOUSSA A.A. (1982). - Trace elements in recent sediments of the Nile Delta continental shelf : their accumulation and significance, VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M..

OLAUSSON E. (1972). - Water sediment exchange and recycling of pollutants through biogeochemical processes, in : Marine Pollution and Sea Life. F.A.O., ed. Mario Ruivo, England, pp. 158.

REGIONI B. and R. FUKAI (1980). - Distribution of different chemical forms of lead in Mediterranean sediments, Ves Journées Etud. Pollutions, C.I.E.S.M., Cagliari, 243 - 250.

RITTENBERG S.C., K.O. EMERY and W.L. ORR (1955). - Regeneration of nutrients in sediments of shallow marine basins, Deep Sea Res., 3 : 23 - 45.

SAAD M.A.N., O.A. EL-RAYIS and F.E. EL-NADY (1980). - Occurrence of some trace metals in bottom deposits from Abu Qir Bay, Egypt, Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. 555 - 560.

WAHBY S.D. (1979). - Spectrographic determination of trace metals in lake Maryut sediments, Rapp. Comm. Int. Mer. Médit., 25/9 : 77 - 78.

MINERAL MAGNETIC STUDIES FOR A POLLUTION MONITORING OF MARINE AND ESTUARINE SEDIMENTS

by

M. SCOULLOS* and F. OLDFIELD**
* Dept. of Chemistry, Univ. of Athens
13A Navarinou St., Athens 106-80
** Dept. of Geography, Univ. of Liverpool
P.O. Box 169, Liverpool L69 3BX

Abstract

Measurements of magnetic susceptibility coupled with those of the "quadrature" or frequency dependent component in sediments taken from coastal marine and estuarine areas provide a very useful rapid tool to study the transport mechanisms and origin of suspended particles.

Two areas were chosen having distinctly different characters and showing elevated magnetic susceptibility at the surface layer when compared with the deeper strata of the sediment column.

Using the quadrature and frequency dependent susceptibility it has been demonstrated very simply that magnetically active spherules in the gulf of Elefsis are of industrial origin whereas in the Louros estuary the magnetic minerals derive from the weathering of soil profiles.

*
* *

Introduction

In an earlier work SCOULLOS et al., (1979) suggested that in marine environments and particularly where magnetically active iron species are important components of industrial and urban discharges to coastal waters, particulate pollution monitoring may be carried out by using magnetic parameters such as magnetic susceptibility (x,k) and Saturation Isothermal Remanent Magnetization (S.I.R.M.). Subsequent results summarized by SCOULLOS (1981, 1984) show that not only particulate iron but also zinc, lead, copper and other metals are positively correlated with magnetic properties in the water and/or the sediment column.

OLDFIELD et al., (1981) and JONES (1984) have also indicated a link between magnetic properties and trace metal concentrations in recent ombrotrophic peats whereas other authors confirmed that several transition metals are enriched in particles derived from fossil fuel burning processes (GOLDBERG et al., 1981), suspended solids in storm waters (REVITT et al., 1982) and contemporary atmospheric dusts (HUNT et al., 1983);

The magnetic parameter used by SCOULLOS et al., (1979) to characterize the magnetic mineral assemblages in the Gulf of Elefsis, which were largely deriving from an iron and steel works, was the coercivity of S.I.R.M.

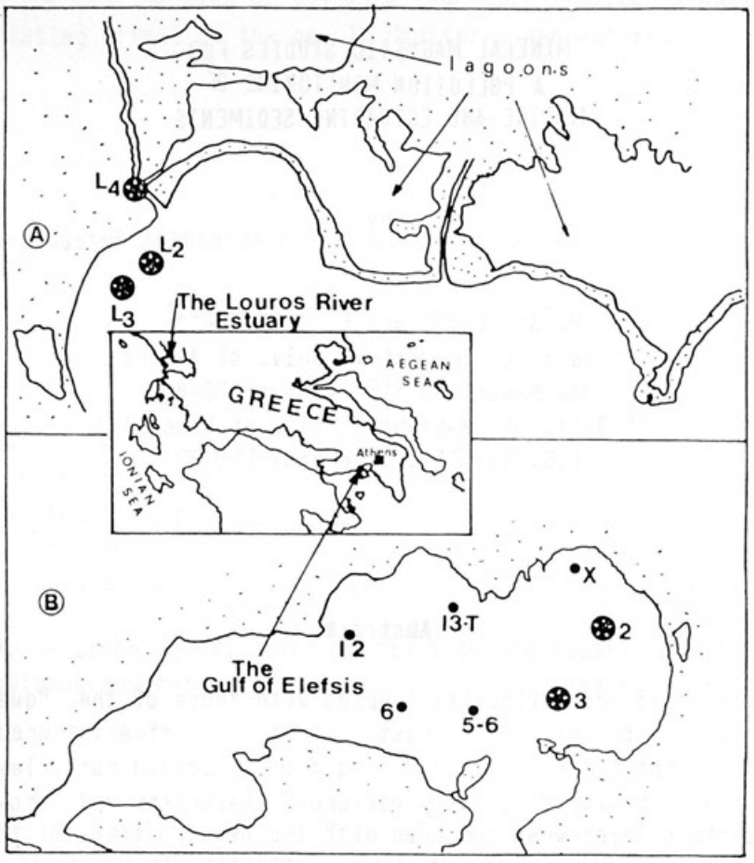


Figure 1 : The two areas from where cores have been taken: A, the Louros River estuary; B, the Gulf of Elefsis.
● core measured for the total magnetic susceptibility,
⊛ core measured also for the "quadrature" component.

Coercivity curves indicated that the major magnetic mineral present was predominently a ferrimagnetic spinel presumably "magnetite". However coercivity curves fail to distinguish between the anthropogenic magnetic fracti/n present in the industrial discharges from that washed into the gulf as a result of the erosion of surface soils rich in secondary magnetic minerals. In order to overcome this significant limitation particularly for marine or estuarine areas where the magnetic concentrations arising from industrial sources are not high, OLDFIELD and SCOULLOS (1984) introduced what they termed "quadrature" susceptibility and a new set of magnetic measurements. Quadrature susceptibility on this context reflects the presence of the ferrimagnetic crystals at the stable single domain/-superparamagnetic size transition (diameters around 0.03 um), this can also be detected by measuring magnetic susceptibility at different frequencies. Using the Bartington magnetic susceptibility meter, values comparable to quadrature readings are obtained by subtracting the high frequency (10 KHz) from the low frequency (1 KHz) values. This approach has become our standard procedure and consequently we now use the term frequency dependent susceptibility (x_{fd}) rather than quadrature (x_q)

In the present paper we present the results of the application of magnetic particulate monitoring in two areas distinctly different in character, namely the gulf of Elefsis and the estuary of Louros river within the Amvrakikos gulf (see figure 1).

The gulf of Elefsis is a small heavily industrialized bay near Athens which has received particular attention (SCOULLOS, 1973, 1979, 1979a; SCOULLOS and RILEY, 1978). The estuary of Louros river has a very narrow intermixing zone with an abrupt change from riverine to marine environment. The Louros river estuary is a typical rural area where heavy industry is virtually absent.

Methods and measurements

Cores 0.4 to 1 m long were taken with perspex tubes of 6 cm internal diameter submerged into the sediment, either by using a MACKERETH (1969) minicorer or manually. Some cores were scanned for volume susceptibility variations using a loop sensor attachment of the Bartington susceptibility meter.

Several soil samples from typical limestone sites were collected and measured for comparison.

The samples were characterized in terms of the following parameters:

- Total magnetic susceptibility either i) volume (k) or ii) mass (x) specific:
- Frequency dependent susceptibility as a volume or mass concentration (k_{fd} or x_{fd} respectively) or as a percentage determined by dividing x_{fd} x 100 by x.

Parameters k and x were measured using low frequency circuits of standard dual frequency sensors; k_{fd} and x_{fd} were calculated using the same sensors to measure both the high (10 KHz) and low (1 KHz) frequency susceptibility. Since in both volume and mass specific samples the component is expressed as a percentage of the total susceptibility, x_{fd} and k_{fd} are directly comparable.

Results and discussion

Figure 2 shows typical total susceptibility (x) scans from the Gulf of Elefsis. The major characteristic is the prominent sharp peak in total (low frequency) susceptibility in the top 15 - 20 cm of all these cores.

Figure 3 presents down core volume susceptibility (k) scans for three cores taken from the Louros river estuary.

It is noteworthy that the k values of the uppermost 10 - 20 cm samples, although different in absolute units from those of the gulf of Elefsis, are clearly higher than those of the deeper layers of the sediment column. This "similarity" in the pattern between the two sets of cores is not attributable to the same reasons.

MULLINS and TITE (1973) have shown that the quadrature or frequency dependent component in soils results almost entirely from the presence of fine-grained secondary magnetic minerals. Such fine crystals whether of maghemite or magnetite are very rarely present either in anthropogenic combustion-derived particles or in common parent geologic materials. They derive almost exclusively from the weathered part of soil profiles (OLDFIELD and BARTINGTON, 1982; THOMPSON and OLDFIELD, in press).

Therefore, considering the down core pattern of the frequency-dependent susceptibility in cores collected from both areas (figures 4 and 5) one can immediately distinguish the difference. In the two cores of the gulf of Elefsis the total susceptibility peak corresponds to a very steep decline in the percentage of the frequency-dependent component which drops to near zero values (figure 4), particularly in core 2 which is closer to the source of the anthropogenic particles. Below the recent industrial deposition horizon, the frequency-dependent component is higher and compares well with the x_{fd}, values of soils developed on the Greek limestones (10-15 per cent of total susceptibility).

From figure 5 it is apparent that the uppermost samples of the Louros estuary cores show relatively high frequency-dependent susceptibility values ranging between 7 and 11 per cent. The lower samples have much lower k_{fd} values, mostly less than 3 per cent. The soil samples of the area have a range of k_{fd} values from 7 to 12.5 per cent.

These results show that the high total susceptibility values in the case of the uppermost sediments of the Louros estuary coincide with increased contribution of the soil-derived fraction and are not due to industrial anthropogenic influences.

In order to gain more information about the different mineral components of these sediments, other magnetic parameters can be used such as the Saturation Isothermal Remanent Magnetization S.I.R.M.), reverse field (i.e. DC demagnetization) measurements in fields of -20 mT, -100 mT etc.

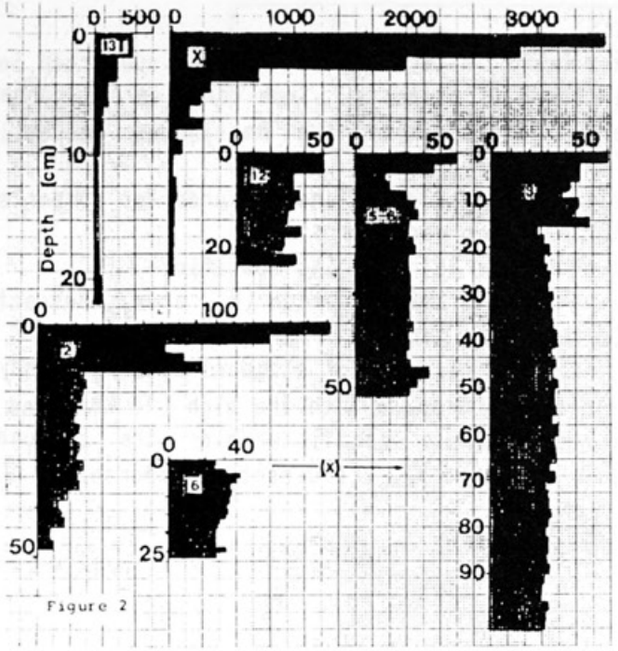


Figure 2

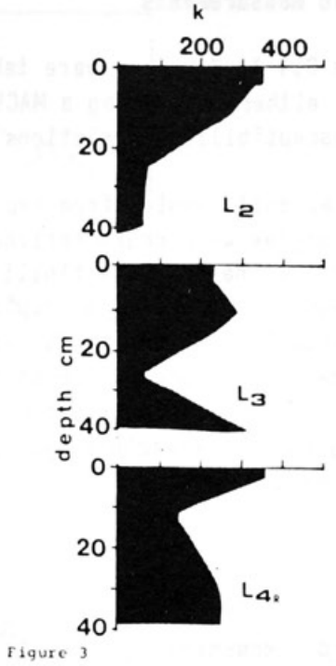


Figure 3

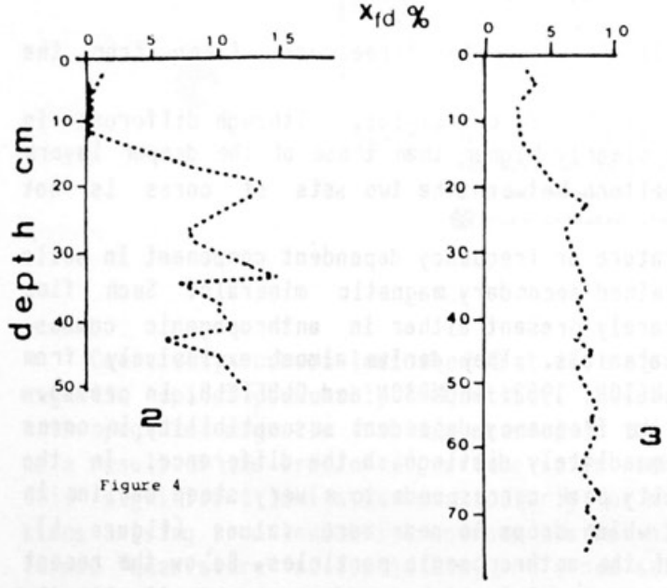


Figure 4

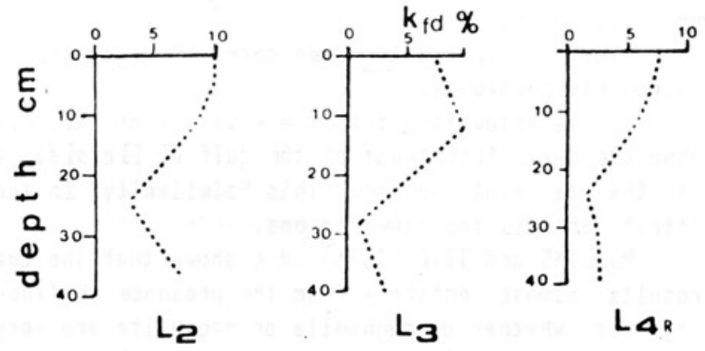


Figure 5

Figure 2 : Specific susceptibility (x) in selected cores taken from the Gulf of Elefsis (in 10^{-6}.G.Oe^{-1}.cm^{3}g^{-1}).

Figure 3 : Down core scans of volume susceptibility (k) in three cores taken from the estuary of Louros River, (in 10^{-6}.G.Oe^{-1}

"frequency dependent" susceptibility :

Figure 4 : as a percentage of the total in two cores taken from the Gulf of Elefsis.

Figure 5 : as a percentage of the total in three cores taken from the estuary of Louros River.

These show that the lower sediments in the Louros cores derive from unweathered parent material rich in antiferromagnetic (probably haematite) minerals coating coarse sand particles.

Conclusions

The use of magnetic susceptibility measurements at low and high frequencies, has made possible the simple magnetic pollution monitoring envisaged in SCOULLOS et al., (1979) in a more rapid and confident way. Frequency-dependent susceptibility measurements were used in two areas of distincly different character in order to distinguish between their "similar" down-core profiles of total magnetic suceptibility and to attribute the observed peaks to magnetically active particles of industrial origin (in the case of the gulf of Elefsis) and soil erosional origin, in the case of the surface sediments of the Louros river estuary.

Ackowledgements

This work was carried out as part of an ongoing project supported by U.N.E.P./I.O.C./MED-POL (GRE-04) and the Commission of the European Communities (ENV 560 GR-B).

References

GOLDBERG E.D., V.F. HODGE, J.J. GRIFFIN, M. KOIDE and D.N. EDGINGTON (1981). - Impact of fossil fuel combustion on the sediments of Lake Michigan Evironmental Science and Technology, 15, 466-471.

HUNT A., F. OLDFIELD and J.M. JONES (1984). - Magnetic measurements and heavy metals in atmospheric particulates of anthropogenic origin. The Science of the Total Environment, 33, 129-139.

JONES J.M. (1984). - Heavy metal magnetic mineral linkage in ombrotrophic peat Journal of Applied Ecology (in press).

MACKERETH F.J.H. (1969). - A short core sampler for sub-aqueous deposits. Limnology and Oceanography, 14, 145-151.

MULLINS C.E. and M.S. TITE (1973). - "Magnetic viscosity, quadrature susceptibility and frequency dependence of susceptibility in single-domain assemblies of magnetite and maghemite" Journal of Geophysical Rec., 78, 804-809.

OLDFIELD F., K. TOLONEN and R. THOMPSON (1981). - History of particulate atmospheric pollution from magnetic measurements in dated Finnish peat Profiles Ambio, 10, 185-188.

OLDFIELD F. and G.W. BARTINGTON (1982). - "Sediment source identification: high and low frequency susceptibility sensors" U.K. Geophysical Assembly Abstracts. Geophysical Journal of the Royal Astronomical Society, 293.

OLDFIELD F. and M. SCOULLOS (1984). - Particulate pollution monitoring in the Elefsis Gulf: The role of mineral magnetic studies Marine Pollution Bulletin. 15, 229-232.

REVITT D.M., J. BRYAN-ELLIS and F. OLDFIELD (1981). - Variations in heavy metals of stormwater pollution research in: B.C. YEN ed. "Urban Storm Drainage", Procedings of the IInd Intern. Conference on urban Storm Drainage, Pentech Press, New York, 49-58.

SCOULLOS M. and J.P. RILEY (1978) . - Water circulation in the Gulf of Elefsis, Greece, Thalassia Jugoslavia, 14, (3/4), 357-370.

SCOULLOS M. (1979). - Chemical Studies of the Gulf of Elefsis Ph. D. Thesis Department of Oceanography, The University of Liverpool, Liverpool, U.K.

SCOULLOS M., F. OLDFIELD and R. THOMPSON (1979). - Magnetic monitoring of marine particulate pollution in the Elefsis Gulf, Greece. Marine Pollution Bulletin, 10 287-291.

SCOULLOS M. (1979). - The Gulf of Elefsis from the viewpoint of the systematic research, Ecologica, 1, 9-13.

SCOULLOS M. (1981). - Zinc in sea-water and sediments, Water, Air and Soil Pollution,16, 187-207.

SCOULLOS M. (1984). - Preliminary results of trace metal and magnetic studies of Greek estuaries and enclosed gulfs, VII International Symposium "Chemistry of the Mediterranean" on Residence Time of Microconstituents in coastal waters May 1984, Primosten, Yugoslavia.

THOMPSON R. and F. OLDFIELD (in press). - Environmental Magnetism. George Allen and Unwin.

TRACE ELEMENT CONTENT IN MUSCLE LIVER AND HEART OF BOOPS BOOPS AND TRACHURUS MEDITERRANEUS FROM THE AEGEAN SEA

by

Catherine PAPADOPOULOU, John ANDREOTIS
Maria VASSILAKI-GRIMANI* and C. YANNOPOULOS**
* Radionalytical Laboratory,
Nuclear Research Center "Democritos"
Athens (Greece)
** Zoological Laboratory
Athens University
Athens 621 (Greece)

Abstract

The study of the trace element content of different species of edible pelagic fish could be of value not only for public health, but also for providing basic information on the flux of pollutants in this trophic level of the marine ecosystem. Moreover the fact that certain elements can be accumulated by individual tissues might be proved useful in the case of characterization of a pollution "indicator organism".

In this work Instrumental Neutron Activation Analysis was used for the determination of the trace elements Se, Co, Cs, Zn, Rb, Cr, Ag, Fe, Sc, and Sb in the flesh, liver and heart of B. boops and T. mediterraneus, both collected in the Aegean Sea.

Comparisons were made in trace element concentration between tissues of each separate species.

The plankton composition of the stomach content of the above fish species, mainly Anchialina and Haplostylus, was also considered in terms of its contribution to the trace element load of the fish.

*
* *

Introduction

The issue of marine pollution from trace metals is of such a complicated nature that scientific extrapolations from a few measurements in a few locations at one or two points in time are inadequate for a reasonable assessment of the situation.

Trace elements of environmental concern may have an adverse effect on marine biota or could represent a human health problem if accumulated to high concentrations in marine biota consumed by people.

Closed seas, such as the Aegean, pose particular problems because they are the habitat of a large number of marine organisms both coastal and pelagic. This characteristic necessitates comparison of data for markedly different species to appreciate the overall pollution status. The extension of trace element measurements to various tissues of a certain species may provide additional data in

characterizing a species as an "indicator organism" because of its ability to selectively concentrate one or more elements.

On the other hand, the feeding habits of fishes in particular, should not be neglected. Previous reports (ZAFIROPOULOS and GRIMANIS 1977 ; FOWLER, 1977 ; PAPADOPOULOU et al., 1978) indicate that marine plankton has a relatively high content of trace metals. This is of particular interest especially as regards zooplankton which has a long life and therefore is expected to accumulate higher levels of metals. Fish which feed on zooplankton are possibly exposed to increased levels of chemical pollution. Thus, the examination of their stomach content could also contribute to the exploration of a different approach to the methodology of trace element distribution patterns in sea-water and in their habitat.

The object of the study reported here was to determine the trace element concentration of Se, Cs, Cr, Ag, Sc, Rb, Fe, Zn, Co and Sb in plankton and in the muscle, liver and heart of the pelagic fish Boops boops and Trachurus mediterraneus, both collected in the Aegean Sea. However, a qualitative and quantitative assessment of the plankton found is the stomach of selected B. boops and T. mediterraneus specimens, was also attempted.

Materials and methods

Twelve B. boops and eleven T. mediterraneus were used in this study. The fishes were collected in the Aegean Sea, east of Evia Island, in October, 1983. Plankton was also collected in the same sampling area with a 0.5 mm mesh standard plankton net. Fish samples were stored at -4 °C for about 72 hours and then were processed at the laboratory. From each fish a portion of skeletal muscle, the heart and the liver were taken and freeze-dried prior to analysis. The size and weight of each fish was also recorded. The stomachs of nine T. mediterraneus and two B. boops were reserved for microscopic examination of their content. The plankton sample was also lyophilized prior to analysis.

Instrumental Neutron Activation Analysis (I.N.A.A.) and γ-spectroscopy were applied for the determination of Se, Cr, Ag, Cs, Sc, Rb, Fe, Zn, Co and Sb. The samples, along with aliquots of mixed standards, were sealed in quartz tubes, irradiated for 30 h. at a thermal neutron flux of 2.7 x 10^{13} $n.cm^{-2}.sec^{-1}$ and then, following a 20 day decay, were counted with a $37cm^3$ Ge(Li) detector connected to a 4,000 multi-channel pulse height analyser. All data were processed using a routine N.A.A. computer programme which was developed in our laboratory (BOCK-WERTHMANN et al., 1974).

Results and discussion

Total length of B. boops specimens varied from 18.2 to 22.2 cm, head length from 3.4 to 4.0 cm and weighed from 82.11 to 117.30 g. These parameters for T. mediterraneus were as follows : total length 21.5-29.0 cm ; head length 4.7-6.2 cm and weight 73.8-103.2 g.

Concentrations of trace elements in tissues of B. boops and T. mediterraneus are given in tables 1 and 2, respectively.

Mean Se concentrations were highest in the heart, as compared to the muscle and liver, in both fish species.

Significant differences were not observed for Cr between muscle and liver in B. boops. However, mean Cr levels in the heart were significantly higher than in the other two tissues. In T. mediterraneus both liver and heart Cr concentrations were significantly higher as compared to muscle.

Significant differences between tissues were not observed for Ag, Cs and Sc in B. boops. Because of the unequal number of observations per tissue for Ag in T. mediterraneus we did not attempt a comparison between means. The differences in Cs, Sc and Rb concentrations between tissues were not significant.

Element	TISSUE		
	Muscle	Liver	Heart
Se	6.44[a] (1.3-12)	24.92[b] (12-38)	35.08[c] (18-72)
Cr	0.461[a] (0.13-1.1)	0.231[a] (0.025-0.46)	9.33[b] (2.8-23)
Ag	0.0449[a] (0.019-0.16)	0.0367[a] (0.0054-0.11)	0.0593[a] (0.035-0.11)
Cs	0.0436[a] (0.024-0.076)	0.0338[a] (0.010-0.049)	0.0462[a] (0.019-0.086)
Sc	0.00057[a] (0.00013-0,0011)	0.00132[a] (0.00024-0.0045)	0.00527[a] (0.0010 - 0.017)
Rb	3.14[a] (1.4-4.8)	1.53[b] (0.23 - 3-3)	2.54[a] (1.5-4.2)
Fe	17.13[a] (8.8-26.0)	563.4[b] (302 - 849)	889.6[c] (127 - 1678)
Zn	15.52[a] (9.3-19)	75.73[b] (50-103)	51.08[c] (30-67)
Co	0.0399[a] (0.025-0.060)	0.890[b] (0.34-1.4)	0.6825[c] (0.41-1.2)
Sb	0.015 (0.0067-0.25)	0.057 (0.0080-0.49)	ND

[a,b,c] Means in a row with different superscript letters differ significantly ($P<0.05$) as determined by analysis of variance.

Values in parentheses indicate extreme concentrations.

ND = Not detectable

Table 1

Trace element concentration (ug/g d.w.) in muscle, liver and heart of Boops boops.

Rubidium concentration in the liver of B. boops was significantly lower than in both muscle and heart.

Tissue concentrations for Fe were significantly different in both fish species. The highest values were observed in the heart and the lowest in the muscle.

The tendency for Zn was to concentrate mainly in the liver. Mean Zn values between tissues were significantly different in B. boops ($p < 0.05$) and in I. mediterraneus ($p < 0.001$).

Mean Co values were greater ($p < 0.05$) in the liver than in the muscle or heart in B. boops. In I. mediterraneus mean Co concentration in the liver and heart did not differ appreciably ($p > 0.05$) but were significantly different as compared to the muscle value.

In both fish species Sb mean values were higher in the liver than in the muscle.

In a previous work, PAPADOPOULOU et al., (1978) reported mean Zn, Co, Se, Cs and Rb concentrations in the flesh of I. mediterraneus as follows : Zn 19, Co 0.030, Se 2.9, Cs 0.067 and Rb 0.32 ug/g d.w. for specimens collected from Skiathos island and Zn 19, Co 0.027, Se 2.1, Cs 0.067 and Rb 0.024 ug/g d.w., for specimens collected from Karystos. These values, with the exception of Rb, are comparable to those reported in the present study.

GRIMANIS et al., (1980) reported the following trace element concentrations in the flesh of B. boops : Zn 24, Co 0.092, Se 1.72, Cr 0.224, Rb 4.4 and Fe 33.2 ug/g d.w. for specimens sampled in Kissamos Gulf and Zn 22.4, Co 0.06, Se 4.12, Cr 0.14, Rb 3.12 and Fe 36.8 ug/g d.w. for specimens

Element	TISSUE		
	Muscle	Liver	Heart
Se	1.5[a] (1.1-2.0)	21.09[b] (10-29)	30.73[c] (11-66)
Cr	0.3511[a] (0.072-0.74)	1.507[b] (0.37-2.9)	2.322[b] (0.46-6.5)
Ag	0.0951[1] (0.0028-0,21)	0.2712[2] (0.037-0.98)	0.3072[3] (0.0058-1.99)
Cs	0.134[a] (0.084-0.18)	0.0968[a] (0.026-0.16)	0.199[a] (0.010-0.96)
Sc	0.00162[a] (0.00032-0.0067)	0.00307[a] (0.00045-0.0091)	0.00147[a] (0.00035-0.0041)
Rb	3.35[a] (2.5-4.4)	2.82[a] (1.9-3.7)	2.93[a] (1.1-4.6)
Fe	30.28[a] (13.8-97)	727.1[b] (354-1263)	997.9[b] (341-1730)
Zn	14.62[a*] (11.5-25.5)	118.18[b] (74-163)	53.36[c] (42-63)
Co	0.0916[a] (0.030-0.33)	1.96[b] (0.36-3.0)	1.74[b] (0.75-3.5)
Sb	0.0568 (0.0067-0.25)	0.1112 (0.0080-0.16)	ND

[a,b,c] Means in a row with different superscript letters differ significantly ($P<0.05$) as determined by analysis of variance.

* $P<0.001$

1. n=5 2.n=11 3. n=7

ND: Not detectable

Values in parentheses indicate extreme concentrations.

Table 2

Trace element concentration (ug/g d.w.) in muscle, liver and heart of Trachurus mediterraneus.

from Antikyra Gulf. For T. mediterraneus sampled in Kissamos Gulf the concentrations reported for the same trace elements were : Zn 17.2, Co 0.056, Se 1.48, Cr 0.224, Rb 2.6 and Fe 36.8 ug/g d.w. These values are also in agreement with those reported in this study.
The trace element concentration of the plankton collected in the same sampling area is given in table 3.

The stomach content of T. mediterraneus was characterized by the almost exclusive presence of mysids (table 4). Other species included Isopoda, not identified (6 per cent) and Copepoda.

Element	Concentration	Element	Concentration
Se	1.3	Rb	3.2
Cr	13.0	Fe	1500
Ag	0.083	Zn	1100
Cs	0.032	Co	1.2
Sc	0.076	Sb	0.31

Table 3

Trace element content (ug/g d.w.) of the plankton sample.

Weight of stomach content (mg d.w.)	Total number of organisms	Mysids % Anchialina	Haplostylus	Isopoda %	Other species %
T. mediterraneus					
178.9	360	70	15	5	10
89.2	192	80	10	5	5
152.1	172	80	10	-	10
190.2	272	85	5	5	5
168.3	140	90	-	5	5
302.0	520	85	10	5	-
213.0	248	80	15	5	-
241.0	180	80	15	5	-
255.8	176	80	18	2	-
B. boops					
42.4	88	35	60	5	-
78.8	112	50	45	5	-

Table 4

Identification and quantitative assessment of the plankton found in the stomach of selected I. mediterraneus and B. boops specimens.

As regards mysids, two species were identified · Anchialina agilis and Gastrosaccus spp (Haplostylus). The presence of mysids in the stomach of these pelagic fish and their unknown behaviour in the marine environment does not allow us to draw definite conclusions on the influence of anthropogenic pollution on the marine biota associated with these plankton species.

It is also worth mentioning a selective feeding habit of I. mediterraneus regarding Anchialina agilis. Its presence in the stomach content is almost eight times greater than that of Haplostylus. In B. boops, however, Haplostylus and Anchialina appear in almost equal quantities. Although these mysids account for over 90 per cent of the species found in the stomach of the fish studied, their presence in zooplankton samples is extremely rare (HATZAKIS, 1982).

The trace element concentration of B. boops and I. mediterraneus coupled with their peculiar feeding habits necessitates the extension of similar studies to involve more marine biota in many sampling areas in the Aegean Sea, at various points in time. Such a task, although complicated, will allow scientists to study the influence of trace elements in the different trophic levels and make valid assessments on chemical marine pollution.

Acknowledgements

The authors wish to thank Mrs M. Draina for technical assistance and Mrs P. Ketse for typing the manuscript.

References

BOCK-WERTHMANN W., G. PAPACOSTIDIS, A.P. GRIMANIS, J. PETROU, D. GEORGIOU and M. VASSILAKI-GRIMANI (1974). - ACTANAL. A comprehensive computer programme for routine activation analysis using Ge(Li) detectors. N.R.C. "Demokritos", Athens, Greece. REP. DEMO 74/15.

FOWLER S. (1977). - Trace elements in zooplankton particulate products. Nature (Lond.) 269, 5,623, pp. 51-53.

GRIMANIS A.P., D. ZAFIROPOULOS, C. PAPADOPOULOU and M. VASSILAKI-GRIMANI (1980). - Trace elements in the flesh of different fish species from three Gulfs of Greece. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. pp. 407-412.

HATZAKIS A. (1982). - The mysids of the Greek Seas. Ph. D. Thesis University of Athens.

PAPADOPOULOU C, D. ZAFIROPOULOS, I. HADZISTELIOS, M. VASSILAKI-GRIMANI and C. YANNOPOULOS (1978). - Trace elements in pelagic organisms and a pelagic food chain of the Aegean Sea. IVes Journées Etud. Pollutions Antalya, C.I.E.S.M. pp. 231-232.

ZAFIROPOULOS D. and A.P. GRIMANIS (1977). - Trace elements in Acartia clausi from Elefsis Bay of the upper Saronicos Gulf, Greece. Mar. Pollut. Bull., 8 (4), pp. 79-81.

DISTRIBUTION OF ARSENIC IN WATER COLUMNS WATER PARTICULATES AND SEDIMENT CORES FROM THE NORTHERN SARONIKOS GULF (GREECE)

by

A.P. GRIMANIS, D. ZAFIROPOULOS
N. KALOGEROPOULOS and Maria VASSILAKI-GRIMANI
Radioanalytical Laboratory
Nuclear Research Center "Demokritos"
Athens (Greece)

Abstract

The combined domestic and industrial wastes entering the northern Saronikos Gulf, Greece, have resulted in considerably increased concentrations of arsenic in the waters and sediments of the area.

Total, particulate and dissolved arsenic was determined by Neutron Activation Analysis in seawater from the northern Saronikos Gulf. Most of the arsenic was found in dissolved form (93.5-99.8 per cent). Concentrations of total arsenic ranged from 2.4-5.0 ug/l in surface water and a trend of increasing concentrations with depth was observed at some stations. Levels of arsenic in water particulates of surface water ranged from 0.04-0.27 ug/l with a trend of decreasing concentrations with depth.

Total arsenic was also determined in sediment core samples ranging for the silt-clay fraction (particle diameter < 55 um) of surface sediments from 11-218 ug/g dry weight. A trend of increased concentrations of arsenic in silt-clay fraction of sediments with sediment depth was also observed.

* * *

Introduction

Saronikos Gulf, Greece, receives the combined untreated domestic and industrial effluents of the greater Athens area through a main sewage outfall at Keratsini Bay. Increased concentrations of total arsenic in surface waters and sediments of northern part of the gulf have been previously reported (GRIMANIS et al., 1977 ; ZAFIROPOULOS, 1983).

Elevated concentrations of arsenic have also been found in the flesh of Pagellus erythrinus, Sargus annularis, Gobius niger and Mullus barbatus from Saronikos Gulf (PAPADOPOULOU et al., 1972 ; GRIMANIS et al., 1978 ; GRIMANIS et al., 1980).

The objective of this study was to investigate the distribution of arsenic in water column and sediment core samples from the northern Saronikos Gulf.

FIGURE 1
Sampling Stations

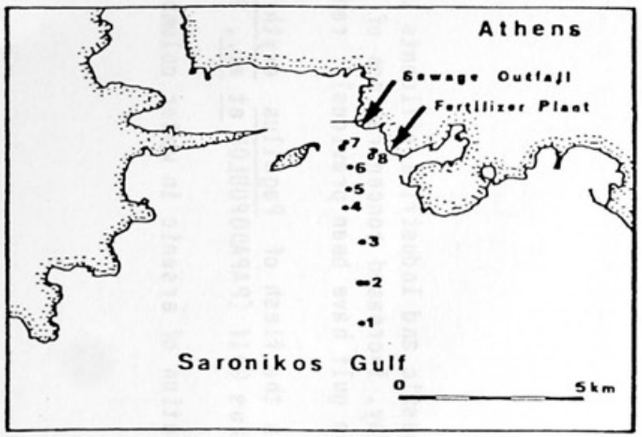


FIGURE 2
Concentrations (μg/g d.w.) of Arsenic in Sediment Core Samples from Saronikos Gulf

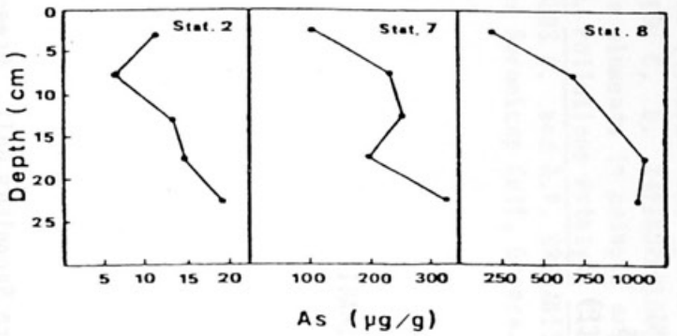


TABLE 1. Concentrations (μg/l) of arsenic in sea water

Area	Form	Concentration	Comments	Reference
Georgia Bight	Dissolved	0.62-1.65 (1.1)	Slight increase with depth at some stations	Waslenchuk, 1978
Coastal Waters of Japan	Total Particulate	1.56-2.45 0.01-0.03	No increase with depth	Ochida 1974
Southern California Bight	Total	0.3-1.45	Increase with depth at some stations. Particulate negligible in relation to dissolved	Andreae, 1978
World Oceans	Total	2-3	Typical concentrations	Robertson and Carpenter, 1976
Mediterranean Sea	Total	1.5-4.0 1.2-3.4	Afterwet digestion Direct determination	M. Stoeppler et al., 1980
Northern Saronikos Gulf	Total Particulate	2.2-5.0 0.04-0.27	Slight increase with depth at some stations Decrease with depth	Present work

Materials and methods

Sampling

Sea-water samples were collected by Go-Flo (General Oceanic) sampler from an inflatable boat using a plastic hydrowire and a PVC (polyvinyl chloride) coated messenger, on March, 1984. Sampling stations are shown in figure 1. Only surface water samples were collected from stations 6, 7 and 8. Sea-water samples were stored in polyethylene (AZLON) bottles which were precleaned to avoid contamination by shaking with 8M HNO_3 for three days, washing five times with tridistilled water and rinsing three times with clean sea-water containing a very low level of trace elements. Bottles were filled up with clean sea-water and left to equilibrate for two weeks.

After conditioning, the bottles were emptied and rinsed again with clean sea-water, and then put into precleaned polyethylene bags to protect them from dust during storage and transport.

Sediment

Sediment core samples were collected by a corer, lined with plexiglass tubes, from three stations (2, 7, 8) (figure 1).

Pretreatment and analysis

Sea-water samples were filtered using a precleaned Sartorius SM 16,510 polycarbonate filtering apparatus in a Laminar-flow hood. Filters used were Sartorius cellulose nitrate SM 11,306, 0.45 um pore size, 47 mm diameter and subjected to a precleaning procedure (MART, 1979). After the filtration of about 1 litre of each sea-water sample in each filter. filters were subsequently washed with 30 ml of tri-distilled water and dried at room temperature in the laminar flow hood. Filters were pelletized, wrapped in aluminium foil and placed in polyethylene tubes.

Five ml of each filtered sea-water sample were transferred by pipette into identical polyethylene tubes. Aliquots of 5 ml of a standard arsenic solution, containing 0.1 ug As/ml were pipetted into similar polyethylene tubes. Tubes were heat-sealed and neutron-irradiated for 30 min in the swimming-pool reactor of the Nuclear Reasearch Center Demokritos of Greece, using the rotation system (thermal neutron flux density 2.2 x 1013 n.cm-2sec-1).

For the determination of arsenic in filters and filtered sea-waters a modified radiochemical neutron activation analysis method was used (GRIMANIS, 1969). Modification consists in using toluene as a solvent instead of benzene which is equally active but not toxic like benzene.

Each sediment core sample was divided into several subsamples of 5 cm length each. Subsamples of sediments were placed in precleaned plastic vials and frozen. Prior to analysis, samples were thawed and then wet-sieved through a 55 um plastic screen net sieve. The silt clay fraction (particle diameter < 55 um) of sediments were freeze-dried and subsequently homogenized by grinding. For the determination of arsenic in the silt clay fraction of sediment core samples from the northern Saronikos Gulf an instrumental Neutron Activation Analysis Method was used (GRIMANIS et al., 1977).

Intercalibration and standards

The accuracy of the N.A.A. methods used was checked by analysing for arsenic simultaneously with samples of sea-water, filters and sediments, standard reference materials (N.B.S. orchard leaves N° 1,571, I.A.E.A. lake sediment SL-1 and I.A.E.A. SOIL-5). Values found were in good agreement with those in the literature. Variations expressed as overall relative standard deviation for arsenic was less than 10 per cent.

Results and discussion

Arsenic in sea-water

Concentrations of total, dissolved and particulate arsenic in northern Saronikos Gulf sea-water are summarized in table 2. Total concentrations range from 2.2 ug/l at station 1. farthest away from the outfall, to about 4.6 ug/l at stations 7 and 8 close to the outfall. Compared with concentrations of arsenic in sea-water reported in the literature and listed in table 1, levels of arsenic in

TABLE 2. Concentrations (μg/l) of arsenic in sea water of the Northern Saronikos Gulf

Station	Depth (m)	Total	Dissolved	Particulates
8	0.5	4.26	3.99	0.27
7	0.5	5.01	4.92	0.09
6	0.5	3.64	3.58	0.06
5	0.5	3.27	3.20	0.07
	20	1.94	1.93	0.01
	40	2.31	2.30	0.01
4	0.5	4.80	4.74	0.06
	20	3.26	3.23	0.03
	45	7.25	7.23	0.02
3	0.5	2.37	2.33	0.04
	25	2.13	2.11	0.02
	50	3.48	3.47	0.01
2	35	3.34	3.32	0.02
1	0.5	2.66	2.60	0.06
	35	1.92	1.91	0.01

TABLE 3. Concentrations (μg/g)* of arsenic in sediment core samples (the silt and clay fraction of sediment; particle diameter <55 μm)

Depth of sediment (cm)	Station 2 (Background)	Station 7 (Sewage outfall)	Station 8) (Fertilizer factory)
0-5	11.0	103	218
5-10	6.3	229	681
10-15	13.1	251	-
15-20	14.7	198	1130
20-25	18.7	323	1060

*Dry weight

Saranikos waters are increased at least by factor of 2. STOEPPLER et al., (1980) have reported total arsenic in Mediterranean waters to range between 1.5-4.0 ug/l. Arsenic concentrations in Saronikos Gulf are comparable with these values.

Most of the arsenic was found to be in the dissolved form (93.5-99.8 per cent). GOHDA (1974) and ANDREAE (1978) who have studied concentrations and physicochemical forms in oceanic waters also report that dissolved arsenic is the predominant form.

At stations 3 and 4 an increase of arsenic concentrations with depth can be observed. Such an increase with depth has been reported by WASLENCHUK (1978) in the Georgia Bight (US) and by ANDREAE (1978) in the southern California Bight (US).

WASLENCHUK (1978) considers the mixing of arsenic-rich gulf stream waters with arsenic-poor coastal waters to be the reason for virtual variations of arsenic concentrations. ANDREAE (1978) however considers the decomposition of plankton in the deeper waters as the reason for the increased concentrations of arsenic observed.

The number of samples analysed from the northern Saronikos Gulf does not allow us at this stage to reach any concrete conclusions but the decomposition of organic material in the sediments and the release of dissolved arsenic in the water might be a mechanism that is responsible for the increased concentrations of arsenic observed in water samples close to the bottom. This mechanism however needs to be further investigated.

Arsenic is released from the sediments to the overlying water possibly as a result of the decomposition of organic material and/or by geochemical processes.

Arsenic in water particulates

Concentrations of arsenic in water particulates from 8 stations of the northern Saronikos Gulf are also listed in table 2. Levels of arsenic in water particulates of surface waters ranged from 0.04-0.27 ug/l with a trend of decreasing concentrations with depth.

A possible explanation of this phenomenon might be the following : the particulates retained on the filter (0.45 um) may contain a high percentage of phytoplankton cells, detritus and other organic and inorganic particles. In the eutrophic area of the northern Saronikos Gulf the concentration of the particulates is higher in the upper layer (1-4 m) of the sea-water column (IGNATIADES, 1979). Therefore, higher levels of arsenic found in the particulates of the surface sea-waters, compared with those in deep waters, might be connected with the higher concentration of the particulate matter present in this upper layer.

Arsenic in sediment cores

Concentrations of total arsenic in the silt-clay fraction of sediment cores from three stations of Saronikos gulf are summarized in table 3. The highest concentrations were observed at station 8 close to a fertilizer factory outside Piraeus Harbor. At station 7 concentrations are increased by a factor of about 10 as compared with concentrations at station 2 situated about 4.7 kilometres from the main sewage outfall.

Arsenic concentrations in sediments of the Adriatic have been reported to range from 10-28 ug/g (STEGNAR et al., 1980). ROBERTSON and CARPENTER (1976) report as typical oceanic concentrations 2-20 ug/g. Compared with these values arsenic concentrations in Saronikos sediments are considerably increased.

Increase in arsenic concentrations with depth in the sediment cores can be observed from table 3 as well as from figure 2.

At station 8 close to the fertilizer factory, concentrations increase from 218 ug/g at the surface to 1,060 ug/g at 20-25 cm. This might be the result of the fact that sludge from the factory operations, mainly pyrites, is no longer discharged into the area.

The increase of arsenic levels with depth at station 7 close to the outfall is less pronounced. A tentative explanation is that inputs of arsenic through the main sewage outfall have decreased in recent years.

At station 2 concentrations of arsenic increase from 11.0 ug/g at the surface to 18.7 ug/g at 20-25 cm. The release of arsenic from the sediments to the overlying water due to decomposition of organic material is a tentative explanation for the trend observed.

A trend of increasing arsenic concentrations with sediment depth has been reported in the Hayle estuary. The author relates this trend with high mining activities in the past (YIM, 1979). A similar trend was also reported by STEGNAR et al., (1980).

Conclusions

The input of arsenic through the main sewage outfall in Saronikos Gulf resulted in increased concentrations of this element in both sea-water ans sediments of the northern part of the Gulf. Although the sewage outfall is an important source of suspended particulates in Saronikos Gulf, most of the arsenic found in sea-water was in dissolved form. Levels of arsenic found in water particulates of surface waters were less than 2 per cent of the total with a trend of decreasing concentrations with depth. A trend of increasing concentrations of arsenic in sea-water with depth at some stations was observed. The same trend was also observed in sediments with surface sediments showing the lowest values.

Although from these preliminary data only tentative conclusions can be drawn, it is postulated that arsenic is released from the surface sediments due to decomposition of organic materials.

Acknowledgements

The partial financial support of this work from the European Economic Community (Research Contract N° ENV-696-G-(B) is gratefully acknowledged.

We also wish to thank Mrs. E. HATZELLI-HANDRINOU for her valuable technical assistance.

References

ANDREAE M.O. (1978). - Distribution and speciation of arsenic in natural waters and some marine algae. Deep Sea Research, 25, 391-402.

GOHDA S. (1974). - The content and oxidation state of arsenic and antimony in coastal water of Japan. Journal of the Oceanographical Society of Japan, 30, 163-168.

GRIMANIS A.P. (1969). - Simultaneous determination of arsenic and copper in wines and biological materials by Neutron Activation Analysis. Nat. Bur. Stand. (U.S.A.) Spec. Publ. 312, 1, 197-202.

GRIMANIS A.P., M. VASSILAKI-GRIMANI and G.B. GRIGGS (1977). - Pollution studies of trace elements in sediments from the Upper Saronikos Gulf, Greece. J. Radioanal. Chem., 37, 761-773.

GRIMANIS A.P., D. ZAFIROPOULOS and M. VASSILAKI-GRIMANI (1978). - Trace elements in the flesh and liver of two fish species from polluted and unpolluted areas of the Aegean Sea. Environmental Science and Technology, 12, (6), 723-726.

GRIMANIS A.P., D. ZAFIROPOULOS, C. PAPADOPOULOU and M. VASSILAKI-GRIMANI (1980). - Trace elements in the flesh of different fish species from three gulfs of Greece. Ves Journées Etud. Pollution, Cagliari, C.I.E.S.M., 407-412.

IGNATIADES L. (1979). - The influence of water stability on the vertical structure of a phytoplankton community Marine Biology, 52, 97-104.

MART L. (1979). - Prevention of contamination and other accuracy risks in voltametric trace metal analysis of natural waters. Fresenius Z. Anal. Chem., 296, 350-357.

PAPADOPOULOU C., A.P. GRIMANIS and I. HADJISTELIOS (1972). - Mercury and arsenic in a fish collected in polluted and non-polluted sea-waters. Journées Etud. Pollutions, Athènes, C.I.E.S.M., 141-144.

ROBERTSON D.E., R. CARPENTER (1976). - Activation Analysis in E.D. Goldberg (Ed.), Strategies for Marine Pollution Monitoring, Wiley Interscience, New York.

STEGNAR P., I. VUKADIN, B. SMODIS, A. VAKSELJ and A. PROSENC (1980). - Trace elements in sediments and organisms from Kastela Bay. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M., 595-600.

STOEPPLER M., C. MOHL and H.W. NURNBERG (1980). - Total arsenic in sea-water and marine organisms : a comparative study from the Mediterranean Sea and selected regions of the Oceans. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M., 281-284.

WASLENCHUK D.E. (1978). - The budget and geochemistry of arsenic in a continental shelf environment. Marine Chemistry, 7, 39-52.

YIM W.W.-S. (1976). - Heavy metal accumulation in estuarine sediments in a historical mining of Cornwall. Marine Pollution Bulletin, 7, 147-150.

ZAFIROPOULOS D. (1983). - Application of the analytical method of neutron activation in the study of trace elements in Saronikos Gulf. Ph. D. Thesis, University of Patras.

STUDIES OF NINE TRACE ELEMENTS IN FLESH AND LIVER OF THE FISH GOBIUS NIGER FROM VARNA BAY, BULGARIA AND SARONIKOS AND PETALION GULFS GREECE (FIRST PART)

by

D. APOSTOLOV, M. IOVCHEV, Lydia KINOVA
I. PENEV, E. TASKAEV (1)
and
A.P. GRIMANIS, G. KANIAS, Catherine PAPADOPOULOU
Maria VASSILAKI-GRIMANI, D. ZAFIROPOULOS (2)
(1) Activation Analysis Group,
Institute of Nuclear Research and Nuclear Energy,
Sofia, Bulgaria
(2) Radioanalytical Laboratory,
Nuclear Research Center "Demokritos",
Athens, Greece

Abstract

Within the framework of the scientific collaboration between the Institute of Nuclear Research and Nuclear Energy of Sofia, Bulgaria and the Nuclear Research Center "Demokritos" of Athens, Greece, nine trace elements (As, Co, Cr, Cu, Hg, Rb, Se, V and Zn) were determined by neutron activation analysis in the flesh and liver of the edible fish Gobius niger caught from Varna Bay, Bulgaria and Saronikos and Petalion Gulfs, Greece.

The purpose of this study is to find out if marine organisms and especially common edible fish species from Varna and Saronikos and Petalion Gulfs are suitable or unsuitable for human consumption due to toxic trace elements pollution from anthropogenic activities. No dangerous -for the human health-concentrations of the nine trace elements under investigation were found in all samples of the fish Gobius niger.

*
* *

Introduction

In the framework of the scientific collaboration between the Activation Analysis Group of the Institute of the Nuclear Research and Nuclear Energy of Sofia, Bulgaria and the Radioanalytical Laboratory of the Nuclear Research Center "Demokritos", Athens, Greece in the project entitled: "Methodology and Applications of Neutron Activation Analysis on Ecology" toxic and other trace elements were determined in marine organisms and sediments from Varna Bay of Bulgaria and Saronikos and Petalion Gulfs of Greece.

The purpose of this joint project was to investigate whether marine organisms and especially common edible fish species from these areas are suitable or not for human consumption due to toxic trace element pollution from anthropogenic activities.

Figure 1
Sampling Stations from Varna Bay, Bulgaria

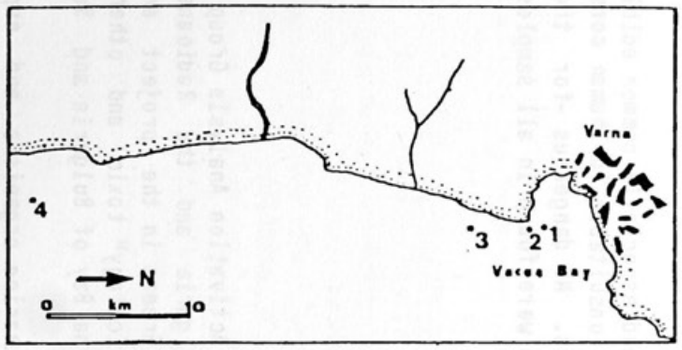


Figure 2
Sampling Stations from Saronikos and Petalion Gulfs, Greece

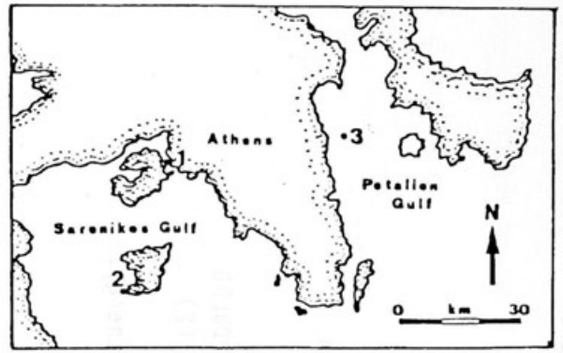


Table 1
Average concentration (µg/kg wet weight) of As, Co, Cr in the flesh and liver of Gobius niger from Varna Bay, Bulgaria and Saronikos and Petalion Gulfs, Greece

Station	Specimen per composite sample	Mean Length (cm)	Arsenic		Cobalt		Chromium	
			flesh	liver	flesh	liver	flesh	liver
Varna Bay								
1	20	10	913 (±54)	1610 (±120)	19.5 (±1.7)	71.4 (±7.1)	115 (±17)	220 (±46)
2	20	10.5	1090 (±63)	1140 (±90)	32.2 (±3.1)	48.2 (±4.8)	71.7 (±15)	108 (±24)
3	19	10	761 (±50)	2050 (±140)	21.5 (±2.1)	54.2 (±5.4)	113 (±24)	78 (±14)
4	18	9.5	760 (±47)	-	36.7 (±3.7)	-	111 (±22)	-
Saronikos Gulf								
1	10	10	3710 (±250)	3860 (±240)	4.6 (±0.65)	30.1 (±3.0)	62.2(±14)	120 (±23)
2	12	9.5	6440 (±310)	7230 (±470)	6.67(±0.82)	24 (±2.4)	66.7(±15)	78 (±15)
Petalion Gulf								
3	20	~~10~~.5	5000 (±250)	4430 (±280)	4.54(±0.7)	35 (±3.5)	77.3(±16)	150 (±30)

Varna Bay receives domestic and possibly industrial wastes from Varna, a city with a population of 600.000 inhabitants and industrial activities including metal processing, electrical appliances, textile, food and beverage industries.

Saronikos Gulf receives domestic sewage and industrial effluents from the greater Athens, an area with most of Greece's industrial activity and a population of over 3.000.000 inhabitants.

Coastal waters of Petalion Gulf receive domestic effluents from coastal cities especially in summer time.

In this part of the work nine trace elements (As, Co, Cr, Cu, Hg, Rb, Se, V and Zn) were determined in the flesh and liver of Gobius niger. This benthic fish is very common not only in the Mediterranean Sea but also in the Black Sea. It can be found in areas receiving high loads of industrial effluents and domestic sewage where other benthic fish are difficult or impossible to be found. BUSSANI and PRINCI (1978) suggest Gobius niger as an indicator of mercury contamination of the marine environment, also in view of its wide geographical distribution.

Materials and methods

Sampling

Specimens of Gobius niger were collected during summer 1980 by bottom trawlers from 4 stations of Varna bay (Fig. 1) and 3 stations of Saronikos and Petalion Gulfs (Fig. 2).

Pretreatment

Samples identification and preparation were carried out according to the method recommended by the FAO Fisheries Technical Paper N°. 158 (BERNHARD 1976).

Analytical procedure

For the determination of all nine trace elements Neutron Activation Analysis (NAA) has been applied. For the simultaneous determination of arsenic and copper, as well as for the determination of mercury, radiochemical NAA methods have been used (GRIMANIS 1969; GRIMANIS, KANIAS 1982). For the rest of the elements under investigation Instrumental NAA has been applied.

Intercalibration and standards

The accuracy of the NAA methods used were checked by analysing for all elements, samples of Gobius niger simultaneously with standard reference materials (NBS orchard leaves N°. 1571 and Monaco Intercalibration sample MA-M-1). Values found were in good agreement with those in the literature. Variations expressed as overall relative standard deviation for As, Co, Cr, Cu, Hg, Rb, Se, V and Zn were less than 12 % .

Results and discussion

Average concentrations of As, Co, Cr, Cu, Hg, Rb, Se, V and Zn in the flesh and liver of Gobius niger from sampling stations of Varna Bay and Saronikos and Petalion Gulfs determined by NAA are summarized in tables 1, 2 and 3. Results of at least duplicate analyses are given in ug/kg wet weight. The $\pm$ limits shown in the same tables denote standard deviations of the NAA methods used. Specimens per composite samples as well as mean length of specimens are also included in the tables.

In general, no indication of any pollution from toxic trace elements was found in Gobius niger collected from Varna Bay and Saronikos and Petalion Gulfs. However significant differences of some trace elements were observed between the flesh and liver of Gobius niger specimens, as well as among Gobius niger specimens collected from Varna Bay and Saronikos and Pelation Gulfs.

Table 2
Average concentration (µg/kg wet weight) of Cu, Hg and Rb in the flesh and liver of Gobius niger from Varna Bay, Bulgaria and Saronikos and Petalion Gulfs, Greece

Station	Specimen per composite sample	Mean Length (cm)	Copper		Mercury		Rubidium	
			flesh	liver	flesh	liver	flesh	liver
Varna Bay								
1	20	10	326 (±29)	10700 (±1070)	56 (±6.7)	161 (±16)	863 (±78)	464 (±42)
2	20	10.5	956 (±86)	7780 (±780)	71.7(±8.6)	185 (±8.6)	806 (±73)	295 (±26)
3	19	10	1040 (±94)	12500 (±1250)	82.7(±10)	114 (±11)	802 (±72)	326 (±29)
4	18	9.5	587 (±53)	-	-	-	741 (±67)	-
Saronikos Gulf								
1	10	10.0	228 (±23)	6950 (±690)	41.5(±5.0)	102 (±12)	817 (±73)	430 (±38)
2	12	9.5	180 (±18)	6680 (±670)	37.7(±4.5)	78.3(±9.4)	747 (±67)	434 (±39)
Petalion Gulf								
3	20	10.5	202 (±20)	8110 (±810)	22.7(±2.7)	59.6(±7.2)	768 (±69)	458 (±41)

Table 3
Average concentration (µg/kg wet weight) of Se, V and Zn in the flesh and liver of Gobius niger from Varna Bay, Bulgaria and Saronikos and Petalion Gulfs, Greece

Station	Specimen per composite sample	Mean Length (cm)	Selenium		Vanadium		Zinc	
			flesh	liver	flesh	liver	flesh	liver
Varna Bay								
1	20	10	922 (±78)	1370 (±96)	54.3 (±6.5)	59.5 (±7.1)	10000 (±600)	59350 (±3560)
2	20	10.5	686 (±40)	1210 (±85)	47.8 (±5.7)	47.9 (±5.8)	10600 (±640)	47900 (±2870)
3	19	10	464 (±33)	1180 (±83)	45.6 (±5.5)	39.1 (±4.7)	9370 (±560)	36100 (±2160)
4	18	9.5	526 (±37)	-	45.6 (±5.5)	-	7740 (±460)	-
Saronikos Gulf								
1	10	10.0	100 (±6.9)	960 (±72)	11.9 (±1.4)	54.2 (±6.5)	4840 (±280)	12600 (±880)
2	12	9.5	275 (±19)	740 (±52)	10.4 (±1.3)	50.6 (±6.1)	3820 (±229)	10900 (±760)
Petalion Gulf								
3	20	10.5	227 (±16)	1000 (±70)	14.5 (±1.7)	59.0 (±7.1)	4140 (±248)	11900 (±830)

Arsenic: Higher values of arsenic were found in the flesh and liver of Gobius niger collected from stations of Saronikos and Petalion Gulfs than those found from stations of Varna Bay. However these values are considered normal and are much lower than those found for other benthic fish species collected from Saronikos and other Gulfs of Greece (GRIMANIS et al., 1978, 1980).

Cobalt: Increased concentrations of cobalt were found in the flesh and liver of Gobius niger from Varna Bay compared with those from Saronikos and Petalion Gulfs. The highest value of cobalt found in the flesh of Gobius niger from station 3 of Varna Bay is similar or lower than cobalt values found in several fish species from East Mediterranean (GRIMANIS et al., 1978, UYSAL, 1980; VOUTSINOU-TALIADOURI, 1982). Concentrations of cobalt in livers of Gobius niger from Varna Bay are much lower than those found in livers of Sargus annularis from Petalion Gulf (GRIMANIS et al., 1978).

Chromium: Similar concentrations of chromium were found in the flesh and liver of Gobius niger collected from all stations which were within the natural background limits reported by several investigators for edible benthic fish species.

Copper: Gobius niger specimens collected from Varna Bay have higher values of copper in their flesh than those from Saronikos Gulf. Copper values found in Varna were lower than those found in several fish species collected from Turkish coasts (UYSAL, 1980) and from Gera Gulf of the island of Lesvos, Greece (GRIMANIS et al., 1980). Comparable concentrations of copper were found in the liver of samples of Gobius niger from all stations, which are considered "normal".

Mercury: Slightly increased concentrations of mercury in the flesh and liver of Gobius niger of Varna Bay were found, compared with those of Saronikos and Petalion Gulfs. All mercury values found are much lower than the maximum permissible level for mercury in marine organisms which is given by the WHO as 500 ug/kg wet weight.

Rubidium: Similar concentrations of rubidium were found in Gobius niger collected from both sea areas.

Selenium: Levels of selenium found in the flesh of Gobius niger from Varna Bay were higher than those found in the flesh of Gobius niger from Saronikos Gulf. However these increased concentrations of selenium from Varna were lower than the ones found in mesopelagic and coastal fish species from Adriatic (STEGNAR et al., 1978). Comparable concentrations of selenium were found in the liver of Gobius niger specimens from all stations.

Vanadium: Concentrations of vanadium in the flesh of Gobius niger from Varna Bay were higher than those found from stations of Saronikos and Petalion Gulfs. These values of vanadium are probably associated with ship activity and oil pollution. These increased values are comparable with those reported for fish species from Aegean Sea (PAPADOPOULOU et al., 1978). Similar concentrations of vanadium were found in the liver of Gobius niger specimens from all stations.

Zinc: Concentrations of zinc in both the flesh and liver were higher in specimens collected from Varna Bay compared to specimens collected from Saronikos and Petalion Gulfs. However these concentrations are similar to concentrations reported for benthic fish species from other areas of the Mediterranean (MAJORI et al., 1978; GRIMANIS et al., 1978; VOUTSINOU-TALIADOURI, 1982).

Conclusions

In general no indication of any pollution from toxic trace elements was found in Gobius niger collected from Varna Bay and Saronikos and Petalion Gulfs. Arsenic, cobalt, chromium, copper, mercury, selenium and zinc concentrations were higher in the liver than in the flesh of Gobius niger sampled from Saronikos and Petalion Gulfs and Varna Bay. Vanadium concentrations do not show any clear trend whereas rubidium concentrations were higher in the flesh.

Cobalt, copper, selenium, vanadium and zinc concentrations were higher in the flesh and liver of specimens collected from Varna Bay as compared to specimens collected from Saronikos and Petalion Gulfs.

Arsenic concentrations were higher in Gobius niger specimens collected from Saronikos and Petalion Gulfs. Rubidium and mercury concentrations were comparable in fish from all areas.

These variations do not indicate pollution since all concentrations of toxic trace elements under investigation were comparable to concentrations reported for the same or other fish species from the Mediterranean.

All mercury concentrations found in all Gobius niger specimens were lower than the WHO maximum permissible level of 500 ug/kg wet weight.

References

BERNHARD M. (1976). - FAO Fish. Techn. Pap. N°. 518. Manual of methods in aquatic environment research. Part 3. Sampling and analysis of biological material.

BUSSANI M., M. PRINCI (1978). - Gobius niger as indicator of Hgtot mean concentration in the marine environment. IVes Journées Etud. Poll., Antalya, C.I.E.S.M. 671-672.

GRIMANIS A.P. (1969). - Simultaneous Determination of Arsenic and Copper in Wines and Biological Materials by Neutron Activation Analysis. Nat. Bur. Stand. (U.S.A.) Spec. Publ. 312, 1, 197-202.

GRIMANIS A.P., D. ZAFIROPOULOS, and M. VASSILAKI-GRIMANI (1978). - Trace Elements in the flesh and liver of two fish species from polluted and unpolluted areas of the Aegean Sea. Environmental Science and Technology, 12 Number 6, 723-726.

GRIMANIS A.P., D. ZAFIROPOULOS, C. PAPADOPOULOU and M. VASSILAKI-GRIMANI (1980). - Trace elements in the flesh of different fish species from three Gulfs of Greece. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M., 407-412.

MAJORI L., G. NEDOCLAN, G.B. MODONUTTI, F. DARIS (1978). - Levels of metal pollutants in sediments and biota of the Gulf of Trieste: a long-term survey. IVes Journées Etud. Poll. Antalya, C.I.E.S.M., 237-243.

PAPADOPOULOU C., D. ZAFIROPOULOS, I. HADJISTELIOS, M. VASSILAKI-GRIMANI and C. YANNOPOULOS (1978). - Trace elements in pelagic organisms and a pelagic foodchain of the Aegean Sea. IVes Journées Etud. Pollutions, Antalya, C.I.E.S.M., 231-232.

STEGNAR P., I. VUKADIN, B. SMODIS, A. VAKSELJ and A. PROSENC (1980). - Trace elements in sediments and organisms from Kastela Bay. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M., 595-600.

UYSAL M. (1980). - Levels of trace elements in some food chain organisms from the Aegean Coasts. Ves Journées Etud. Poll. Cagliari, C.I.E.S.M., 503-512.

VOUTSINOU-TALIADOURI F. (1982). - Monitoring of some metals in some marine organisms from the Saronikos Gulf, VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M., 329-333.

GRIMANIS A.P. and G.D. KANIAS (1982). - Rapid determination of mercury in biological materials by radiochemical neutron activation analysis. J. Radioanal. Chem. 72, 587-595.

OCCURRENCE OF SOME HEAVY METALS IN SURFICIAL SEDIMENTS FROM THE DAMIETTA ESTUARY OF THE NILE

by

MASSOUD A.H. SAAD* and M.A. FAHMY**
* Oceanography Department, Faculty of Science
Alexandria University (Egypt)
** Institute of Oceanography and Fisheries
Alexandria (Egypt)

Abstract

The Damietta estuary of the Nile extending between Faraskour's Dam and its sea-mouth, has a maximum depth of 12.5 m. Surface sediments were collected at five transects along this estuary. Three stations were selected at each transect, representing the western, middle and eastern regions of the estuary. The samples were analysed for metals (Cu, Zn, Cd, Mn, and Fe) and grain size. The average percentages for mud, sand and gravel varied from 23.05-99.1, 0.9-56.4 and 0.0-25.6, respectively. The mud fraction generally comprised most sediment content. The average concentrations of metals ranged from 37.2-158.1, 46.0-300.7, 0.65-1.65, 78-657 and 310-612 ppm for Cu, Zn, Cd, Mn, and Fe, respectively. The maximum averages for Cu, Zn and Cd obtained at transect II coincided with the discharge of sewage wastes. The highest averages for Mn and Fe recorded at and near the mouth of the estuary were associated with the highest average percentages of mud. The data show that the eastern side of the estuary was exposed to more pollution than the western side.

*
* *

Introduction

Faraskour's Dam, constructed on the Damietta branch of the Nile, separates two different water bodies, the fresh and the mostly marine water environments. Small amounts of the Nile water are transported via a narrow canal at the western side of the dam to the marine region, which represents the Damietta estuary. It seems that external factors affect the environment of this estuary, which has a maximum depth of 12.5 m. The present study, representing a part of a research programme, deals with the occurrence and distribution of some heavy metals in the surface sediments of the Damietta estuary in an attempt to show the effect of metal pollution on this estuary.

Material and methods

Surface sediment samples were collected at five transects along the Damietta estuary. Three stations were chosen at each transect, representing the western, middle and eastern regions of the estuary (fig. 1). A part of the sample was analysed for texture or grain size, according to the wet

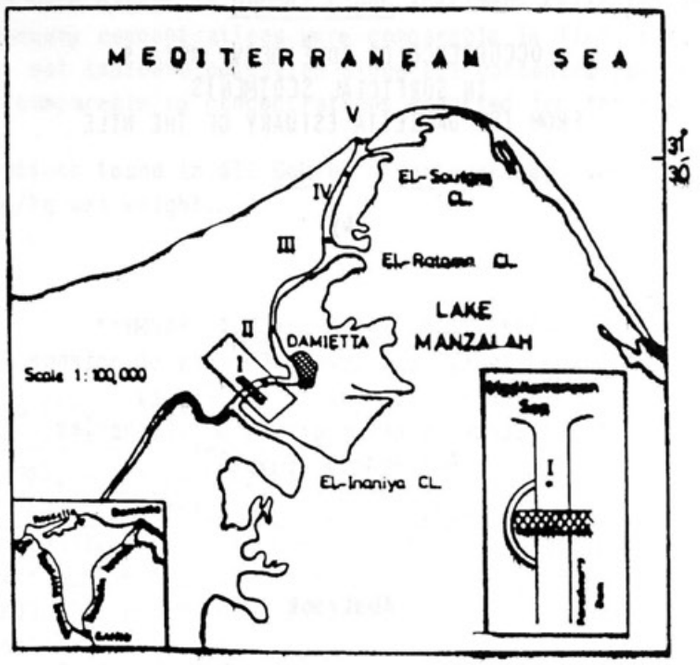


Fig. 1. - Map of Damietta Estuary showing position of transects.

sieving technique and the other part was used for extraction of the metals. The samples were digested with 2N nitric acid (ABDULLAH and ROYLE, 1974). Measurements of the elements (Cu, Zn, Cd, Mn, and Fe) were carried out in duplicate using a Shimadzu AAS. Estimation of the accuracy of the analyses was difficult, due to the absence of calibration standards. However, the precision of the method is: ± 2 per cent for Fe and Cd and ± 4 per cent for Cu, Zn and Mn.

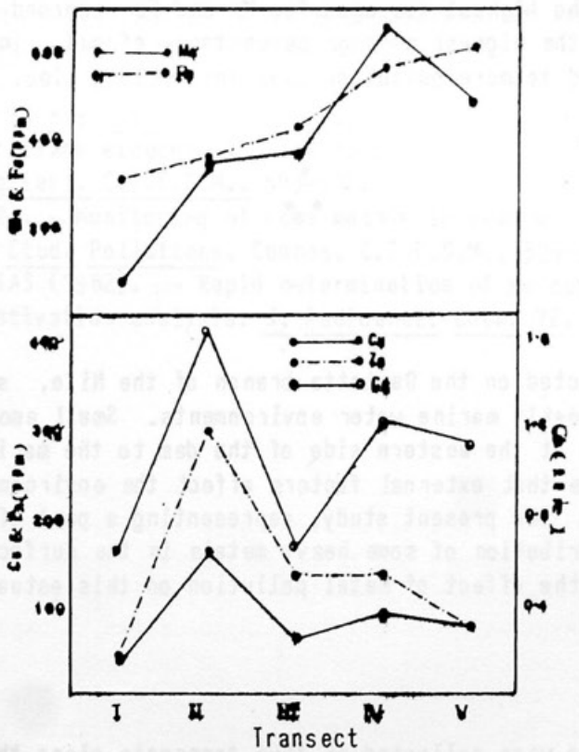


Fig. 2. - Variations of the average values (averages of the three stations at each transect) of heavy metals in the sediments of Damietta Estuary.

Results and discussion

The results of the sediment analyses are listed in table 1. The average percentages of mud, sand and gravel ranged from 23.05-99.1, 0.9-56.4 and 0.0-25.6, respectively. The mud fraction (particle < 61 μm) of the sediments generally comprised most sediment content. However, the coarse particles of the sediments, sand (particle diameter 61 μm-2 mm) and gravel (particle diameter > 2 mm) dominated in the sediments of the western side of transects I, II and III, as well as in the sediments of the middle region of transect II. The increase in mud content might have resulted from the Nile water entering the Damietta estuary via the western canal (fig.1), the discharge of untreated sewage wastes and the mud derived from the estuarine banks by current erosion. There was a general increase in the mud content towards the sea. This coincided with transportation of mud along the estuary by the water flow.

As shown in fig. 2, the average values of Cu, Zn and Cd showed irregular local variations along the Damietta estuary. However, the averages for Fe and Mn increased in the direction of the sea. The Cd content was the lowest compared with the other metals. The minimum average concentrations of 37.2, 46.0, 0.65, 78.0 and 310.0 ppm for Cu, Zn, Cd, Mn and Fe, respectively were found at transect I. The maximum average values of 158.1, 300.7 and 1.65 ppm for Cu, Zn and Cd, respectively, obtained at transect II, coincided with the discharge of sewage wastes from the city of Damietta. This evidence is supported by the maximum value of dissolved organic matter (SAAD and ABDEL-MOATI, 1984), the low average values of dissolved oxygen and pH, as well as the maximum average value of H_2S in the sea-water at transect II (ABDEL-MOATI, 1981).

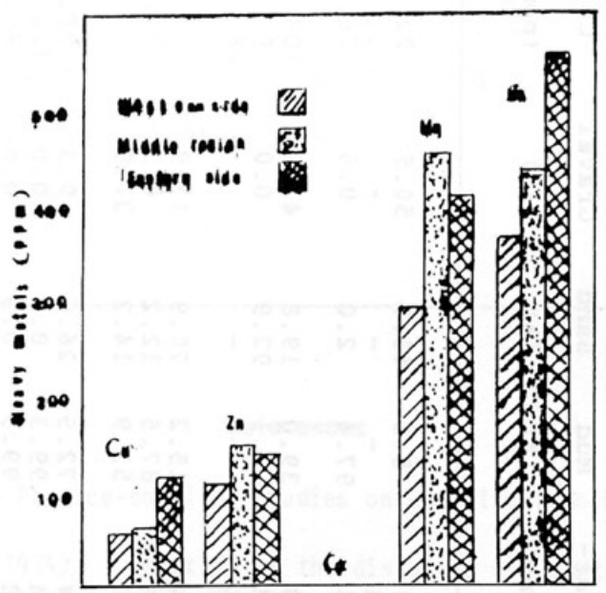


Fig. 3. - Histograms for the average values of heavy metals in the sediments calculated along the Western, Middle and Eastern regions of Damietta Estuary.

The highest average concentrations of Mn and Fe obtained at transects IV and V were associated with the highest average percentages of mud and the lowest average percentages of sand and gravel. This implies that Fe and Mn correlate well with the mud content of the sediments (table 2). Fe and Mn give a positive correlation with each other (r = 0.61), indicating an incorporation of more or less similar proportions of the two elements within the sediments. A similar observation was found in the sediments of Abu Kir Bay (SAAD et al., 1980) and the El-Mex region (SAAD et al., 1981). The correlation coefficient of Cu with Zn (r = 0.86) is noticeably higher than that for Cu with Cd (r = 0.58). High correlation between Cu and Zn was also reported by EL-SAYED et al. (1980), SAAD et al., (1980, 1981) in other Egyptian marine sediments affected by pollution. Both metals are thought to be biologically important (SAAD et al., 1980).

TABLE 1

Percentages of mud, sand and gravel, as well as values of heavy metals in the sediments of Damietta estuary

Transects	Depth (m)	Locat-ions	Mud (%)	Sand (%)	Gravel (%)	Cu (ppm)	Zn (ppm)	Cd (ppm)	Mn (ppm)	Fe (ppm)
		W	5.8	43.7	50.5	22.8	20.3	0.16	77	134
I	5.45	M	-	-	-	12.6	32.6	0.40	81	205
		E	97.4	2.0	0.6	76.2	85.2	1.38	77	592
		W	39.0	19.8	41.2	108.6	263.3	2.06	360	353
II	12.5	M	7.1	92.9	0.0	85.3	214.2	0.93	251	233
		E	-	-	-	280.4	424.5	1.96	419	484
		W	15.2	70.9	13.9	29.1	55.2	0.96	225	203
III	5.85	M	87.5	12.2	0.3	91.4	283.1	0.32	438	645
		E	53.9	14.2	31.9	70.3	66.4	0.74	443	437
		W	72.5	26.6	0.9	90.5	149.8	1.99	458	517
IV	4.80	M	99.3	0.7	0.0	102.2	164.7	0.93	966	587
		E	99.5	0.5	o.o	85.8	85.2	0.77	547	600
		W	-	-	-	77.0	92.1	1.80	353	626
V	4.80	M	99.1	0.9	0.0	61.2	76.2	0.55	540	531
		E	-	-	-	91.0	72.1	1.00	566	680

W = west, M = middle, E = east.

As shown in fig. 3, the average values of Cu, Zn, Mn and Fe calculated along the western side of the Damietta estuary were markedly lower than the corresponding averages obtained from the eastern side. However, the average concentration of Cd showed the opposite trend. This indicates that the eastern side of the estuary was exposed to more pollution than the western side. The high average values of metals in the sediments of the main channel reflect the influence of pollution, mainly from the eastern side.

TABLE 2

Correlation coefficients between metals and mud content in the sediments of Damietta estuary

	Mud (%)	Cu	Zn	Cd	Mn	Fe
Fe	0.97	0.36	0.19	0.25	0.61	1.0
Mn	o.58	0.33	0.23	0.09	1.0	
Cd	0.04	0.58	0.48	1.0		
Zn	0.08	0.86	1.0			
Cu	0.49	1.0				
Mud (%)	1.0					

References

ABDEL-MOATI M.A., (1981). - Physico-chemical studies on Damietta branch of the Nile. M. Sc. Thesis, Alex. Univ.

ABDULLAH M.I. and ROYLE, (1974). - A study of the dissolved and particulate trace elements in the Bristol Channel. Mar. Biol. Ass. U.K., 54; 581.

EL-SAYED M.KH., M.A. EL-SAYED and A. MOUSSA, (1980). - Anthropogenic material in sediments from the eastern harbour of Alexandria, Egypt. Ves journées Etud. Pollutions Cagliari, C.I.E.S.M., 215-222.

SAAD M.A.H., O.A. EL-RAYIS and F.E. EL-NADY (1980). - Occurrence of some trace metals in bottom deposits from Abu Kir Bay, Egypt. Ves Journées Etud. Pollutions Cagliari, C.I.E.S.M., 555-560.

SAAD M.A.H., O.A. EL-RAYIS and F.E. EL-NADY (1981). - Occurrence of organic matter and heavy metals in sediments from the Mediterranean. Proc. Internat. Symp. Manag. Indust. Wastewater in Developing Nations, Alex. Egypt. 127-139.

SAAD M.A.H. and M.A. ABDEL-MOATI, (1984). - Dissolved organic matter status in Diametta estuary of the Nile. Archs. Inst. Pasteur Tunis, 61(4) : 453-462.

ETUDE DES TENEURS GLOBALES EN METAUX LOURDS CHEZ LA MOULE PERNA PERNA (L) DANS LA REGION D'ALGER. VARIATIONS DE CES TENEURS EN FONCTION DE QUELQUES PARAMETRES BIOLOGIQUES.

par

André ASSO
Institut des Sciences de la Mer
et de l'Aménagement du Littoral
BP 90, Alger 1er novembre (Algérie)

Résumé

La distribution de six métaux lourds, le Zinc, le Mercure, le Cadmium, le Plomb, le Cuivre et le Manganèse a été étudiée chez la moule Perna perna (L) au cours de prélèvements saisonniers dans la période novembre 1978-octobre 1980, pour cinq stations de la région d'Alger. Les variations des concentrations en fonction de la taille, des saisons et du sexe ont été envisagées. Pour la majorité des éléments métalliques, l'accumulation est supérieure chez les individus de petites tailles, elle est maximale au printemps et minimale en automne.

Les individus mâles accumulent plus de métaux excepté le Cadmium également réparti entre les deux sexes.

*
* *

Introduction

Cette étude complète les données sur la pollution par les métaux lourds en région d'Alger (ASSO, 1980), zone particulièrement soumise à un processus de développement industriel récent, ainsi qu'à une forte densité démographique. Le matériel et les méthodes sont les mêmes que ceux précédemment décrits (ASSO, 1980-1982).

Résultats et commentaires

a) Intercalibrations

Parallèlement à chaque série de mesures sur les échantillons composés de dix moules, nous avons effectué une analyse sur du matériel standard distribué par l'A.I.E.A. (Monaco) :

- par le Mercure et le Cuivre, les écarts entre les mesures sont non significatifs avec le test de Student ;

- par le Zinc, les écarts varient de 0 à 36 % ;
- Cadmium, Plomb et Mercure enregistrent des écarts variables, parfois importants justifiant un coefficient de correction.

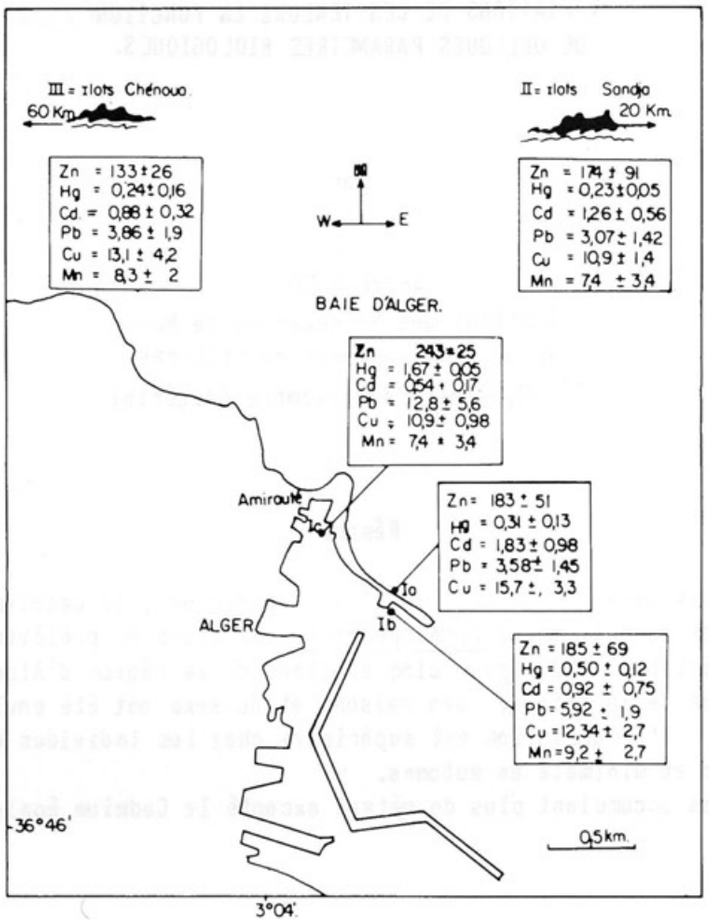


Figure 1. - Concentrations moyennes en p.p.m. poids sec pour six métaux étudiés chez la Moule Perna perna (L) dans la région d'Alger, de novembre 1978 à octobre 1980.

b) Evaluation des teneurs globales

La représentation sous forme de carte (Fig. 1) pour les six métaux lourds étudiés permet de comparer les différentes stations, trois dans le port d'Alger, une à l'est (Sandja) une à l'ouest (Chenoua). Ces valeurs moyennes correspondent à cinq ou dix prélèvements différents pour chaque station.

LE ZINC : les deux stations de l'avant-port Ia et Ib ont des valeurs faibles, comparables à celles de l'étang de Thau (France) (FOWLER et OREGIONI , 1976). On note un gradient de concentration à l'intérieur du port en Ic ; la situtation dans les deux autres stations, pourtant éloignées d'Alger, est identique à celle de la station Ia.

LE MERCURE : un gradient très marqué se rencontre pour ce métal de l'extérieur vers l'intérieur du port, avec des teneurs préoccupantes dans cette station. Nous dépassons 1 p.p.m. poids sec qui est la valeur maximale obtenue par ARNOUX et al. (1980) à Marseille-Cortiou, zone réputée polluée. Les concentrations en II et III sont comparables. On pouvait s'attendre à des valeurs plus faibles au Chenoua où il n'existe aucune pollution locale. La présence du métal, certes à des concentrations faibles, peut-être expliquée par son transport au sein des masses d'eau du courant atlantique.

LE CADMIUM : les concentrations sont faibles pour l'ensemble des stations étudiées, avec des valeurs souvent inférieures à 1 p.p.m. poids sec, qui est une valeur difficile à rencontrer dans d'autres régions de la Méditerranée. Comme pour le Mercure, la présence du Cadmium au Chenoua est expliquée par le transport des eaux atlantiques qui reçoivent les déchets de l'usine de traitement des minerais de Zinc (et de Cadmium) de Ghazouet, à l'ouest du pays.

LE PLOMB : se répartit de manière comparable au Mercure . Un gradient de concentration existe dans le port d'Alger, avec des valeurs élevées et préoccupantes en Ic. La dilution intervient efficacement au niveau de l'avant-port qui retrouve des valeurs voisines aux stations II et III.

LE CUIVRE : si l'on considère avec BRYAN (1976) que 10 p.p.m. poids sec est la valeur moyenne du Cuivre des Bivalves, nous constatons que la moule Perna perna (L) de la région d'Alger s'écarte très peu de cette valeur. On peut noter toutefois une répartition en gradient négatif vers l'intérieur du port d'Alger, à l'opposé donc du Mercure, du Plomb, et du Zinc.

LE MANGANESE : comme pour le Cuivre, toutes nos valeurs sont inférieures à 10 p.p.m. poids sec. Il ne semble donc pas y avoir de problèmes de pollution par ce métal par ailleurs beaucoup moins toxique que les précédents.

c) Variations des concentrations en fonction de la taille et du sexe.

Pour chaque prélèvement saisonnier deux classes de tailles différentes ont été sélectionnées pour l'analyse. En supposant la relation concentration-taille, rectiligne dans l'intervalle étudié, nous obtenons une série de droites avec des pentes différentes.

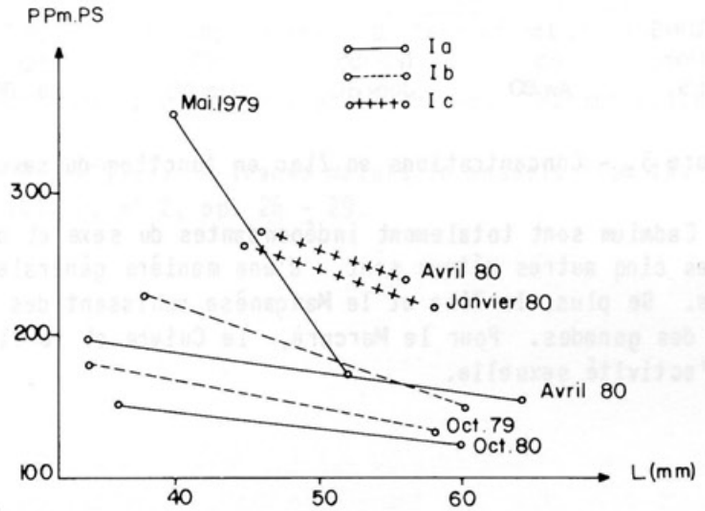


Figure 2. - Concentrations en Zinc en fonction de la taille et des saisons dans 3 stations du port d'Alger.

Exemple du Zinc : fig. 2. Pour ce métal toutes les pentes sont négatives, traduisant une accumulation supérieure chez les individus de petites tailles, résultats en accord avec ceux de BOYDEN (1977). Nous constatons d'autre part un minimum automnal, période de repos sexuel. L'accumulation reprend en janvier et pendant toute la période printanière qui suit, en parallèle avec la gamétogénèse, la ponte intervenant avant l'été (ABADA, BOUDJEMA et MOUEZA 1980).

Pour la majorité des métaux étudiés, la pente est négative, excepté pour le Manganèse. Selon les conditions du milieu et la période de prélèvement, la valeur de cette pente varie et peut, à la limite, s'annuler. Si, pour quelques analyses, des pentes positives ont pu être obtenues, elles sont rares et toujours compensées par une série de pentes négatives.

En ce qui concerne les variations saisonnières, la majorité des éléments analysés présente un minimum automnal et un maximum printanier que nous avons mis en rapport avec les phénomènes de la gamétogénèse, toutes choses étant égales par ailleurs. Seul le Manganèse semble faire exception à ce processus.

d) Variations des concentrations liées au sexe.

Nous constatons que les mâles ont des concentrations en Zinc supérieures aux femelles pour deux prélèvements. Cette différence s'annule en novembre, date à laquelle la gamétogénèse est stoppée (fig. 3).

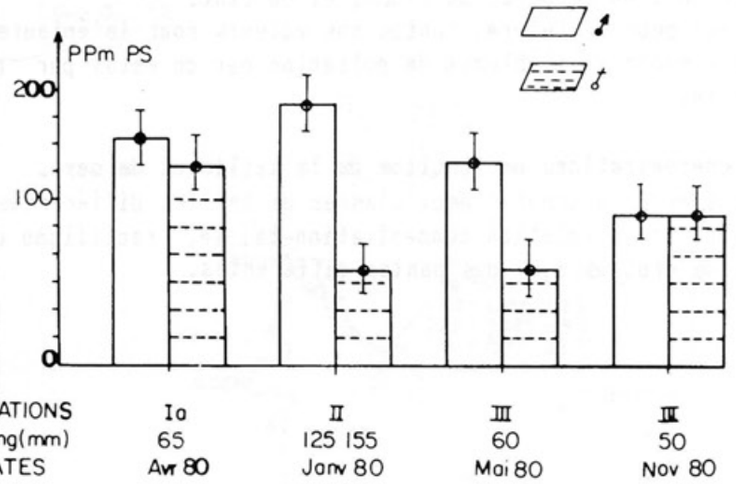


Figure 3. - Concentrations en Zinc en fonction du sexe.

Les concentrations en Cadmium sont totalement indépendantes du sexe et de l'activité gamétogénique. Les concentrations des cinq autres métaux sont, d'une manière générale, plus élevées chez les mâles que chez les femelles. De plus, le Zinc et le Manganèse subissent des fluctuations en relation avec l'état de remplissage des gonades. Pour le Mercure, le Cuivre et le Plomb, ces relations sont difficiles à relier avec l'activité sexuelle.

Conclusions

L'étude du suivi des concentrations pour six métaux sur une période de deux ans nous a permis de définir le niveau de ces polluants chez la moule Perna perna (L) et de la comparer avec d'autres régions de la Méditerranée. Les concentrations sont faibles pour les îlots Sandja à l'est de la baie d'Alger ainsi que pour les ilôts Chenoua situés à 60 kilomètres à l'ouest d'Alger. La station située à l'extérieur du port d'Alger nous a permis de tester la dilution rapide qui peut intervenir à partir d'une zone polluée comme celle de l'intérieur du port d'Alger. Ainsi, le Mercure passe de 1,67 à 0,31 p.p.m. poids sec. Dans la zone enclavée du port, le Plomb à 12,7 p.p.m. poids sec et le Mercure à 1,67 p.p.m. poids sec semblent atteindre des valeurs préoccupantes. La situation est, par contre, normale pour les trois métaux, le Cadmium, le Cuivre et le Manganèse.

Au regard des variations saisonnières des concentrations, nous constatons que la surveillance continue des polluants ne pourra être simplifiée à l'avenir, car avec un minimum automnal et un maximum printanier, ces deux mesures sont suffisantes pour tester l'état d'une station donnée.

Le problème des liaisons de concentrations avec la taille a été mené en parallèle avec celui des variations saisonnières.

L'interprétation empirique menée sur deux classes de tailles différentes permet de conclure à une accumulation en général supérieure chez les petits individus.

Notre méthode peut conduire à une détermination de la pente et du coefficient de régression après changements d'origine.

Enfin, l'étude des concentrations en fonction du sexe propose une analyse globale du métabolisme de ces oligo-éléments. Elle ouvre la porte à toute une série d'analyses plus fines, en fonction des organes, des tissus et même des organites cellulaires qui doivent déboucher sur une meilleure connaissance de la biologie de cette espèce indicatrice.

Références

ABADA, BOUDJEMA (G.) et MOUEZA (R.) (1981). - Structure des populations d'une moulière naturelle en baie d'Alger. Acta ecologica, aecol. gener., Vol. 2, n° 2, pp. 183-194.

ARNOUX (A), TATOSSIAN (J.), MONOD (J.L.), BLANC (A.) (1980). - Etude des teneurs en métaux lourds et composés organo-chlorés dans des organismes marins prélevés dans le secteur de Cortiou (Marseille. Ves Journées Etud. Pollutions, pp. 471-482, Cagliari - C.I.E.S.M.

ASSO (A.) (1980). - Etude des métaux lourds chez Perna perna (L) dans la région d'Alger. Ves Journées Etud. Pollutions, pp. 163-168, Cagliari - C.I.E.S.M.

ASSO (A.) (1982). - Contribution à l'étude des polluants métalliques chez la moule Perna perna (L) dans la région d'Alger. Thèse 3è cycle - Aix-Marseille II, 138 p.

BOYDEN (B.L.) (1977). - Effect of size upon metal content of shellfish. J. mar. biol. assoc. U.K. - 57 (3), 675 - 714.

BRYAN (G.W.) (1976). - Heavy metal contamination in the sea. Marine Pollution - Academic Press, pp. 185 - 302.

FOWLER (S.W.), OREGIONI (B.) (1976). - Traces metals in mussels from the N.W. Mediterranean. Marine Pollution Bulletin, Vol. 7, n° 2, pp. 26 - 29.

PARTICULARITES DE LA COMMUNAUTE PHYTOPLANCTONIQUE IMPLIQUEE PAR LES FACTEURS CHIMIQUES D'UN MILIEU PUISSAMENT TROPHIQUE

par

Pia-Elena MIHNEA et Elvira CUINGIOGLU
Institut Roumain de Recherches Marines, Constantza (Roumanie)

Abstract

The main modifications both of the chemical factors and the quantitative features of the phytoplanktonic community during 1960-1980 are briefly presented.

As it was observed some positive correlations between Euglenophyta and Chrysophyta groups and marine chemical compounds, a trial was done to reckon the correlation between the most frequent phytoplanktonic species and P-PO4; N-NO2; N-NO3; BOD5.

An outlook on the principal aspects of the phytoplanktonic community in highly eutroficated waters are discussed.

*
* *

Matériel et méthode

Nous avons prélevé les échantillons pour les analyses chimiques et biologiques sur 12 stations situées devant où à proximité des principaux déverseurs, tout au long de la partie sud du littoral roumain de la mer Noire. Dans l'intervalle 1975 à 1977, les échantillons ont été prélevés seulement sur l'isobathe de 5 m, tandis qu'à partir de 1978 jusqu'en 1980, nous les avons aussi prélevés sur l'isobathe de 10 m. En vue de mettre en relief les modifications des eaux de mer dans la zone littorale, nous avons établi une station témoin en haute mer, à 5 Mm (1975-1977) ou à 10 Mm (1978-1980) de la côte. Tous les échantillons provenaient de l'horizon 0-1 m.

Les échantillons ont été prélevés mensuellement, au printemps, en été et en automne prolongé. On a déterminé les concentrations du P-PO4, N-NO2, N-NO3, N-NH4, Si-SiO3, DBO5, ainsi que la densité du phytoplancton. Les paramètres chimiques ont été définis selon STRICKLAND et PARSONS (1972), tandis que les échantillons de phytoplancton ont été analysés par la méthode de la sédimentation.

Résultats et discussions

Le milieu marin étudié a été caractérisé par un très important potentiel trophique. L'état de l'écosystème soumis à la pollution avec des effets eutrophisants peut être divisé, jusqu'à présent, en deux étapes :

1. l'étape de l'augmentation progressive de la concentration des anions trophiques, ainsi que d'autres fractions, impliquées directement dans la nutrition du phytoplancton ;
2. l'arrivée à un degré élevé d'eutrophisation et le maintien de celui-ci.

Pendant la première étape, le milieu marin s'est modifié par la croissance du taux des éléments trophiques (Fig. 1-2), ainsi que par l'apparition et le maintien, dans un certain degré de concentration, des détergents, pesticides, métaux lourds, N-NH4, la diversité des produits organiques résultant de la décomposition de la matière organique, etc. Les effets produits par ces modifications se sont manifestés à travers l'apparition de quelques particularités nouvelles, qualitatives et quantitatives de la communauté phytoplanctonique (MIHNEA, 1978; MIHNEA, 1980; MIHNEA, CUINGIOGLU et BILAL, 1980; MIHNEA, 1982) .

Les aspects qualitatifs nouveaux concernent le rapport entre les représentants des embranchements qui forment le phytoplancton, le temps affecté au déroulement du cycle biologique des espèces dominantes, l'augmentation de la fréquence de certains taxons rares pendant les décennies antérieures, l'installation de nouvelles espèces qui sont devenues dominantes, etc.

Du point de vue quantitatif, les modifications sont exprimées par la croissance de la quantité totale de phytoplancton (Fig. 1), et, par conséquent, de la biomasse totale.

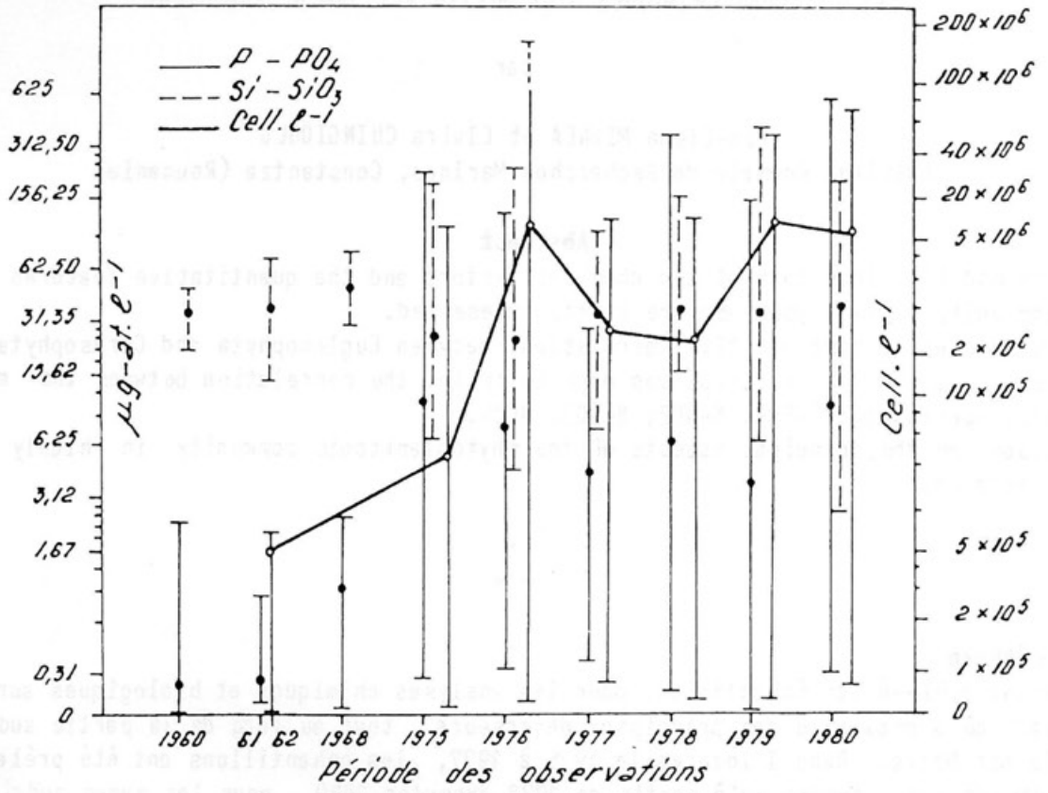


Fig. 1 - Valeur moyenne et limites de variation de la concentration de P-P04, du Si-Si03 et du phytoplancton* pendant l'intervalle 1960-1980.
* pendant l'intervalle 1960-62 d'après BODEANU (1967)

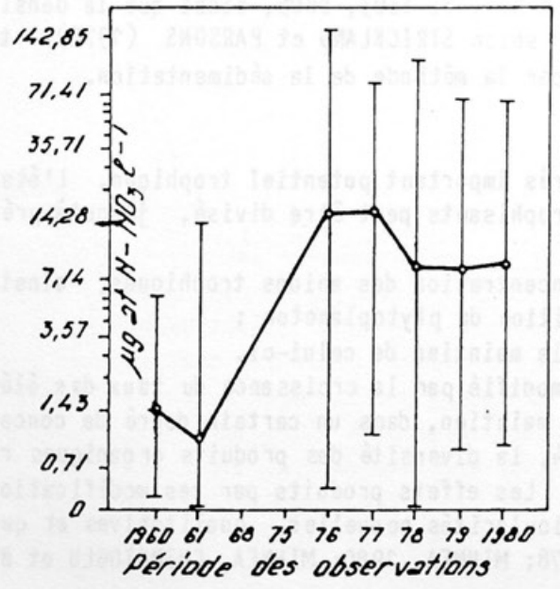


Fig. 2 - Valeur moyenne et limites de variation de la concentration de N-N03 pendant l'intervalle 1960-1980.

Les phénomènes de "marée rouge" ont eu une fréquence accrue et leurs amplitudes ont atteint des niveaux sans précédent dans cette mer.

Dans les conditions d'un milieu intensément eutrophisé (Fig. 3-4 ; à titre d'exemple, on utilise exclusivement des données de l'année 1980), on observe, du point de vue du chimisme, ce qui suit :

- la tendance du maintien permanent des principaux anions trophiques dans le milieu ;
- les moyennes calculées pour chaque station, ainsi que les limites de variation des concentrations déterminées au cours d'un an, prouvent le haut niveau de concentration des anions trophiques dans toute la zone étudiée ;
- la réduction au zéro analytique a lieu seulement pendant quelques-unes des floraisons, étant donc un phénomène éphémère.

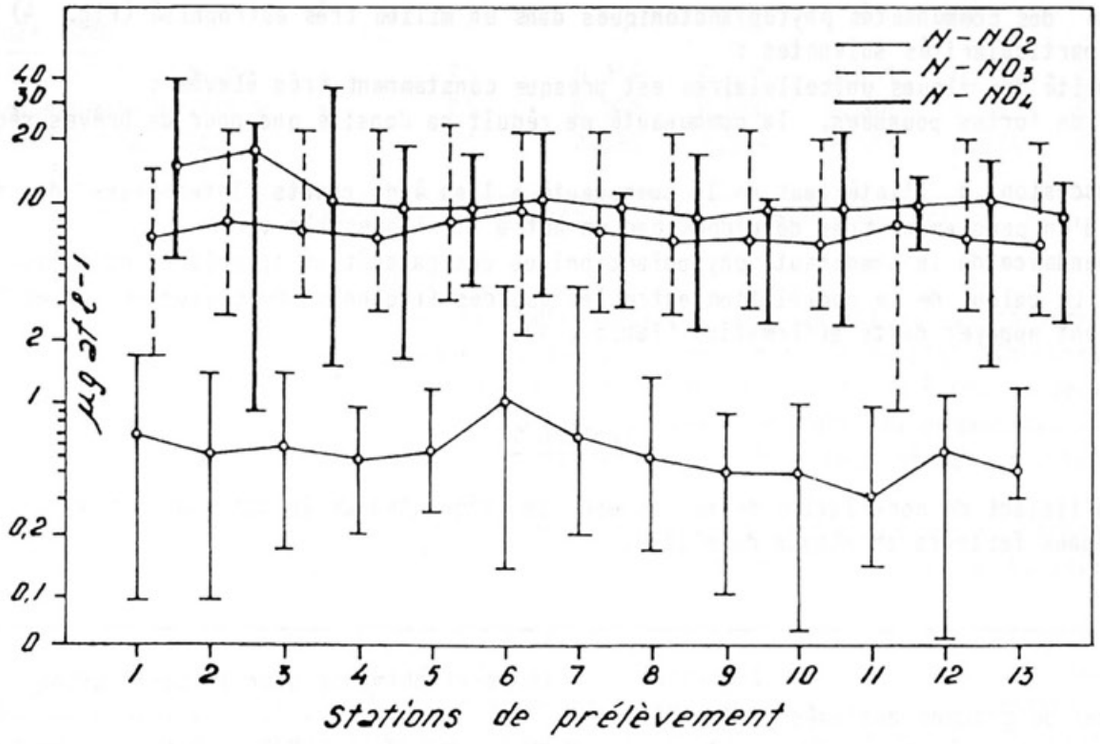


Fig. 3. - Concentration de l'azote (N-N02, N-N03 et N-NH4) au cours de l'année 1980 dans les points de prélèvement ($\bar{X}$ pour 8 mois) (Les stations de prélèvement du Nord au Sud : 1. Navodari ; 2. Mamaia ; 3. I.R.C.M. ; 4. Ion Ratiu ; 5. Modern ; 6. Constanta-Sud ; 7. Eforie Nord ; 8. Eforie Sud ; 9. Tuzla (témoin 5-10 Mm) ; 10. Costinesti ; 11. Neptun ; 12. Mangalia ; 13. Vama Veche).

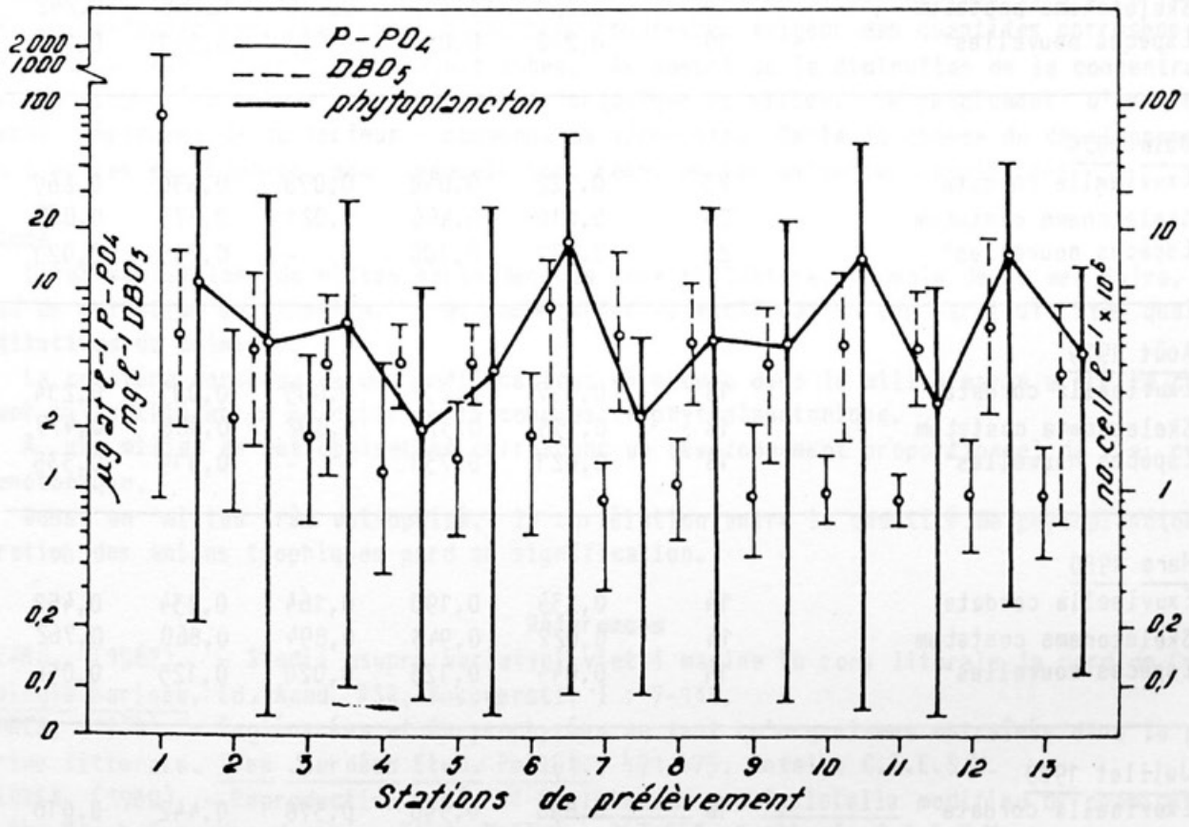


Fig. 4. - Concentration du phosphore (P-P04), du silicium (Si-Si03) et densité du phytoplancton au cours de l'année 1980 ($\bar{X}$ pour 8 mois).

L'analyse des communautés phytoplanctoniques dans un milieu très eutrophisé (Fig. 4) a mis en évidence les particularités suivantes :

- la densité des algues unicellulaires est presque constamment très élevée ;
- après de fortes poussées, la communauté ne réduit sa densité que pour de brèves séquences de temps ;
- la succesion à l'intérieur de la communauté a lieu à de courts intervalles de temps, le remplacement d'un peuplement très développé par un autre étant possible ;
- la dépendance de la communauté phytoplanctonique des paramètres trophiques du milieu commence à s'effacer. Le calcul de la corrélation entre les espèces fréquentes du phytoplancton et les anions trophiques vient appuyer cette affirmation (Tableau 1).

Tableau 1

Valeur du coefficient de corrélation entre les espèces fréquentes de la communauté phytoplanctonique et les principaux facteurs chimiques du milieu.

Espèces ou groupes analysés	Degrès de liberté (n-2)	facteurs chimique pour la corrélation				
		P-P04	N-N02	N-N03	N-NH4	DB05
Mai 1979						
Exuviaella cordata	19	0,567	0,216	0,030	0,078	0,607
Skeletonema costatum	19	0,160	0,397	0,120	0,056	0,242
Espèces nouvelles*	19	0,278	0,027	0,553	0,051	0,257
Juin 1979						
Exuviaella cordata	23	0,122	0,048	0,076	0,530	0,867
Skeletonema costatum	23	0,010	0,466	0,021	0,373	0,082
Espèces nouvelles*	23	0,021	0,100	-	0,205	0,021
Août 1979						
Exuviaella cordata	18	0,077	0,237	0,095	0,007	0,214
Skeletonema costatum	18	0,153	0,375	0,092	0,088	0,479
Espèces nouvelles*	18	0,021	0,731	-	0,019	0,335
Mars 1980						
Exuviaella cordata	14	0,134	0,190	0,164	0,134	0,452
Skeletonema costatum	14	0,022	0,943	0,894	0,860	0,762
Espèces nouvelles*	14	0,044	0,123	0,020	0,125	0,056
Juillet 1980						
Exuviaella cordata	18	0,898	0,536	0,378	0,442	0,010
Skeletonema costatum	18	0,062	0,246	0,096	0,080	0,473
Espèces nouvelles*	18	0,088	0,120	0,301	0,443	0,443

Août 1980						
Exuviaella cordata	21	0,059	0,035	0,031	0,616	0,494
Skeletonema costatum	21	0,068	0,480	0,188	0,193	0,456
Espèces nouvelles*	21	0,087	0,354	0,128	0,034	0,239

* Le groupe inclut : Chlorophyta, Euglenophyta, Chrysophyta, Cryptophyta.

Ayant la possibilité d'un apport continuel d'ions trophiques, le milieu est en permanence en état de "saturation", étant capable de supporter des développements massifs de phytoplancton. Au moment où les émissaires diminuent ou réduisent complètement leurs débits, certains éléments peuvent se remettre en état par leur libération sur le compte des quélateurs. Les matières organiques susceptibles de ces fonctions existent dans le milieu étudié tout au long de l'année, dépassant d'habitude 40 mg $KMnO4^{-1}$.

La diminution du degré de signification de la corrélation entre les anions trophiques et le phytoplancton peut donc être considérée comme une particularité d'un milieu où la pollution mène à l'eutrophisation.

Dans un milieu où l'eutrophisation résulte d'une pollution permanente par les eaux usées domestiques, on peut maintenir certains composants à des niveaux satisfaisants de concentration, mais certainement pas tous les composants. Théoriquement, la grande majorité des composants organiques résulte des processus de décomposition de la matière organique ; par la suite, la présence ou leur degré de concentration dans l'eau de mer dépend des conditions physico-chimiques ou biologiques caractéristiques du milieu à un moment donné. L'instabilité quantitative et qualitative des eaux déversées, les modifications produites par les fluctuations des paramètres météorologiques, contribuent à la variation quantitative des fractions organiques.

Comme nous pouvons le constater dans les milieux extrêmement eutrophisés il y a d'importantes quantités de phytoplancton. Les communautés très nombreuses exigent des quantités correspondantes de composants organiques trophiques ou auxotrophes. Au moment de la diminution de la concentration ou lors de la disparition d'un facteur de nature organique du milieu, le peuplement d'une espèce - strictement dépendant de ce facteur - commence sa déchéance. Reste la chance du développement d'un autre ou d'autres peuplements, pour lesquels sont remplies les exigences spécifiques.

Conclusions

1. L'eutrophisation du milieu marin dans la zone du littoral roumain de la mer Noire, par un processus de pollution continuelle, a eu comme effet la modification des particularités qualitatives et quantitatives du chimisme.

2. La première conséquence des modifications chimiques dans le milieu marin a été le changement concernant la qualité et la quantité de la communauté phytoplanctonique.

3. A un milieu en eutrophisation correspond un développement proportionnel de la communauté phytoplanctonique.

4. Dans un milieu très eutrophisé, la corrélation entre la quantité de phytoplancton et la concentration des anions trophiques perd sa signification.

Références

N. BODEANU, (1967). - Studii asupra variatiei vietii marine în zona litorala la nord de Constanta. Ecologie marinae, Ed. Acad. RSR, Bucuresti, I : 7-138.

P.E. MIHNEA, (1978). - Euglénacées et Chrysophycées en tant qu'organismes entraînés dans la pollution marine littorale. IVes Journées Etud. Pollut., 471-475, Antalya C.I.E.S.M.

P.E. MIHNEA, (1980). - Reproductive cycle of Skeletonema and Cyclotella modified by chemical changes in the Black Sea. Ves Journées Etud. Pollut., 863-868, Cagliari, C.I.E.S.M.

P.E. MIHNEA, E. CUINGIOGLU et I. BILAL, (1980). - Modifications des communautés phytoplanctoniques littorales sous l'influence du phénomène de pollution. Ves Journées Etud. Pollut., 869-876, Cagliari, C.I.E.S.M.

P.E. MIHNEA, (1982). - Poluarea apelor marine costiere la litoralul românesc al Marii Negre si fenomenul de eutrofizare (The pollution of the nearshore romanian marine waters and the eutrophication phenomenon). Pontus Euxinus, Studies and Researches, II : 297-304.

J.D.H. STRICKLAND and T.R. PARSONS, (1972). - A practical handbook of seawater analysis. Fisheries Research Bd. of Canada Bulletin, 167-310.

CONCENTRATIONS DE METAUX LOURDS DANS LES SEDIMENTS SUPERFICIELS D'UNE ZONE MARINE SOUMISE A L'INFLUENCE D'EAUX DOMESTIQUES USEES TRAITEES ET DECHARGEES DANS LA MER

par

Radu MIHNEA et Ioan PECHEANU
Institut Roumain de Recherches Marines
Constantza (Roumanie)

Résumé

Les eaux domestiques usées de la zone nord de Constantza sont épurées dans une station de traitement puis déchargées, dans une zone marine peu profonde.

Dans les sédiments superficiels prélevés en cette zone d'influence ont été déterminés les métaux lourds Cu, Pb, Zn, et Cd.

Les résultats obtenus indiquent des valeurs inférieures à celles mentionnées par certains auteurs pour d'autres eaux océaniques de faible profondeur.

Les facteurs qui déterminent ces valeurs plus basses sont largement commentés.

*
* *

Introduction

PECHEANU (1982), MIHNEA et al. (1982) et SERBANESCU et al. (1980) ont effectué des études sur le littoral roumain, concernant le rôle et l'importance de certaines sources anthropogènes, sur la teneur des ions métalliques de certains composants du milieu marin.

Les recherches concernant la protection de la qualité des eaux marines sur le littoral roumain sont basées sur un vaste et complexe programme, à long terme, dont l'un des objectifs prévoit de suivre l'évolution du contenu des métaux lourds dans les sédiments.

Méthodologie

Dans la zone de faible profondeur située au nord de Constantza, s'effectue le déchargement des eaux domestiques usées provenant des activités socio-urbaines, après avoir été traitées dans une station d'épuration spécialement aménagée.

Durant toute la saison estivale, dès le début du mois de mai jusqu'au début de septembre, afin que les mesures de protection soient plus efficaces, ces eaux usées sont dirigées vers l'agriculture et utilisée à l'irrigation de certaines cultures, leur évacuation en mer étant complètement interdite.

Pour évaluer les aspects les plus divers concernant le contenu et la dynamique des métaux lourds dans les sédiments de cette zone (environ 3.500 m, vers le nord, l'est et l'ouest) furent prélevés des échantillons sur un réseau fixe de 12 stations (fig. 1), situées de telle manière que toute la zone d'influence soit couverte.

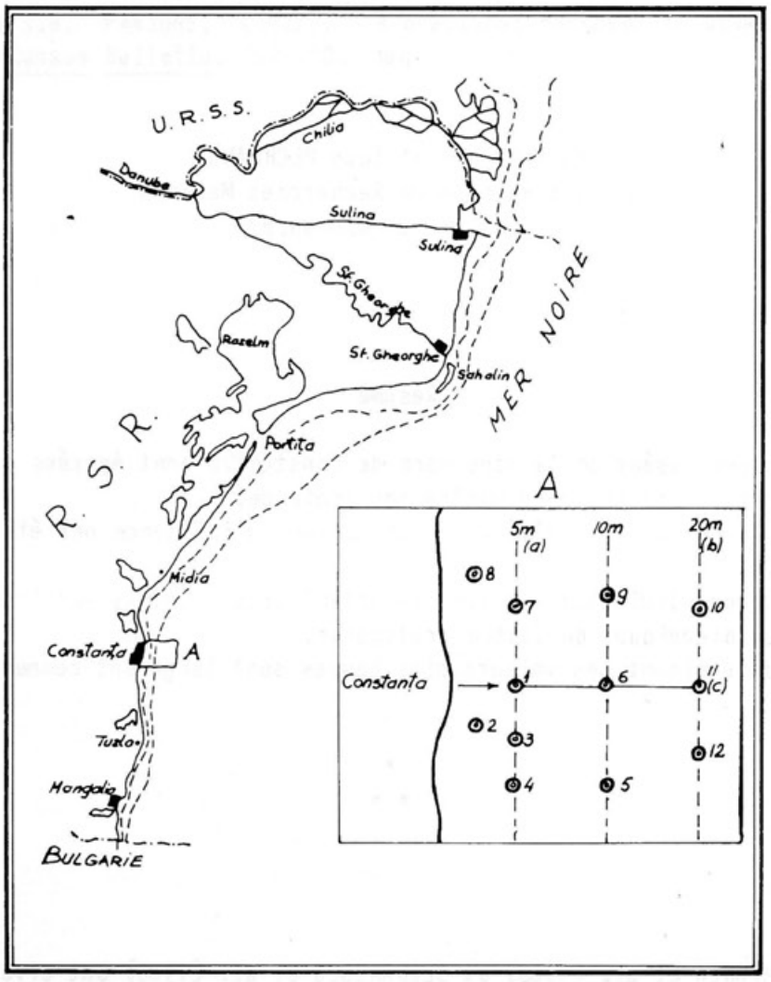


Figure 1. - Le littoral roumain avec la localisation de la zone et des stations de prélèvement.

Les échantillons ont été prélevés depuis la surface à l'aide d'une benne type VAN VEEN, en avril, mai et septembre.

La conservation, le transport et la préparation ont été effectués conformément aux techniques et selon les méthodes indiquées dans la littérature spécialisée.

L'extraction et le passage des métaux lourds en solution ont été réalisés avec HCl et HNO3 1N, et les mesures à l'aide d'un spectrophotomètre à absorption atomique PYE UNICAM type SP 2.900.

Résultats et discussions

Le tableau ci-après présente les résultats obtenus, exprimés en ug/g de sédiment sec à 20° C.

Contenu en métaux lourds des sédiments superficiels de la zone d'influence

Station	Cu ug/g			Moy.	Pb ug/g			Moy.	Zn ug/g			Moy.	Cd ug/g		
mois	IV	VI	IX		IV	VI	IX		IV	VI	IX		IV	VI	IX
1	12,98	11,89	1,67	8,84	5,33	12,01	3,39	6,91	21,60	36,45	2,96	20,24	N.D.	0,18	N.D.
2	-	1,45	3,96	2,70	-	3,85	4,62	4,23	-	15,84	19,56	17,70	N.D.	N.D.	N.D.
3	1,93	16,41	-	9,17	4,67	13,98	-	9,32	23,10	46,60	-	34,85	N.D.	0,33	N.D.
4	1,94	25,65	1,66	9,75	6,13	20,42	5,25	10,60	16,39	62,10	8,15	28,88	N.D.	N.D.	N.D.
5	20,83	4,05	3,02	9,03	23,46	6,62	6,56	12,21	55,89	17,94	12,61	28,81	0,42	N.D.	N.D.
6	2,73	17,91	2,62	7,75	5,91	16,33	4,95	9,06	21,61	41,46	8,74	23,93	N.D.	N.D.	N.D.
7	1,86	2,26	1,92	2,01	4,27	4,95	5,65	4,95	14,80	16,16	9,97	13,64	N.D.	N.D.	N.D.
8	1,83	2,67	1,90	2,13	4,14	4,71	4,87	4,57	15,29	18,02	3,92	12,41	N.D.	N.D.	N.D.
9	2,52	2,93	2,89	2,78	5,44	5,42	6,91	5,92	18,92	19,00	8,31	15,41	N.D.	N.D.	N.D.
10	9,06	4,05	3,73	5,61	9,69	6,94	5,90	7,51	36,53	19,45	11,86	22,61	N.D.	N.D.	N.D.
11	4,84	4,39	3,59	4,26	7,38	6,90	6,72	7,00	23,56	17,27	10,29	17,04	N.D.	N.D.	N.D.
12	-	1,76	22,12	11,94	-	5,44	19,70	12,57	-	17,68	39,10	28,39	N.D.	N.D.	0,44

N.D. = non décelable
Moy. = moyenne

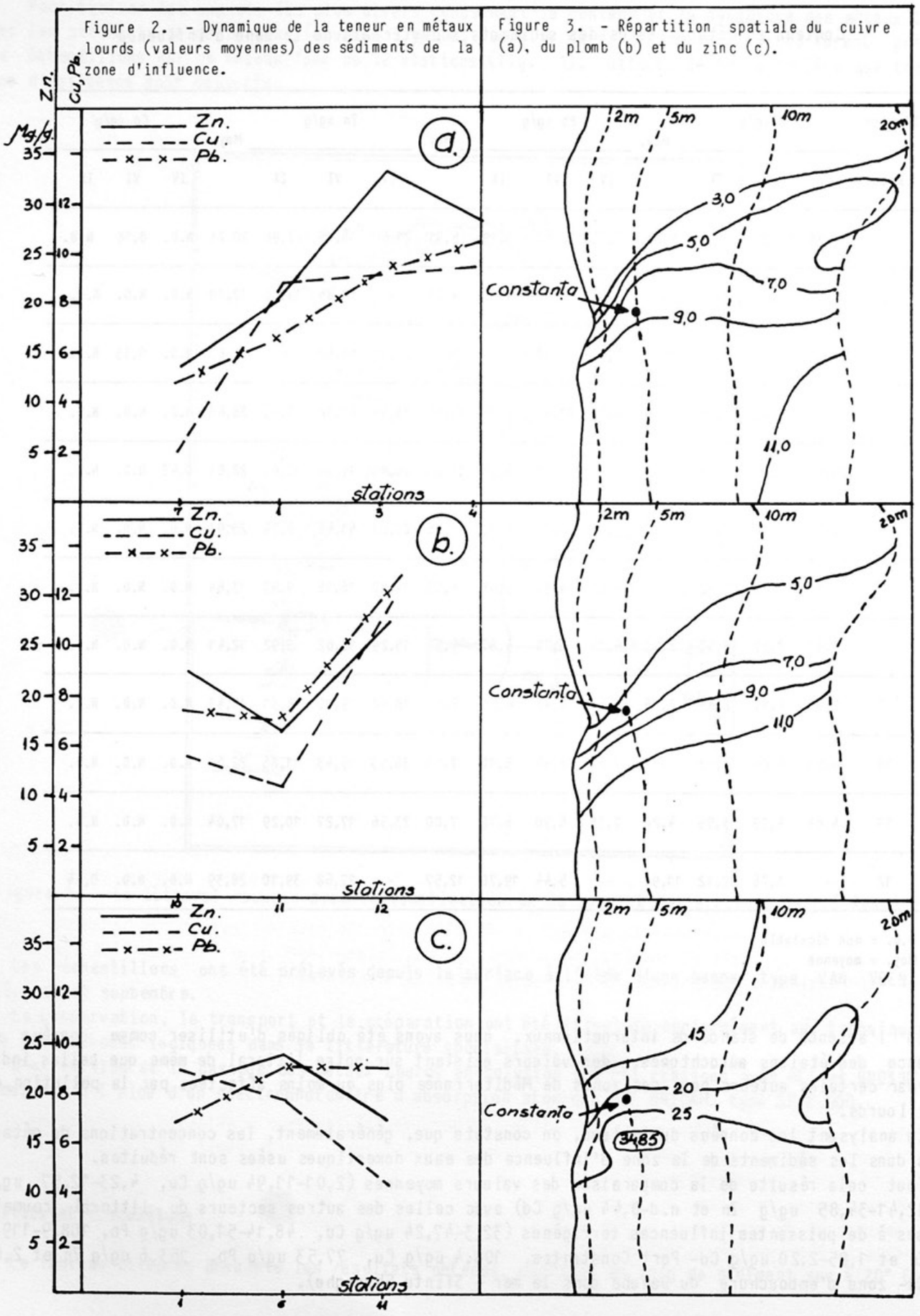
En l'absence de standards internationaux, nous avons été obligés d'utiliser comme données de référence des étalons autochtones, des valeurs existant sur notre littoral de même que celles indiquées par certains auteurs pour des zones de Méditerranée plus ou moins affectées par la pollution de métaux lourds.

En analysant les données du tableau, on constate que, généralement, les concentrations de métaux lourds dans les sédiments de la zone d'influence des eaux domestiques usées sont réduites.

Tout cela résulte de la comparaison des valeurs moyennes (2,01-11,94 ug/g Cu, 4,23-12,57 ug/g Pb, 12,41-34,85 ug/g Zn et n.d-0,44 ug/g Cd) avec celles des autres secteurs du littoral roumain soumises à de puissantes influences terrigènes (32,3-47,24 ug/g Cu, 48,14-51,03 ug/g Pb, 108,9-119,7 ug/g Zn et 1,85-2,20 ug/g Cd- Port Constantza, 108,4 ug/g Cu, 77,53 ug/g Pb, 153,6 ug/g Zn et 2,67 ug/g Cd- zone d'embouchure du Danube dans la mer - Sfîntu Gheorghe).

Figure 2. - Dynamique de la teneur en métaux lourds (valeurs moyennes) des sédiments de la zone d'influence.

Figure 3. - Répartition spatiale du cuivre (a), du plomb (b) et du zinc (c).



Les valeurs identifiées dans la zone marine du nord de Constantza sont beaucoup plus réduites que les concentrations indiquées par M. BERNHARD (1980), concentrations trouvées par divers auteurs dans les sédiments de certaines zones de Méditerranée affectées par ce type de pollution.

Les quelques valeurs plus élevées rencontrées dans certaines stations de la zone d'influence doivent être, généralement, attribuées aux conditions spécifiques, telles que le fond pierreux et l'abondance de nourriture qui déterminent le dévéloppement d'une riche faune de Mollusques.

Celles-ci accumulent d'importantes quantités d'ions métalliques qu'elles libèrent seulement après leur mort.

Bien que la plupart des valeurs soient basses et leur intervalle de variation assez restreint, on constate pourtant une certaine répartition spatiale des contenus (fig. 2 et 3).

On observe une tendance d'augmentation des valeurs du nord vers le sud, favorisée par les courants et les vents dominants nord-sud, qui transportent les masses d'eaux impurifiées vers le sud de la zone recherchée.

Bien que nous ayons analysé des sédiments pendant les trois étapes de l'évacuation, c'est-à-dire avant, pendant et après celle-ci, il n'est pas possible de constater une dynamique temporelle des valeurs, spécifique à chaque étape.

La persistance prolongée des métaux lourds dans les sédiments même après l'interdiction de déchargements, explique l'absence de dynamique saisonnière corrélée à la présence ou à l'absence des eaux usées dans la zone.

L'existence de certaines concentrations réduites de métaux lourds dans les sédiments de la zone d'influence du déverseur d'eaux domestiques usées démontre que le chargement organique prédominant, le fond pierreux, la présence d'une grande quantité d'organismes marins, les conditions hydrométéorologiques favorisant la dispersion, la dilution et le transport à de grandes distances des eaux impurifiées, l'interdiction de l'évacuation durant l'été, sont les principaux facteurs expliquant la présence des teneurs réduites de ces ions dans les sédiments superficiels de cette zone.

Conclusions

1. Bien que dans la zone marine du nord de Constantza s'effectue l'évacuation des eaux domestiques usées, on n'y observe pas encore une pollution par métaux lourds des sédiments superficiels.

2. Le chargement organique, le fond pierreux, et l'abondance de nourriture qui déterminent la présence d'un filtre naturel, les conditions hydrométéorologiques et l'interdiction de l'évacuation pendant l'été, sont les facteurs qui influent sur la dynamique et la répartition spatiale des concentrations de métaux lourds dans les sédiments.

Références

BERNHARD M. (1980). - Sources and levels of contamination in the Mediterranean. Ves Journées Etud. Pollution, Cagliari, C.I.E.S.M. 139-144.

PECHEANU I. (1982). - Contenu de certains métaux lourds dans les sédiments superficiels du bassin du port de Constantza. VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M., 435-438.

SERBANESCU O., I. PECHEANU, R. MIHNEA (1980). - Le Zinc, le Cuivre et le Fer dans quelques sédiments et organismes du Danube inférieur et de la zone marine du littoral roumain de la mer Noire. Cercetari marine, 13, 219-225.

LEVELS OF HEAVY METALS AND ORGANOCHLORINE PESTICIDES IN MARINE SEDIMENTS AND ORGANISMS. VALENCIA-CASTELLON, SPAIN.

by

Julio MEDINA ESCRICHE, Agustin PASTOR GARCIA
Félix HERNANDEZ HERNANDEZ
Juan B. BEFERULL BLASCO and Juan C. BARBERA UBEDA.
Department of Analytical Chemistry
Faculty of Chemistry
University of Valencia Environmental Laboratory
University College of Castellon (Spain)

Abstract

The level of metallic pollutants (total Hg, Cd, Pb and Cr) and organochlorine pesticides (PCBs and DDTs) is determined in marine sediments and organisms from Cullera, Valencia, Sagunto and Castellon.

This work is part of the Mediterranean Pollution Monitoring Programme MEDPOL II, and was carried out during 1983.

The metals were analysed by AAS, and the organochlorine components by GC with an electron capture detector. Intercalibration samples from the national programme were used as control in the procedure followed to analyse the metal and pesticide levels in the living organisms.

The degree of metallic pollution in the sediments and organisms studied can be considered low, except the Hg content in Parapenaeus longirostris, Mullus barbatus and Mullus surmuletus. The organochlorine content of the sediments is similar in the various stations, and comparable to other not very polluted areas of the Mediterranean. However the living organisms present a higher level, in particular Mytilus galloprovincialis.

*
* *

Introduction

The level in sediments and organisms of heavy metals and organochlorine pesticides of high molecular weight is an important means of measuring the degree of pollution of the marine environment. This is mainly caused by man's industrial and agricultural wastes which, carried by wind and water, are finally precipitated or absorbed in the sediment and sea-water and to a greater or a lesser extent enter the food-chain. In the long run the create a serious problem, owing to their toxicity, their persistence and the extent of their diffusion, with consequent risk to the marine environment and its fauna and flora, and to public health.

As part of the Mediterranean Pollution Monitoring Programme MEDPOL II, which comes under the Mediterranean Action Plan coordinated by the United Nations Environment Programme, during the months of June, September and December 1983 the metal (total Hg, Cd, Pb and Cr) and organochlorine pesticide

(PCBs and DDTs) levels in the muscle tissues of marine organisms (Mytilus galloprovincialis, Mullus barbatus, Mullus surmuletus, Parapenaeus longirostris, Venus gallina and Tellina sp.) were determined, as were also the levels in sediments from the continental shelf of the provinces of Valencia and Castellon.

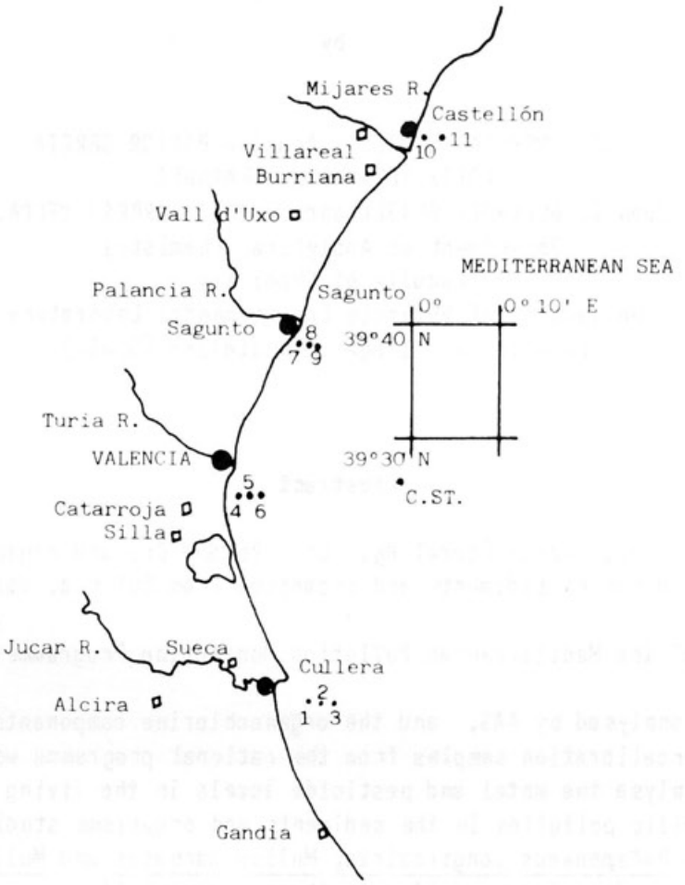


Figure I. Map of the area with location of the sediment sampling station.

The metal analysis was carried out using AAS, and the organochlorine pesticides were analysed by GC with electron capture detector. Homogenized fish samples for intercalibration (obtained from the Escuela Nacional de Sanidad in Madrid) were used as control for the analytical methods by centres collaborating in the 1983 Programme.

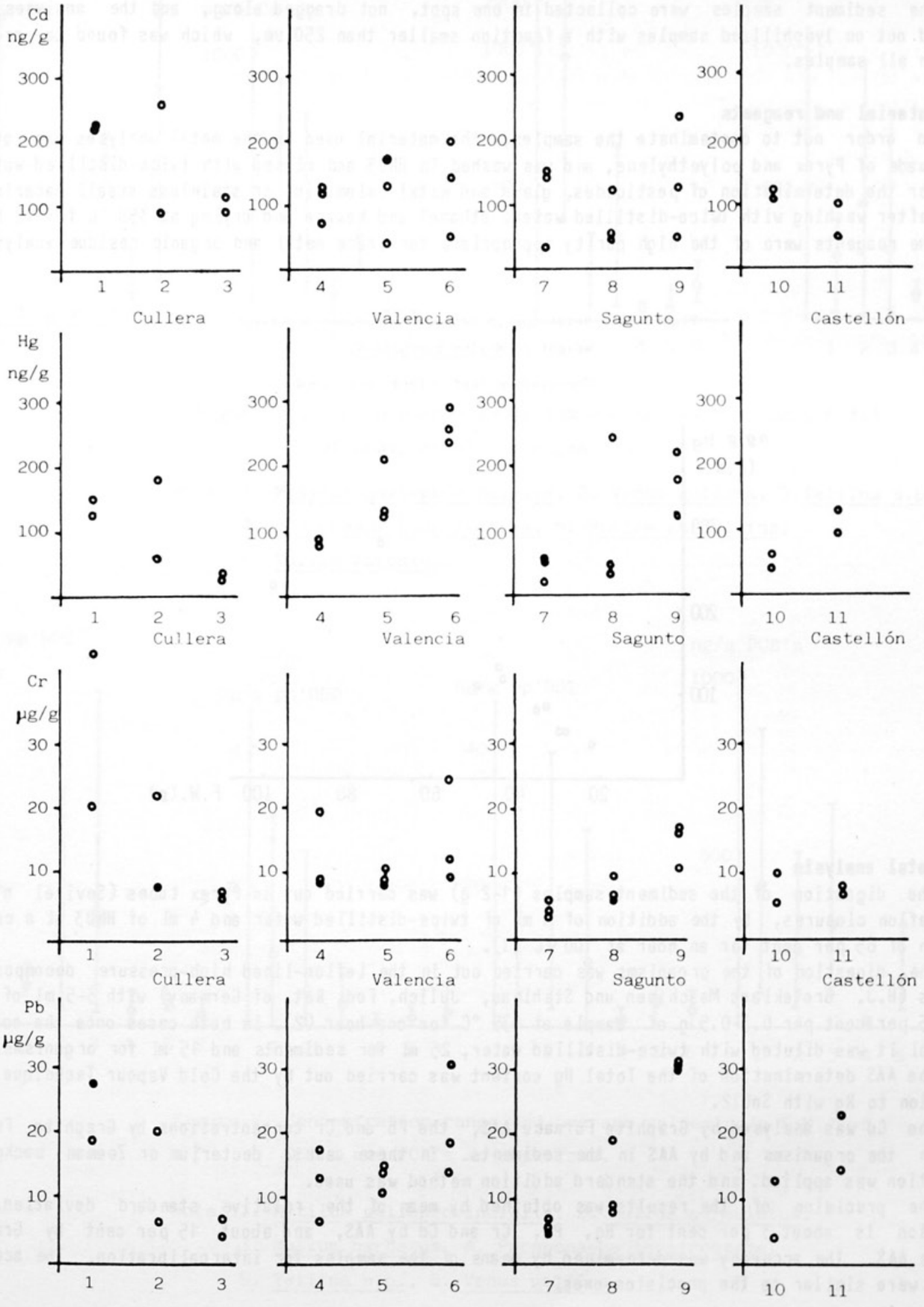
Experimental

Sampling and preparation

The samples were collected in the areas of Cullera, Valencia, Sagunto and Castellon by the Spanish Oceanographic Institute, and stored in a freezer at -18 °C up to the moment of preparation and analysis.

Each sample was divided into two from the moment it was collected. One part was placed in polythene bags for metal analysis and the other in aluminium foil for determination of organochlorine pesticides.

Figure II. Heavy metal content of lyopholised sediments in relation to their distance from the coast.



The organisms were classified, weighed and their lenght measured, and the muscle tissues was separated, lyophilized and homogenized for use in the analyses.

The sediment samples were collected in one spot, not dragged along, and the analyses were carried out on lyophilized samples with a fraction smaller than 250 um, which was found to predominate in all samples.

Material and reagents

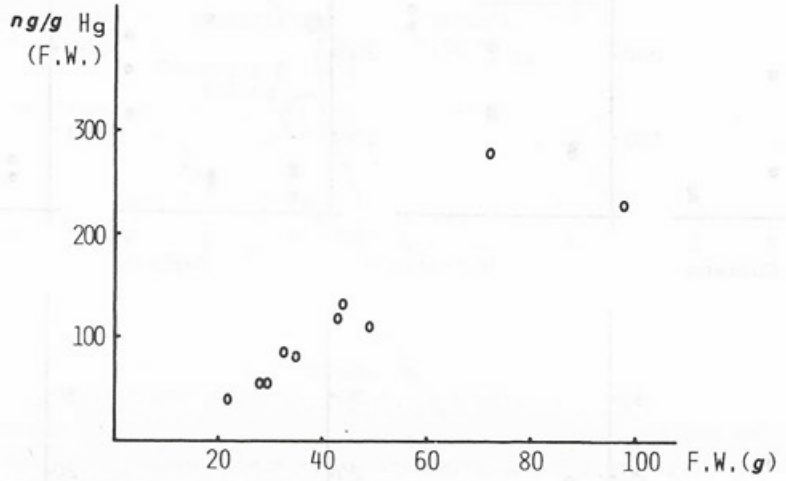
In order not to contaminate the samples, the material used in the metal analyses was at all times made of Pyrex and polyethylene, and was washed in HNO_3 and rinsed with twice-distilled water.

For the determination of pesticides, glass and metal (aluminium or stainless steel) material was used, after washing with twice-distilled water, ethanol and hexane and drying at 350 °C for 12 hours.

The reagents were of the high purity appropriate for trace metal and organic residue analyses.

Figure III. Relationship between Hg content and fresh weight of Mullus surmuletus.

Correlation coefficient r = 0.904



Metal analysis

The digestion of the sediment samples (1-2 g) was carried out in Pyrex tubes (Sovirel n° 20) with Teflon closures, by the addition of 1 ml of twice-distilled water and 4 ml of HNO_3 at a concentration of 65 per cent for an hour at 100 °C (1).

The digestion of the organisms was carried out in the teflon-lined high-pressure decomposition vessels (H.J. Groteklaes Maschinen und Stahlbau, Julich, Fed. Ret. of Germany) with 3-5 ml of conc. HNO_3 65 per cent per 0.3-0.5 g of sample at 135 °C for one hour (2). In both cases once the solution was cool it was diluted with twice-distilled water, 25 ml for sediments and 15 ml for organisms.

The AAS determination of the Total Hg content was carried out by the Cold Vapour Technique after reduction to Hg with $SnCl_2$.

The Cd was analysed by Graphite Furnace AAS, the Pb and Cr concentrations by Graphite Furnace AAS in the organisms and by AAS in the sediments. In these cases, deuterium or Zeeman background correction was applied, and the standard addition method was used.

The precision of the results was obtained by mean of the relative standard deviation. The precision is about 3 per cent for Hg, Pb, Cr and Cd by AAS, and about 15 per cent by Graphite Furnace AAS. The accuracy was determined by means of the samples for intercalibration. The accuracy values were similar to the precision ones.

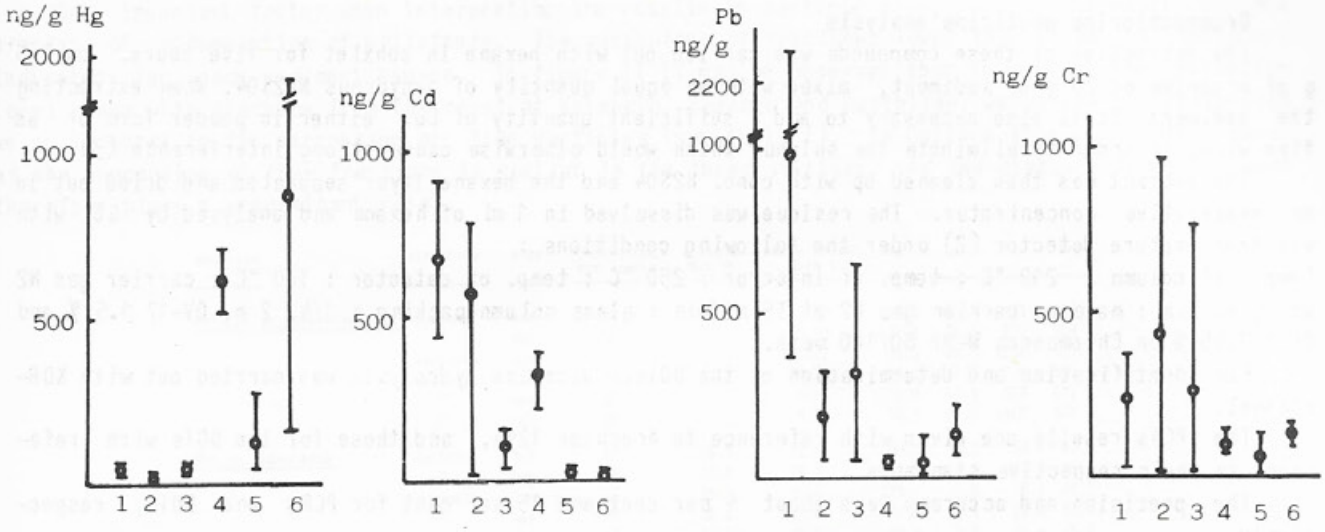


Figure IV. Concentration range and average values (ng/g F.W.) of heavy metals in organisms.

Note: 1. Mytilus galloprovincialis, 2. Venus gallina, 3.Tellina s.p. 4.Parapenaeus longirostris, 5. Mullus surmuletus, 6. Mullus barbatus.

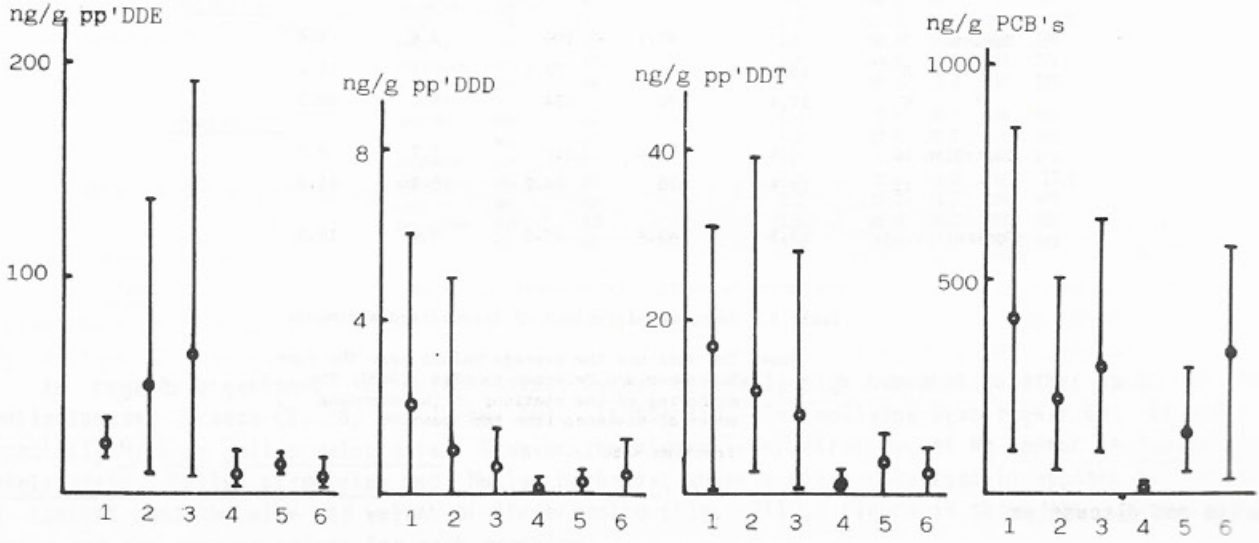


Figure V. Concentration range and average values(ng/g F.W.) of DDT's and PCB's in organisms.

Note: 1. Mytilus galloprovincialis, 2. Mullus surmuletus, 3. Mullus barbatus, 4. Parapenaeus longirostris, 5. Tellina s.p., 6. Venus gallina.

Organochlorine pesticide analysis

The extraction of these compounds was carried out with hexane in sohxlet for five hours, on 2-5 g of organism or 20 g of sediment, mixed with an equal quantity of anhydrous Na_2SO_4. When extracting the sediment it is also necessary to add a sufficient quantity of Cu, either in powder form or as fine wire, in order to eliminate the sulphur which would otherwise cause strong interference (3).

The extract was then cleaned up with conc. H_2SO_4 and the hexane layer separated and dried out in an evaporative concentrator. The residue was dissolved in 1 ml of hexane and analysed by GC with electron capture detector (2) under the following conditions :

Temp. of column : 210 °C ; temp. of injector : 250 °C ; temp. of detector : 300 °C ; carrier gas N_2 at 25 ml/min ; make up carrier gas N_2 at 35 ml/min : glass column packing : 1/4, 2 m, OV-17 1.5 % and QF-1 1.95 % on Chromosorb W-HP 80/100 mesh.

For identification and determination of the DDTs, alcaline hydrolysis was carried out with KOH-ethanol.

The PCBs results are given with reference to Arochlor 1254, and those for the DDTs with reference to their respective standards.

The precision and accuracy were about 4 per cent and 15 per cent for PCBs and DDTs, respectively.

STATION		% fraction <63 µm	Hg (ng/g)	Cd (ng/g)	Cr(µg/g)	Pb (µg/g)
Cullera	1	16.7	138	217	32.5	23.6
"	2	12.6	117	171	15.5	13.4
"	3	17.5	31.2	99	6.8	5.7
Valencia	4	4.0	181	106	12.5	12.4
"	5	6.5	155	113	9.8	13.5
"	6	34.6	261	128	15.3	21.2
Sagunto	7	1.7	40.1	109	4.8	5.6
"	8	16.5	106	72.1	7.5	12.2
"	9	17.4	170	134	15.2	28.0
Castellón	10	3.8	51.4	112	7.7	8.3
"	11	13.9	108	94.2	15.2	11.3
Control	c.st.	13.3	43.4	37.6	8.8	19.5

Table I. Heavy metal content of lyopholised sediments

Note: The data are the average values over the June, September and December samples (1983). The numbering of the stations is in ascending order of distance from the coast.

Fraction <250 µm

Results and discussion

Figure I shows the map of the area studied and the location of the stations where the sediment samples were taken.

The metal content of the sediments is given in table I. The results obtained are relatively low compared to other parts of the Mediterranean (4), Cataluna (1), Thermaikos Gulf (5) and the values are similar to the Alboran Sea, Nice, Monte Carlo, N. Adriatic Sea (6), E. Aegean Sea (5,6). The control situation is in the Valencia area, at a point sufficiently far from the coast, about 27 km., and at a depth of 90 m. The greater content of Hg and Cd compared with the control station is worth noting, whereas the Cr and Pb are at similar concentrations.

One important factor when interpreting the results is particle size, since this bears on the process of accumulation of pollutants. The percentage of fractions lower than 63 um is therefore indicated for each sediment sample. In figure II it may be observed that in general the pollution level rises with distance from the coast at Valencia, Sagunto and Castellon, which can be interpreted as an increase in the percentage of fine particles (see table I). In Cullera this is not the case, as the proportion of fine fractions is similar in the three stations, and the distance from the coast therefore plays a predominant role.

ORGANISM	SITUATION	DATE	INDIVIDUALS FOR SAMPLE	LENGTH MEAN cm	FRESH WEIGHT MEAN g	Hg	Cd	Pb	Cr
MYTILUS GALLOPROVINCIALIS	CULLERA	SEP	49		3.23	18.9	81.2	491	248
	"	DEC	52		3.20	30.1	76.3	542	208
	VALENCIA	SEP	72		1.11	35.2	66.9	2296	25.9
	CASTELLON	JUN	38		3.18	52.1	47.0	724	381
	"	SEP	27		3.54	58.5	43.5	369	171
	"	DEC	40		3.90	58.3	90.9	1467	370
MULLUS SURMULETUS	CULLERA	SEP	12	10.5	22.4	40.2	2.2	56.4	41.5
	"	DEC	7	11.8	28.3	57.4	1.7	62.0	26.1
	VALENCIA	JUN	3	15.7	72.4	279	1.8	6.1	70.1
	"	SEP	5	12.1	29.6	54.4	0.4	52.8	50.4
	"	DEC	7	12.2	31.5	86.4	3.0	45.4	15.8
	SAGUNTO	JUN	4	14.0	44.1	133	4.3	13.9	79.3
	"	SEP	2	16.8	98.0	228	0.6	19.5	51.4
	"	DEC	6	14.2	49.1	111	2.2	53.6	5.1
	CASTELLON	JUN	2	14.0	43.4	117	2.3	53.3	82.0
	"	SEP	4	13.1	35.0	82.2	4.4	128	135
MULLUS BARBATUS	SAGUNTO	SEP	5	17.2	94.1	1921	0.8	225	136
	CASTELLON	JUN	3	13.5	42.0	165	1.7	145	83.2
	"	SEP	2	16.0	68.0	542	0.7	91	158
	"	DEC	4	14.7	51.0	151	3.5	67.4	39.2
PARAPENAEUS LONGIROSTRIS	VALENCIA	JUN	13	8.0	8.9	520	36.3	38.7	63.7
	CASTELLON	SEP	7	12.1	11.2	617	38.9	35.1	147
	"	DEC	6	15.0	13.5	713	21.4	67.3	73.6
TELLINA S.P.	VALENCIA	JUN	63		2.1	46.6	12.7	50.5	193
	"	SEP	311		1.7	29.3	9.6	508	7.5
	SAGUNTO	JUN	36		3.5	50.7	15.4	239	195
	CASTELLON	JUN	43		3.7	49.5	8.9	653	771
	"	SEP	48		2.6	35.1	3.8	164	120
VENUS GALLINA	CULLERA	SEP	55		6.1	2.5	76.3	148	209
	"	DEC	81		4.1	17.3	75.7	328	969
	VALENCIA	SEP	123		3.5	7.4	78.3	172	275
	SAGUNTO	SEP	85		3.3	20.6	1.2	161	15.0
	"	DEC	69		3.7	17.2	71.1	239	475
	CASTELLON	SEP	63		3.9	16.3	36.9	227	426
	"	DEC	45		4.6	25.9	57.1	52.9	689

Table II. Heavy metal content of organisms.
(ng/g fresh weight)

As regards organisms, the metal content is not generally high compared to other parts of the Mediterranean, Greece (7, 8) and Turkey (9), (table II). The molluscs have higher Cd, Pb and Cr, especially Mytilus galloprovincialis. However the highest concentrations of Hg appear in Parapenaeus longirostris, Mullus surmuletus and Mullus barbatus, where a clear relationship appears between the Hg content and the size and weight of the organism (Fig. III). Figure IV shows the concentration ranges and the average values for each organism.

Table III shows the average content of organochlorine pesticides in the various sediments. There are no significant variations according to the time of year.

In the sediments the DDT levels are slightly higher in the Cullera and Castellon samples, although, generally speaking, the levels of DDTs and PCBs are lower than in the other areas of the Mediterranean, Cataluna (3, 10) and industrial areas of Venice (11).

The concentration of organochlorine pesticides in the living organisms is given in table IV, where it can be seen that there are no significant variations either according to place or to time of year. In consequence the ranges and average values of these compounds are presented in figure V for

STATION		% fraction < 63 µm	pp'DDE	pp'DDD	op'DDT	pp'DDT	PCB's
Cullera	1	19.9	10.6	1.02	1.18	6.25	23.2
	2	18.6	4.73	0.34	1.47	3.58	15.8
	3	9.5	1.40	-	0.10	0.52	7.40
Valencia	4	5.6	1.36	-	-	0.37	19.9
	5	13.0	1.39	0.12	0.10	0.51	19.6
	6	32.1	2.05	0.22	-	0.55	27.6
Sagunto	7	2.11	1.04	-	-	0.36	5.79
	8	15.8	1.23	-	0.10	0.57	15.1
	9	22.5	2.86	0.30	0.53	0.96	13.5
Castellón	10	3.8	3.73	-	0.22	-	15.7
	11	13.6	2.38	0.41	4.33	5.30	25.2
Control c.st.		13.3	1.89	-	0.57	1.14	22.5

Table III. Organochlorine pesticides content of lyopholised sediments. (ng/g fraction < 250 µm)

the different species studied. It should be noted that Parapenaeus longirostris has the lowest levels of DDTs and PCBs.

The level of organochlorine pesticides obtained in this study is, in general, higher than that found in the Ebro Delta (Spain) for Mytillus galloprovincialis, Venus gallina and Mullus barbatus (12).

Finally, apart from certain exceptions in molluscs, the similarity in chromatographic distribution of the PCBs in the sediments and in each organism over the whole area studied must be pointed out.

References

(1) OBIOLS SALVATS J. and L.A. PEIRO MIR (1980). - Heavy metals in marine sediments from the Delta del Ebro, Ves Journées Etud. Poll., Cagliari, C.I.E.S.M., 391.

(2) BERNHARD M. (1976). - Manual of methods in aquatic environment research. Part 3. Sampling and analyses of biological material F.A.O. Fisheries Technical Paper n° 158, U.N.E.P.,Rome.

(3) GASSIOT MATAS M., F. BROTO PUIG and L. DALMAU JUANOLA (1982). - Determinacion de contaminantes organoclorados (PCBs y DDTs) en sedimentos marinos mediante C.G. con detector de captura de electrones. Instituto Quimico de Sarria, Barcelona.

(4) U.N.E.P./ W.G. 91/5 (1983). - II réunion du Groupe de Travail de la Cooperation Scientifique et Technique. Athènes.

(5) VOUTSINOU-TALIADORI F. (1982). - Metal concentration in polluted and unpolluted Greek sediments: a comparative study, VI C.I.E.S.M./I.O.C./U.N.E.P., Workshop on pollution of the Mediterranean, Cannes, 237-243.

(6) BERNHARD M. (1982). - Levels of trace metals in the Mediterranean, VI C.I.E.S.M./I.O.C./-U.N.E.P., Workshop on pollution of the Mediterranean, Cannes, 237-243.

(7) VASILIKIOTIS G., K. FYTIANOS and A. ZOTOU (1982). - Heavy metals in marine organisms of the North Aegean Sea ; Greece, VI C.I.E.S.M./I.O.C./U.N.E.P.; Workhop on pollution of Mediterranean, Cannes, 303-306.

(8) GRIMANIS A.P., D. ZAFIROPOULOS, C. PAPADOPOULOU, T. ECONOMOU, M. VASSILAKI-GRIMANI (1982). - Trace elements in mytilus galloprovincialis from three Gulfs of Greece, VI C.I.E.S.M./I.O.C./-U.N.E.P., Workshop on pollution of the Mediterranean, Cannes, 319-322.

ORGANISM	SITUATION	DATE	INDIVIDUALS FOR SAMPLE	LENGTH MEAN cm	FRESH WEIGHT MEAN g	pp'DDE	pp'DDD	op'DDT	pp'DDT	PCB's
Mytilus galloprovincialis	Cullera	SEP	46	-	3.8	16.0	3.70	30.4	31.4	339
		DEC	78	-	3.3	21.6	6.06	4.58	22.2	103
	Valencia	SEP	63	-	1.3	19.9	1.76	2.97	24.6	322
	Castellón	JUN	24	-	4.5	24.9	0.99	-	23.8	848
		SEP	42	-	4.0	20.2	-	5.98	-	501
		DEC	32	-	3.0	33.8	0.22	2.92	1.21	322
Mullus surmuletus	Cullera	SEP	3	10	18.9	27.5	0.29	0.96	3.20	56.0
		DEC	7	12.1	32.7	70.4	4.94	25.5	34.4	191
	Valencia	JUN	4	14.4	50.8	22.7	0.18	-	3.44	163
		DEC	3	11.3	25.9	38.3	2.44	16.9	38.8	261
	Sagunto	JUN	1	14	46	156	0.17	-	2.32	506
		SEP	3	15.3	77.4	8.81	0.28	-	2.84	124
		DEC	2	14	52	21.6	-	5.91	5.13	178
	Castellon	JUN	3	15.4	58.1	41.2	0.56	-	7.85	222
		SEP	2	13.7	32.2	74.8	0.47	-	7.84	339
Mullus barbatus	Cullera	SEP	10	10.4	20.9	35.8	0.35	0.89	4.94	103
	Valencia	SEP	_	11.5	28	17.9	-	-	0.53	286
		DEC	3	12	31	21.1	1.56	16.2	28.3	195
	Sagunto	JUN	3	14.7	47.3	132	0.21	-	2.3	457
		SEP	4	17.5	93.6	28.2	0.39	-	3.27	99.8
		DEC	4	13.8	47.9	7.77	0.65	4.40	8.36	95.8
	Castellón	JUN	1	12.5	37.1	81.1	1.20	4.05	13.4	593
		SEP	3	15.2	45.9	193	0.87	-	9.72	638
		DEC	3	15.2	44.6	74.3	0.44	2.95	7.35	177
Parapenaeus longirostris	Valencia	JUN	16	9.5	9.3	2.20	-	0.15	0.24	11.2
	Castellón	SEP	5	16	21.7	3.28	-	-	-	12.9
		DEC	6	15	18.8	20.8	0.32	1.08	2.69	14.7
Tellina s p.	Valencia	JUN	65	-	2.5	18.0	0.37	0.56	4.42	51.2
		SEP	166	-	1.7	9.73	-	-	-	294
	Sagunto	JUN	34	-	3.5	15.7	0.50	-	6.96	142
	Castellón	JUN	36	-	2.5	12.5	-	-	-	140
		SEP	83	-	3.0	12.7	0.55	3.89	6.61	75.2
Venus gallina	Cullera	SEP	55	-	5.8	3.25	-	-	-	331
		DEC	114	-	4.1	5.28	1.13	-	3.38	70.8
	Valencia	SEP	86	-	3.5	6.09	-	-	-	526
	Sagunto	SEP	107	-	3.9	2.64	0.18	1.23	1.71	27.2
		DEC	85	-	3.6	11.5	1.34	-	3.49	58.4
	Castellón	SEP	62	-	2.5	14.9	0.24	-	5.49	580
		DEC	52	-	2.0	16.0	0.25	3.39	4.22	82

Table IV. Organochlorine pesticides content of organisms. (ng/g fresh weight)

(9) UYSAL H. and S. TUNCER (1982) . - Levels of heavy metals in some commercial food species in the Bay of Izmir (Turkey), VI C.I.E.S.M./I.O.C./U.N.E.P., Workshop on pollution of the Mediterranean, Cannes, 323-327.

(10) OBIOLS SALVAT J., M. GASSIOT MATAS and F. BROTO PUIG, Programa MEDPOL (1982) Instituto Quimico de Sarria, Barcelona. Personal communication.

(11) FOSSATO V. (1982). - Etude des hydrocarbures chlorés dans l'environnement de la Lagune de Venise, VI C.I.E.S.M./I.O.C./U.N.E.P., Workshop on pollution of the Mediterranean, Cannes, 465-468.

(12) MENDEZ J. M.T. BOMBOI, M. MARIÑO and M.G. MARIÑO (1983). - Hidrocarburos clorados DDTs y PCBs en organismos marinos del Delta del Ebro. Departamento de Fisico Quimica Sanitaria de la Escuela Nacional de Sanidad. Madrid, Personal communication.

ORGANOCHLORINE POLLUTION IN MALLARD AND RED CRESTED POCHARD DUCKLINGS FROM THE EBRO DELTA

by

Gustavo A. LLORENTE
Xavier RUIZ and Jacint NADAL
Catedra de Zoologia Vertebrats
Facultat de Biologia de la Universitat de Barcelona
Av. Diagonal, 645. 08071 Barcelona (Spain)

Abstract

Samples obtained from a pool of tissue and organ subsamples coming from 12 Anas platyrhynchos and 5 Netta rufina ducklings from the Ebro Delta, have been analysed in search of organochlorine pollutants. All specimens were about 1-3 days old. Pooled tissues were: flight muscle, liver, adipose tissue, kidneys, brain and heart. Results obtained show that, in all cases. the pollution levels of samples from Mallard ducklings are higher than those from Red Crested Pochard ducklings.

In spite of this, when the distribution pattern of percentual organochlorine load among the six tissues is considered, we observe a high degree of homogeneity, with the sole exception of medulla,in which the relative pollution load is rather higher in Netta rufina than in Anas platyrhynchos ducklings.

All results were compared to others obtained from the analysis of eggs and adults of the same species from the Ebro Delta. The distribution pattern is also compared with that shown for Bubulcus ibis nestlings. Differences are attributed to alternative modes of development (precocial versus altricial).

*
* *

Introduction

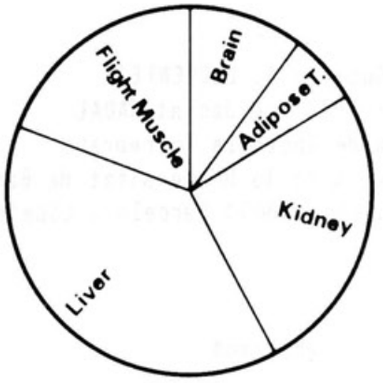
The influence of organochlorines in the first days after hatching may be very important because the accumulation can reach high relative levels mainly due to the concentration factor inherent to the small amount of lipids in the different tissues.

The Ebro Delta is an important agricultural area, and the use of organochlorine pesticides is generalized. If we take into account the important bird fauna that reside at some moment of their life cycle in this zone, the transcendence of the use of organochlorines for the survival of the chicks is clear.

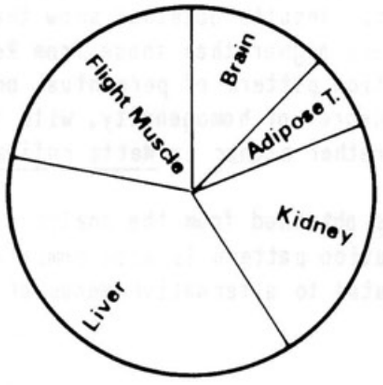
This paper has two goals, on one hand to evaluate the concentration levels of organochlorine pesticides in the duckling stage of the mallard (A. platyrhynchos) and of the Red Crested Pochard (Netta rufina), and on the other, to analyze the distribution of the pollutant load in the different tissues analyzed. This enables us to discriminate the possible influence of the model of development, in the distribution as an expression of the functional maturity of the tissues.

Material and methods

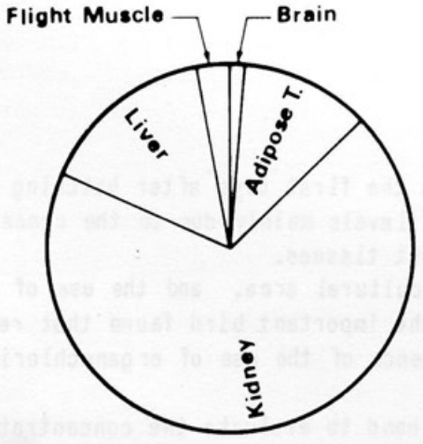
For the study of the impact on the duckling stage, 12 ducklings of A. platyrhynchos and 5 of Netta rufina, approximately 2-3 days old, were captured at the end of May 1982. After the death of the specimens, immediate dissection was undertaken so as to extract the subsamples of muscular, adipose, hepatic, renal, nervous and cardiac tissues. Once extracted, they were frozen at -18 ° C



Anas platyrhynchos



Netta rufina



Bubulcus ibis

Figure 1. - Percentual distribution of the organochlorine pollution load among the five tissues usually analyzed. Both duck species, which are precocial, are compared with a semi-altricial species, the Cattle Egret, inhabiting the same ecosystem. Differences are explained and justified in the text.

until analysis. The analytical procedure, following the suggestions and method of BERNHARD (1976), was based first of all on the preparation of a pool which was the direct average for each tissue. After having obtained the sample, the extraction and analysis of the organochlorine residues was carried out using n-hexane as dissolvent, following the methodology of HOLDEN and MARSDEN (1969) with modifications as specified in LLORENTE et al., (1983). The gas chromatography was done with a Varian Aerograph series 2,700 apparatus equipped with an ECD of Ni 63; the column was 2 m long and 1/4 inch in diameter and filled with 4 % DC.200 - 6 % QF. -1 on chromosorb WAWDMCS of 80-100 mesh. The carrier gas was N2 of high purity with a flow of 30-40 ml.x min-1

The samples were analyzed for the following organochlorinated compounds; hexachloro cyclohexanes (lindane), cyclodienes (heptachlor, heptachlor epoxide, aldrin and dieldrin), DDTs (pp'DDE, pp'TDE, pp'DDT and op'DDT) and PCBs (aroclor 1254 and aroclor 1260). The minimum level of detection used was 10 ppb for cyclodienes and pp'DDE; 20 ppb for pp'TDE, op'DDT and pp'DDT, and 80 ppb for PCBs.

When PCBs were detected in a sample,it was submitted to an alcaline attack in order to transform pp'DDT into pp'DDE, op'DDT into op'DDE and pp'TDE into pp'DDMU. Also the HCHs and cyclodienes are not stable under this attack. Because of this, following the indications of WATTS (1980), pesticide quantification was made before and after the attack. This enables quantification without overlapping either group of compounds (RUIZ , 1982; AGUILAR, 1983; LLORENTE et al., 1983; LLORENTE, 1984).

PCB quantification was made peak by peak, paying attention to the isomeric distribution and weight percentage of PCB represented in each peak. This is following the Webb/McCall method, which is proven to be one of the best by CHAU & SAMPSON (1975).

Periodically a series of blanks were prepared so as to ensure that there was no pollution attributable to the system. The values obtained are not corrected for the recovery, which is near 85 % thus being considered complete by WATTS (1980). The precision of the method has been evaluated through standard deviation calculations referred to an internal standard. Values obtained show a relative standard deviation of less than 1.5 %.

Results

The results obtained for different analyses carried out are offered in tables I and II, where the levels of the different organochlorines in the tissues analyzed are specified. These levels correspond to both ppm in fat basis and ppm in fresh weight basis. All the PCB levels follow the aroclor 1260 pattern.

In figure 1 the percentual contribution to the total pollutant load of each of the five most commonly analyzed tissues in this type of study is specified, enabling comparison with other publications. Figure 2 shows the percentual contribution of all the tissues analyzed in our study.

Discussion

The results obtained reveal that the concentration levels of organochlorines in ducklings are relatively and globally higher than those found in eggs or adults of the same species (LLORENTE et al., 1982; 1983; LLORENTE, 1984). This is attributable to the fact that all the residues contained in the egg yolk have been retained in the scarce lipid material of the duckling at hatching, thus leading to a considerable effect of concentration, as can be observed in the tables of ppm in fresh weight (tables I and II).

The accumulation levels shown by the duckling of Mallard are substantially higher than those of Red Crested Pochard. This coincides with the levels found in both adults and eggs of both species, and can be considered a reflection of the different trophic level exploited by those ducks. However, when considering the percentual accumulations in the five usual tissues (figure 1), it can be seen

Table I

	flight muscle	liver	kidney	Adipose tissue	heart	brain
Lindane	0.25 (0.004)	0.40 (0.04)	0.08 (0.002)	0.06 (0.03)	ND	0.07 (0.003)
Heptachlor	ND	0.14 (0.01)	ND	0.04 (0.02)	ND	0.04 (0.002)
Hept. Epoxyde	0.50 (0.008)	1.37 (0.13)	1.02 (0.03)	0.11 (0.06)	0.70 (0.01)	0.28 (0.01)
Aldrin	ND	ND	ND	ND	ND	ND
Dieldrin	0.79 (0.01)	1.32 (0.13)	5.92 (0.17)	0.08 (0.04)	14.2 (0.28)	0.44 (0.02)
Cyclodienes	1.30 (0.02)	2.82 (0.31)	6.95 (0.2)	0.23 (0.15)	14.9 (0.29)	0.77 (0.03)
pp'DDE	90.96 (1.43)	180.28 (17.64)	103.35 (2.95)	21.08 (10.78)	183.74 (3.62)	42.83 (2.42)
pp'TDE	2.27 (0.03)	14.40 (1.41)	24.95 (0.71)	0.60 (0.31)	4.48 (0.09)	4.66 (0.26)
op' DDT	ND	ND	ND	0.13 (0.07)	ND	0.09 (0.005)
pp'DDT	0.96 (0.01)	1.01 (0.1)	ND	2.08 (1.06)	2.51 (0.05)	1.77 (0.1)
DDTS	94.16 (1.47)	195.63 (19.06)	128.3 (3.66)	23.88 (12.22)	190.73 (3.76)	49.35 (2.78)
PCBS	8.79 (0.14)	15.60 (1.52)	4.57 (0.13)	9.15 (4.68)	23.26 (0.46)	3.77 (0.21)
Total in PPMS	104.53 (1.64)	214.45 (21.03)	139.90 (4.00)	33.32 (17.07)	228.89 (4.51)	53.96 (3.05)
% lipid extraction	1.57	9.78	2.85	51.14	1.97	5.65

Organochlorine compound levels in ppm. Figures given are direct averages obtained from the composite sample made up by pooling the tissue subsamples coming from 12 Anas platyrhynchos ducklings. Ppm are expressed on a fat content basis and on a fresh weight basis (in brackets). ND means not detected.

that the distribution pattern of residues is very similar in both species. The liver is the organ with the highest relative degree of pollution, followed by kidney, muscle, brain and fat tissue. It is logical for the liver to have the highest concentration, being, as it is, the organ responsible for the absorption and metabolization of the last remnants of the vitellum, where a considerable amount of organochlorines have accumulated by differential partitioning while the different structural components that make up the embryo's body are being formed. The existence of this differential partitioning and its relevance in the evaluation of residue levels in eggs has already been proved by PEAKALL and GILLMAN (1979).

While the liver metabolizes and transforms the vitelline lipids into lipids ready for the consumption of the different tissues of the embryo. these are released into the bloodstream carrying with them the different pollutants. Therefore it also makes sense for the kidney, the tissue that expresses the blood lipid and lipophilic pollutant levels (WALKER, 1975). to have the second highest percentage of accumulation.

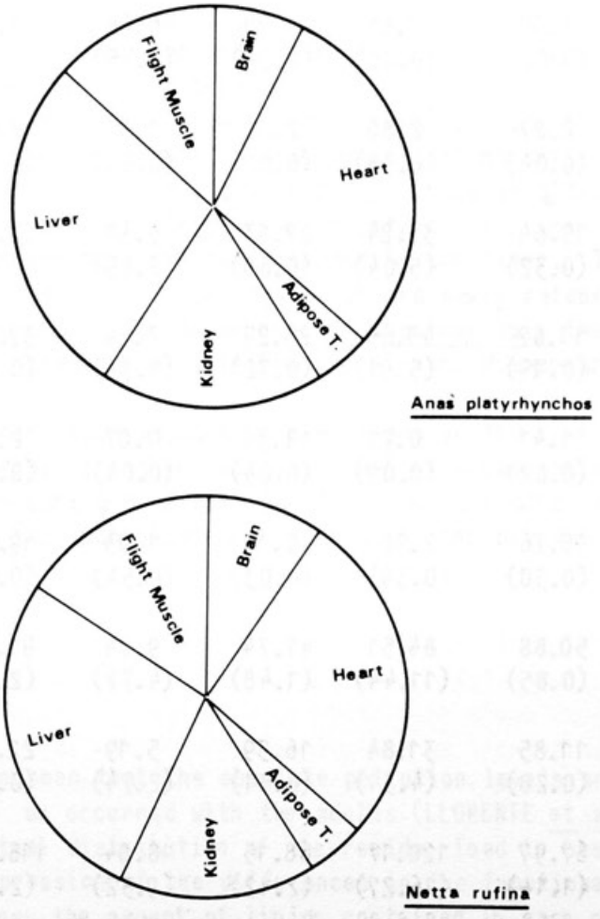


Figure 2. - Percentual distribution of the organochlorine pollution load among six tissues in Anas platyrhynchos and Netta rufina ducklings.

A priori, the residue levels in the fat tissue, surprise us by being lowest among the tissues studied. This is to a certain extent due to a dilution effect, because the quantity of lipidic

Table II

	flight muscle	liver	kidney	Adipose tissue	heart	brain
Lindane	2.97 (0.05)	1.02 (0.14)	1.47 (0.04)	2.51 (1.32)	1.91 (0.04)	0.13 (0.005)
Heptachlor	0.17 (0.003)	0.23 (0.03)	0.38 (0.01)	0.07 (0.03)	0.28 (0.006)	0.16 (0.006)
Hept. Epoxyde	0.62 (0.01)	1.87 (0.25)	0.69 (0.02)	0.45 (0.24)	1.02 (0.02)	0.83 (0.03)
Aldrin	0.09 (0.001)	0.09 (0.01)	0.07 (0.002)	0.12 (0.06)	0.03 (*)	0.02 (*)
Dieldrin	1.39 (0.02)	0.61 (0.08)	1.21 (0.04)	0.66 (0.35)	1.36 (0.03)	0.30 (0.01)
Cyclodienes	2.27 (0.04)	2.80 (0.38)	2.35 (0.07)	1.3 (0.69)	2.69 (0.06)	1.31 (0.05)
pp'DDE	19.54 (0.32)	37.24 (5.04)	22.13 (0.69)	5.40 (2.85)	32.08 (0.73)	11.48 (0.47)
pp'TDE	11.67 (0.19)	43.65 (5.91)	23.27 (0.72)	2.54 (1.34)	37.55 (0.86)	16.12 (0.67)
op'DDT	1.41 (0.02)	0.71 (0.09)	1.33 (0.04)	0.07 (0.04)	2.32 (0.05)	0.46 (0.02)
pp'DDT	18.26 (0.30)	2.90 (0.39)	0.1 (0.03)	1.03 (0.54)	19.62 (0.45)	0.74 (0.03)
DDTS	50.88 (0.85)	84.51 (11.44)	47.74 (1.48)	9.04 (4.77)	91.57 (2.1)	28.80 (1.19)
PCBS	11.85 (0.20)	31.84 (4.31)	16.59 (0.51)	5.19 (2.74)	22.66 (0.52)	9.75 (0.40)
Total in PPMS	67.97 (1.14)	120.17 (16.27)	68.15 (2.12)	18.04 (9.52)	118.83 (2.72)	39.99 (1.64)
% Lipid extraction	1.66	13.54	3.11	52.75	2.29	4.15

Organochlorine compound levels in ppm. Figures given are direct averages obtained from the composite sample made up by pooling the tissue subsamples coming from 5 Netta rufina ducklings. Ppm are expressed on a fat content basis and on a fresh weight basis (in brackets). ND means not detected. * means less than 0.01 ppm.

material is much higher than in the other tissues, (see tables). If we consider these levels in fresh weight, the situation is inverted: the residue levels increase in the adipose tissue. taking second place behind the liver.

The other two tissues, muscle and brain, have pollution levels in proportion to the quantity of lipidic substrates metabolized in their formation and functioning. There is an important difference between both species as far as the accumulation percentage in the brain is considered, since it is higher in the case of N. rufina. This can be explained by the hypothesis established by LLORENTE et al. (1983) for adult specimens, in which an identical situation is observed, stating that the diving habits of the species may be the determinant cause of this higher percentual accumulation. It is important to note that this hypothesis is valid in a general context, because individually it is not necessarily the case. However, it is revealing that already in the initial stages of life the difference is observable.

If, when comparing both species, we add a sixth tissue, the heart muscle, the identity of the distribution pattern of the accumulation levels in the different tissues is maintained, and the heart turns out to be the most polluted organ (fig. 2). The most plausible explanation is that the heart tissue, being functional from the third day of incubation, has received during the whole embryonary and postembryonary life a continued supply of metabolic substrates.

When comparing the distribution of the residue load in these ducklings, which are precocial, with the results of RUIZ et al. (1984) referring to a semialtricial bird (Bubulcus ibis), important differences are observed (figure 1). The variations in the distribution pattern can be attributed to the precocial or altricial condition. In altricial birds, the relative accumulation in muscle is lower than that of the remaining tissues, except for the brain. RICKLEFS (1979) has shown that in precocial birds, the muscle tissue has a much greater functional maturity, being ready to carry out the activities necessary for survival in the environment, while in altricial birds it is barelly active at all. The relative quantity of circulating pollutants is higher in Bubulcus ibis, than in the Anatidae in which most of the residues appear retained in the liver. In this case, the explanation is probably related to the age of the ducklings which are newly hatched, while those studied of the Cattle Egret were 10-14 days old. This means that in Bubulcus ibis the concentration effect due to the incorporation of the last remnants of vitellum had already disappeared, while in the ducklings is still important.

Also, part of the pollutants which would have been found in the liver, have probably gone into the adipose tissue, which reveals a higher relative level of accumulation than in the ducklings. Thus, the circulating residues coming from both the liver and the adipose tissue have an increased relative importance, because the mobilization of lipids in the bloodstream is high in period of active growth.

Conclusions

In conclusion, it can be seen that the absolute pollution levels are related to the trophic niche occupied by each species, as occurred with the adults (LLORENTE et al., 1983; LLORENTE, 1984). On the other hand, the percentual distribution of the residue load is basically conditioned by the model of development, as an expression of the differences in the functional maturity of the tissues, to the extent that it determines the amount of lipids contained in each one. Other factors to be taken into account are age, related to the release of liver lipid reserves from the vitellum, and, to a certain degree, trophic behaviour.

One last consideration is that it is not recommendable to use this kind of sample to estimate the impact of the organochlorines in a given ecosystem, because the concentration effect caused by the small amount of lipids contained in the tissues of chicks distorts reality.

References

AGUILAR A. (1983). - Organochlorine pollution in Sperm Whales, Physeter macrocephalus, from the temperate waters of the Eastern North Atlantic. Marine Pollution Bulletin 14(9): 342-352.

BERNHARD M. (1976). - F.A.O. Fish. Tech. Pap. (158): 124 p. Manual of Methods in aquatic environment research. Part. 3. Sampling and analysis of biological material.

CHAU A.S.Y. and R.C.J. SAMPSON (1975). - Electron capture gas chromatographic methodology for the quantitation of polychlorinated biphenyls. Survey and compromise. Environmental Letters, 8: 89-101.

HOLDEN A.V. and A. MARSDEN (1969). - Single-stage clean-up of animal tissue extracts for organochlorine residue analysis. Journal of chromatography, 44, 481-492.

LLORENTE G.A. (1984). - Contribucion al conocimiento de la biologia y ecologia de cuatro especies de anatidas en el delta del Ebro. Tesis Doctoral. Universidad de Barcelona.

LLORENTE G.A., X. RUIZ and J. NADAL (1982). - Compartimentacion de los plaguicidas organoclorados en tres especies de anatidas procedentes del delta del Ebro (Tarragona). Actas 1er Congreso Iberoamericano de Toxicologia (Seccion Ecotoxicologia). Sevilla. 691-702.

LLORENTE G.A., X. RUIZ and J. NADAL (1983). - Incidence des pesticides organochlorés sur trois espèces d'anatides du delta de l'Ebre (Tarragona, Espagne). VIIes Journées d'Etudes sur les Pollutions marines en Mediterranée. Cannes. 479-486.

PEAKALL D.B. and A.P. GILLMAN (1979). - Limitations of expressing organochlorine levels in eggs on a lipid-weight basis. Bulletin of Environmental Contamination and Toxicology. Vol. 23: 287-290.

RICKLEFS R.E. (1979). - Patterns of growth in birds. V.A comparative study of development in the Starling, Common Tern and Japanese Quail. The Auk. 96: 10-30.

RUIZ X. (1982). - Contribucion al conocimiento de la biologia y ecologia de Bubulcus ibis ibis (L) 1,758 en el delta del Ebro (Tarragona). Tesis Doctoral Universidad de Barcelona.

RUIZ X., G.A. LLORENTE and J. NADAL (1984). - Distribution pattern of organochlorine compounds in five tissues of Bubulcus ibis nestlings (Aves, Ardeidae) from the Ebro Delta, Northeast Spain. Vie et Milieu, vol. 34 (1): 21-26.

WALKER C.H. (1975). - Variations in the intake and elimination of pollutants. In Organochlorine Insecticides: Persistent Organic Pollutants. Ed. by F. Moriarty. Academic Press. London.

WATTS R. (1980). - (Editor). Analysis of pesticide residues in human and environmental samples. A compilation of methods selected for use in pesticide monitoring programs. United States Environmental Protection Agency.

TENEUR EN ORGANOCHLORES DU CANARD COLVERT DANS LE DELTA DE L'EBRE

par

Gustavo A. LLORENTE
Xavier RUIZ et Jacint NADAL
Catedra de Zoologia Vertebrats
Facultat de Biologia
Universitat de Barcelona
Av. Diagonal, 645. Barcelona. 08071 (Espagne)

Abstract

We have studied the organochlorine pollution levels in tissues of mallards from the Ebro Delta during four autumn-winter seasons (1978-79 to 1981-82).

Total number of specimens caught and analysed were 41. Each specimen was dissected in order to take subsamples of the following tissues : flight muscle, liver, adipose tissue, kidney and medulla.

Obtained results are discussed with relation to the trophic niche occupied by this species in Ebro Delta and compared to others obtained on different duck species from the same ecosystem. Also we offer data on pattern distribution of organochlorine load among considered tissues.

*
* *

Introduction

Dans de précédents travaux, on a mis en évidence le besoin d'étudier les différents facteurs qui introduisent une variabilité particulière de l'échantillon, tels que : le rôle joué par un tissu déterminé à un moment donné du cycle biologique, la phénologie d'une espèce dans un système concret, le différent niveau trophique, la condition physiologique de l'exemplaire au moment même d'être capturé ou la différente affinité des divers groupes structuraux pour les lipides auxquels ils sont associés (RUIZ, 1982 : RUIZ et al., 1984 et sous presse ; LLORENTE et al. 1982, 1983 ; LLORENTE, 1984: SCHNEIDER, 1982).

Dans la présente contribution, on expose et discute les résultats concernant la compartimentation et les niveaux des composés organochlorés dans cinq tissus de Anas platyrhynchos capturés dans le delta de l'Ebre durant quatre saisons automno-hivernales consécutives (1978-1979 ; 1979-1980 ; 1980-1981 ; 1981-1982). Ce travail a été préparé afin de mettre en contraste les hypothèses émises à l'occasion d'une contribution précédente, concernant trois espèces d'Anatides du delta de l'Ebre (LLORENTE et al. 1983).

Matériel et méthodes

Le tableau I présente les données morphomètriques correspondant aux exemplaires utilisés pour la réalisation des analyses.

Número	L.C.	L.A.	L.T.	L.TOT.	POIDS	SEXE
1978-79						
1	52,55	258	43,5	525	1.000	♀
2	--	---	--	574	1.100	♂
3	60	273	45,5	567	1.000	♂
4	53	264	46	546	1.050	♀
5	--	--	--	535	905	♀
6	54	244	51	512	905	♀
7	--	---	--	512	835	♀
8	--	---	--	539	1.000	♂
9	55,5	---	--	546	965	♀
10	51,5	264	51,5	534	1.100	♀
11	--	---	--	545	1.100	♂
12	--	---	--	539	940	♀
13	--	---	--	538	1.050	♀
14	--	---	--	540	1.050	♂
1979-80						
1	53	260	43	530	995	♀
2	53,5	249	50	515	950	♀
3	51.5	266	43,5	537	1.040	♂
4	61	275	46,5	570	1.050	♂
1980-81						
1	53,5	233	43,5	521	699	♀
2	55,5	255	46	562	767	♂
3	54	271	46	572	889	♂
4	59,5	265	46,5	576	892	♂
5	57	272	45,5	578	884	♂
6	59	272	43,5	561	970,68	♂
7	54,5	282	44,5	570	1.062,10	♂
8	59	267	45	577	1.200	♂
9	57	261	43,5	555	1.065,80	♂
10	53	257	44,5	515	1.118,32	♀
11	56	264	44	548,5	1.200	♀
12	55,5	231	44,5	553	947	♂
13	57	276	46,5	587	1.111	♂
14	58,5	279	47	583	---	♂
15	59	273	45,5	564	1.191	♂
16	51	265	42,5	527	980	♀
17	54	260	42	549	1.050	♀
1981-82						
1	46,75	247	41	529	986,63	♀
2	55,8	267	44,1	576	1.110,86	♂
3	50,9	261	41,9	542	1.023.34	♀
4	52,5	261	42,6	550	1.145,93	♀
5	56,9	275	45,15	555	1.134,69	♀

Tableau I

Mesures morphométriques des exemplaires analysés de canards colverts (Anas plathyrhynchos) pour chaque saison.

Tous ces exemplaires procèdent de l'activité cynégétique qui a lieu pendant la saison automno-hivernale dans le delta de l'Ebre. Après leur capture les exemplaires étaient transportés au laboratoire où l'on procédait immédiatement à leur dissection afin d'obtenir des sous-échantillons des tissus suivants : muscle de vol, foie, rein, tissu adipeux et moelle épinière.

Les sous-échantillons ont été congelés à -18°C jusqu'au moment de leur analyse à laquelle nous avons procédé selon la méthodologie de HOLDEN et MARSDEN (1969) avec les modifications indiquées à LLORENTE et al. (1983). Une fois les extraits purifiés, ils ont été injectés dans un chromatographe de gaz Varian Aerograph Série 2700 avec un E.C.D. de Ni 63 à colonne de 2 m. de longueur et 1/4 de pouce de diamètre remplie de 4 % de DC 200 et de 6 % de QF1 sur chromosorb WAWDMCS de 50-100 mailles.

Le N2 très pur a été employé comme gaz porteur à un flux de 30-40 ml/mn. Tous les échantillons ont été analysés pour les composés suivants : HCHs (lindane), cyclodiènes (heptachlor, heptachlor époxide, aldrine et dieldrine) DDTs (pp'DDE, pp'TDE, pp'DDT et op'DDT) et PCBs (aroclor 1254 et

aroclor 1260). Le niveau minimum de détection a été de 10 ppb. Sur les tableaux N.D. signifie non détecté. Quand les PCBs ont été détectés dans un échantillon, on a procédé à déshalogéner les DDTs au moyen d'une attaque alcaline qui transforme le pp'DDT en pp'DDE, le op'DDT en op'DDE et le pp'TDE en pp'DDMU. Comme les composés antérieurs les HCHs et les Cyclodiènes ne sont pas stables à cette attaque, la quantification a été réalisée avant et après l'attaque alcaline en suivant les indications de WATTS (1980), ce qui permet de quantifier les différents composés sans imbrication entre les deux groupes de composés (RUIZ, 1982 ; AGUILAR, 1983 ; LLORENTE et al. 1983 ; LLORENTE, 1984).

Les PCBs ont été quantifiés pic par pic en tenant compte de la distribution isomérique et du pourcentage de PCB dans chaque pic. On a suivi la méthode WEBB/McCALL qui est considérée par CHAU & SAMPSON (1975), comme une des meilleures.

Périodiquement une série de blancs ont été préparés pour s'assurer qu'il n'y aurait pas de contamination imputable au système. Les valeurs obtenues n'ont pas été corrigées pour la récupération proche de 85 % et qui est considérée comme complète par WATTS (1980). La précision de la méthode a été évaluée par le calcul de la déviation standard rapportée à un standard interne. Les valeur obtenues montrent une déviation standard relative, inférieure à 1,5 %.

	MUSCLE DE VOL	FOIE	REIN	TISSU ADIPEUX	MOELLE EPINIERE
LINDANE	0.02 (90.91)	0.07 (81.82)	0.01 (90.91)	TRACES (36.36)	N.D.
HEPTACHLOR	0.01 (18.18)	0.08 (54.55)	0.0 (36.36)	TRACES (18.18)	N.D.
ALDRINE	0.14 (63.64)	0.38 (54.55)	0.24 (72.73)	0.06 (27.27)	N.D.
HEPTACHLOR EPOXIDE	0.10 (45.45)	0.32 (54.55)	0.14 (45.45)	0.10 (45.45)	N.D.
pp' DDE	3.75 (100)	3.29 (100)	2.56 (100)	5.64 (100)	0.31 (100)
DIELDRINE	0.07 (63.64)	0.19 (63.64)	0.12 (63.64)	0.12 (54.55)	N.D.
op'DDT	0.02 (9.09)	0.05 (18.18)	0.05 (9.09)	0.06 (9.09)	N.D.
pp'TDE	0.26 (81.82)	0.26 (81.82)	0.22 (72.73)	0.21 (90.91)	N.D.
pp'DDT	0.10 (54.55)	0.16 (45.45)	0.05 (36.36)	0.40 (72.73)	N.D.
PCB 1254	N.D.	N.D.	N.D.	N.D.	N.D.
PCB 1260	N.D.	N.D.	N.D.	N.D.	N.D.

Tableau II

Moyennes en ppm (base lipidique) des différents composés organochlorés dans les tissus analysés. N.D. signifie non détecté. Entre parenthèses, pourcentage de présence. On signale avec traces les moyennes inférieures à 0,01 ppm.
Année 1978-79

Résultats

Les résultats obtenus par les différentes analyses réalisées se trouvent sur les tableaux II à V ; toutes les valeurs sont données en rapport avec la base lipidique. Sur le tableau VI apparaissent

les valeurs moyennes d'accumulation de résidus de composés organochlorés dans les cinq tissus, pour les quatre saisons étudiées.

La figure 1 présente les distributions en pourcentage de la charge de polluants dans les différents tissus pour chacune des saisons échantillonnées.

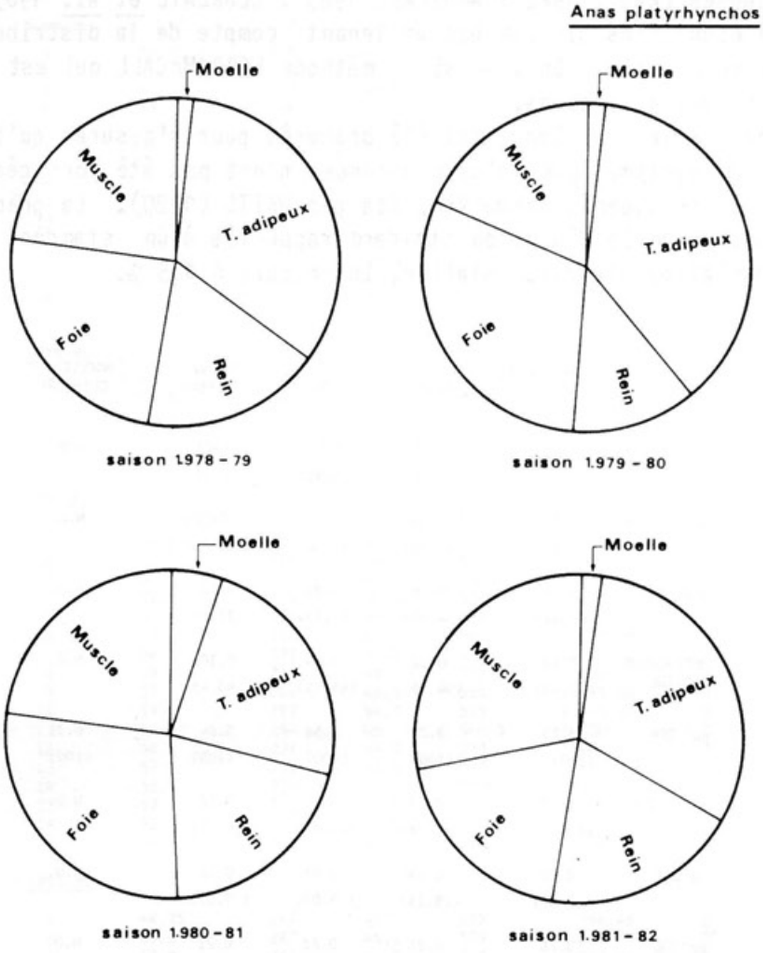


Figure 1. - Distribution en pourcentages de la charge de polluants dans les différents tissus, pour les quatre saisons étudiées.

Discussion

Pour interpréter les résultats obtenus et réaliser les différentes comparaisons, nous avons effectué des preuves statistiques afin d'homogénéiser le mieux possible les échantillons. Nous avons ainsi évité l'introduction de facteurs de variabilité étrangers à ceux qui sont déjà connus.

Tout d'abord, durant chaque saison et pour chaque espèce, un test du U de Mann-Withney a été réalisé, opposant les deux sexes. La différence entre les sexes pour chaque espèce et pour chaque tissu n'a pas été significative, c'est pourquoi cette variable n'a pas été considérée au moment d'établir d'autres comparaisons, et par conséquent on a groupé les individus des deux sexes.

Nous avons ensuite examiné la possibilité d'une différence dans les pourcentages d'accumulation que les divers tissus présentent entre les saisons, l'échantillon automnal d'une part contre l'échantillon hivernal de l'autre, car les études faites par RUIZ (1982) dans le delta de l'Ebre , suivant

un cycle annuel de niveaux de pollution par ces composés dans les tissus de héron garde-boeufs, ont fait soupçonner l'existence de différences significatives dans la quantité de résidus accumulés par l'un ou l'autre tissu selon sa dynamique physiologique. Les résultats obtenus démontrent que chez le Canard colvert les différences ne sont pas significatives ni entre les mois d'octobre et de novembre, ni entre ces deux mois ensemble et la saison hivernale, ce qui permet de réunir les échantillons procédant de n'importe quel sexe ou de n'importe quelle époque en un tout, afin d'établir des comparaisons à d'autres niveaux.

	MUSCLE DE VOL	FOIE	REIN	TISSU ADIPEUX	MOELLE EPINIERE
LINDANE	0.02 (80.00)	0.22 (80.00)	0.06 (50.00)	TRACES (50.00)	TRACES (100)
HEPTACHLOR	0.04 (40.00)	0.51 (80.00)	0.01 (25.00)	TRACES (25.00)	TRACES (100)
ALDRINE	0.01 (40.00)	0.66 (80.00)	0.03 (75.00)	TRACES (50.00)	N.D.
HEPTACHLOR EPOXIDE	0.01 (20.00)	0.48 (60.00)	0.04 (25.00)	0.01 (50.00)	N.D.
pp' DDE	2.34 (100)	2.35 (100)	1.66 (100)	4.02 (100)	0.19 (100)
DIELDRINE	0.02 (20.00)	0.24 (80.00)	0.04 (50.00)	TRACES (25.00)	N.D.
op'DDT	0.03 (20.00)	N.D.	N.D.	0.05 (25.00)	N.D.
pp'TDE	0.11 (100)	0.16 (100)	0.12 (75.00)	0.35 (100)	0.04 (100)
pp'DDT	0.08 (40.00)	0.11 (60.00)	0.07 (50.00)	0.74 (25.00)	N.D.
PCB 1254	N.D.	N.D.	N.D.	N.D.	N.D.
PCB 1260	0.62 (20.00)	0.36 (20.00)	N.D.	1.37 (25.00)	N.D.

Tableau III

Moyennes en ppm (base lipidique) des différents composés organochlorés dans les tissus analysés. N.D. signifie non détecté. Entre parenthèses, pourcentage de présence. On signale avec traces les moyennes inférieures à 0.01 ppm.
Année 1979-1980

Avec les échantillons ainsi groupés on a procédé à la comparaison entre les années, pour chaque tissu et chaque groupe structural. Les résultats témoignent que les niveaux de pollution pour Anas platyrhynchos n'ont pas de différences significatives permettant de signaler aucune direction dans les changements observés.

D'autre part, les modèles de distribution en pourcentage de la charge de polluants totaux dans les cinq tissus étudiés se révèlent très différents pour chacune des années d'échantillonnage, ce qui ne permet pas de tirer des conclusions unitaires cohérentes et généralisables à partir de ces résultats (Figure 1).

Ce résultat apparemment négatif est en réalité d'une importance capitale quand on prépare n'importe quelle étude qui implique l'affirmation ou la déduction de n'importe quel genre de conclusion grâce aux niveaux de pollution de sous-échantillons d'organes ou de tissus. Il faut souligner que les études de RUIZ (1982) et RUIZ et al. (sous presse) démontraient l'existence de fluctuations importantes dans les valeurs de concentration (ppm) des polluants organochlorés dans des sous-

échantillons de tissus tout le long d'une année, dont on tirait la conclusion qu'il était nécessaire de fixer l'époque de capture pour que les échantillons effectués aient le même rôle dans la dynamique physiologique déclenchée par les événements normaux qui se produisent dans le cycle vital des populations. Dans cette étude, on démontre que même ceci n'est pas suffisant, car sous des conditions normales, dans la nature, les facteurs externes peuvent conditionner des déphasages métaboliques à valeur adaptative qui changent d'une façon importante la dynamique physiologique d'une population à une certaine époque, ce qui signifie que l'étude doit se réaliser après avoir vérifié que cet état physiologique est homogène pour tous les échantillons qui sont à comparer.

	MUSCLE DE VOL	FOIE	REIN	TISSU ADIPEUX	MOELLE EPINIERE
LINDANE	0.05 (100)	0.03 (100)	0.02 (92.86)	0.03 (92.31)	0.05 (100)
HEPTACHLOR	0.01 (46.15)	0.08 (85.71)	0.04 (78.57)	0.01 (53.85)	N.D.
ALDRINE	0.19 (61.54)	0.12 (78.57)	0.03 (57.14)	0.01 (69.23)	N.D.
HEPTACHLOR EPOXIDE	0.04 (23.08)	0.15 (64.29)	0.02 (35.71)	0.02 (30.77)	N.D.
pp'DDE	2.90 (100)	3.41 (100)	2.57 (100)	2.94 (100)	0.80 (100)
DIELDRINE	0.03 (38.46)	0.13 (57.14)	0.12 (42.86)	TRACES (30.77)	TRACES (33.33)
op'DDT	0.01 (15.38)	0.02 (14.29)	N.D.	0.02 (15.38)	N.D.
pp'TDE	0.36 (92.31)	0.22 (71.43)	0.27 (64.29)	0.17 (69.23)	0.06 (66.67)
pp'DDT	0.21 (69.23)	0.15 (79.57)	0.41 (50.00)	0.75 (69.23)	N.D.
PCB 1254	N.D.	N.D.	N.D.	N.D.	N.D.
PCB 1260	0.58 (38.46)	1.06 (28.57)	0.58 (21.43)	0.59 (38.46)	N.D.

Tableau IV

Moyennes en ppm (base lipidique) des différents composés organochlorés dans les tissus analysés. N.D. signifie non détecté. Entre parenthèses, pourcentage de présence. On signale avec traces les moyennes inférieures à 0.01 ppm.

Année 1980-1981

Ainsi les conclusions tirées dans le travail chez des Anatides du delta de l'Ebre (LLORENTE, et al. 1983) qui se basaient sur les données obtenues pour une seule saison d'échantillonnage peuvent être considérées substantiellement valables mais non en ce qui concerne le moment précis de leur capture dans l'espace et dans le temps, ces conclusions ne pouvant pas se généraliser à d'autres moments du cycle ou à un échantillon de composition différente, sauf en ce qui concerne les valeurs les plus générales et leur rapport avec le niveau trophique exploité par les différentes espèces.

En définitive, le degré de complication pour l'obtention d'échantillons homogènes dans ce but est si élevé qu'il n'est pas rentable, ni du point de vue scientifique ni du point de vue économique: ainsi que le choix de sous-échantillons de tissus pour établir des comparaisons entre des espèces ou des systèmes ; il est donc préférable de travailler avec des échantillons unitaires, où la quantité de polluants analysée correspond à la quantité absolue de polluants. Ainsi, lorsqu'il s'agit d'éta-

blir des comparaisons entre les populations d'oiseaux, il est beaucoup plus efficace de les établir sur le degré de pollution que les oeufs présentent, car ceux-ci sont à la fois un système presque fermé et unique.

A tout cela on peut ajouter le fait que les différents groupes structuraux qui se réunissent dans les composés organochlorés ont un degré différent d'affinité pour les diverses sortes de lipides, tel que SCHNEIDER (1982), RUIZ, et al. (1984), l'ont démontré récemment, en travaillant respectivement avec des poissons et des oiseaux.

	MUSCLE DE VOL	FOIE	REIN	TISSU ADIPEUX	MOELLE EPINIERE
LINDANE	0.03 (80.00)	0.03 (80.00)	0.03 (80.00)	0.03 (60.00)	0.01 (100)
HEPTACHLOR	0.03 (40.00)	0.01 (40.00)	TRACES (60.00)	TRACES (20.00)	N.D.
ALDRINE	0.01 (80.00)	0.03 (80.00)	0.03 (40.00)	0.01 (60.00)	TRACES (100)
HEPTACHLOR EPOXIDE	TRACES (20.00)	0.01 (20.00)	TRACES (20.00)	TRACES (40.00)	N.D.
pp'DDE	1.31 (100)	1.69 (100)	1.08 (100)	2.23 (100)	0.27 (100)
DIELDRINE	0.05 (60.00)	0.16 (80.00)	0.09 (20.00)	0.11 (60.00)	N.D.
op'DDT	0.03 (20.00)	0.19 (40.00)	0.11 (40.00)	0.33 (40.00)	N.D.
pp'TDE	0.14 (80.00)	0.21 (60.00)	0.11 (80.00)	0.30 (100)	N.D.
pp'DDT	0.18 (80.00)	0.32 (80.00)	0.18 (80.00)	0.39 (100)	N.D.
PCB 1254	N.D.	N.D.	N.D.	N.D.	N.D.
PCB 1260	2.07 (20.00)	0.08 (20.00)	1.00 (20.00)	0.90 (40.00)	N.D.

Tableau V

Moyennes en ppm (base lipidique) des différents composés organochlorés dans les tissus analysés. N.D. signifie non détecté. Entre parenthèses, pourcentage de présence. On signale avec traces les moyennes inférieures à 0.01 ppm.
Année 1981-1982

Il ne faut pas oublier que la plupart des travaux publiés jusqu'à présent sur les niveaux de pollution par ces composés, outre le fait de ne pas réaliser des preuves statistiques, ne tiennent pas compte des considérations que l'on vient d'expliciter ; c'est pourquoi l'interprétation des résultats obtenus en laboratoire sur la diminution et l'augmentation des polluants dans les tissus de la faune vertébrée quant au temps sont très discutables, car on ne peut pas porter un jugement correct d'après des analyses, si l'on ignore les facteurs de variabilité propres à l'échantillon (tissu) ou à l'espèce et à sa phénologie.

Malgré ce que l'on vient de dire, certaines règles générales peuvent être établies. Ainsi, il faut souligner l'absence absolue d'aroclor 1254 chez cette espèce qui peut être considérée comme sédentaire pour le delta de l'Ebre, par rapport avec ce qui se passe chez les espèces migratoires, qui hivernent dans le delta chez lesquelles, bien que la quantité d'aroclor 1254 soit moindre que celle de l'aroclor 1260, est une partie importante du total des polychlorobiphényles (LLORENTE, 1984). Ces résultats sont à mettre en rapport avec ceux obtenus par RUIZ (1982) dans les tissus du Héron garde-boeufs (Bubulcus ibis) du delta de l'Ebre, qui manque aussi de niveaux détectables d'aroclor 1254.

	1978-1979	1979-1980	1980-1981	1981-1982
LINDANE	0.10	0.33	0.18	0.13
HEPTACHLOR	0.12	0.56	0.13	0.05
ALDRINE	0.82	0.71	0.36	0.09
HEPTACHLOR EPOXIDE	0.67	0.54	0.23	0.02
pp'DDE	15.55	10.57	12.63	6.59
DIELDRINE	0.50	0.32	0.20	0.41
op'DDT	0.18	0.08	0.05	0.66
pp'TDE	0.95	0.79	1.08	0.77
pp'DDT	0.71	1.01	1.53	1.07
PCB 1254	N.D.	N.D.	N.D.	N.D.
PCB 1260	N.D.	2.35	2.82	4.06
∑PCBs	N.D.	2.35	2.82	4.06
∑DDTs	17.40	12.45	15.29	8.49
DDTs/PCBs	-	5.29	5.42	2.09
DDE/DDTs	0.89	0.85	0.82	0.77

Tableau VI

Moyennes des composés organochlorés ppm en base lipidique dans les différents tissus analysés dans les quatre saisons considérées.

Il faut d'ailleurs souligner que les niveaux de DDTs sonr toujours supérieurs à ceux de PCBs, ce qui apparaît aussi pour l'oeuf (LLORENTE, op. cit.). Cela semble être une condition typique du delta de l'Ebre, et surtout quand on tient compte du quotient DDE/DDTs, (Tableau VI) qui montre la quantité de DDT métabolisé qui existe dans le système (AGUILAR, 1984); c'est pourquoi plus la valeur obtenue se rapproche de un, plus l'ancienneté de l'entrée de ces polluants dans le système est importante et on peut observer que la valeur du quotient a tendance à diminuer avec le temps, ce qui peut mener à la conclusion de l'existence d'entrées récentes de ces polluants dans le système. Ce qui est en accord avec les observations de GASSIOT(in verbis) en ce qui concerne l'inversion des valeurs dans le quotient DDts/PCBs que l'on observe dans des sédiments marins face aux côtes du delta, par rapport à d'autres points de la côte de Tarragone, où il existe une prédominance évidente des PCBs. Les travaux d'ALBERTO et NADAL (1981), RUIZ et al.(1984 sous presse) et l'observation personnelle sont aussi d'accord avec cette affirmation.

Conclusion

En résumé, il faut considérer que les modèles de distribution en pourcentage de la charge de polluants totaux dans les tissus étudiés sont considérés comme très différents pour chacune des années d'échantillonnage, ce qui démontre que les facteurs externes peuvent conditionner des déphasages métaboliques qui modifient beaucoup la dynamique physiologique d'une population même à un

moment donné du cycle biologique annuel, ce qui fait que l'étude doit se réaliser après avoir vérifié que l'étude physiologique est homogène à tous les échantillons à comparer. Ces prémices mènent l'échantillonnage à un dégré tel de difficulté qu'il devient pratiquement insurmontable, ce qui rend beaucoup plus efficace de se servir d'un autre genre d'échantillon (p.e. oeufs) pour établir des comparaisons entre des populations d'oiseaux soit dans l'espace, soit dans le temps.

Malgré tout ceci, certaines tendances générales peuvent être observées quand on considère les résultats d'une manière globale. Ainsi l'on maintient le rapport entre le degré de pollution et le niveau trophique exploité par chaque espèce.

Finalement, il faut souligner l'absence absolue d'aroclor 1254 dans les échantillons d'Anas platyrhynchos du delta de l'Ebre par rapport à des espèces migratoires qui hivernent dans cette zone et qui viennent du nord de l'Europe (LLORENTE, 1984). D'ailleurs, l'évolution du quotient DDE/DDTs tout au long des quatre saisons indique une légère mais soutenue entrée de ces polluants dans le système.

Références

AGUILAR, A. (1983). - Organochlorine Pollution in Sperm whales. Physeter macrocephalus, from the temperate waters of the Eastern North Atlantic. Marine Pollution Bulletin., 14(9) : 342-352.

AGUILAR, A. (1984). - Relationship of DDE/DDTs in marine Mammals to the Chronology of DDT Input into the Ecosystem. Canadian Journal of Fisheries and Aquatic Sciences; 41 (6) : 840-844.

ALBERTO L.J. & J. NADAL, (1981). - Residuos organoclorados en huevos de diez especies de aves del delta del Ebro. Publicaciones del Departamento Zoologia Barcelona, 6 : 73-83.

CHAU A.S.Y. & R.C.J. SAMPSON (1975). - Electron Capture gas Chromatographic Methodology for the Quantitation of Polychlorinated Biphenils. Survey and Compromise. Environmental Letters, 8 : 89-101.

HOLDEN A.V. & A. MARSDEN, (1969). - Single-Stage Clean-Up of Animal Tissue Extracts for Organochlorine Residue Analysis. Journal of Chromatography, 44 : 481-492.

LLORENTE, G.A. (1984). - Contribucion al conocimiento de la biologia y ecologia de cuatro especies de anatidas en el Delta del Ebro. Tesis Doctoral. Universidad de Barcelona.

LLORENTE G.A. , X. RUIZ & J. NADAL (1982). - Compartimentacion de los plaguicidas organoclorados en tres especies de anatidas procedentes del Delta del Ebro (Tarragona). Actas del 1e Congreso Iberoamericano de Toxicologia (Seccion Ecotoxicologia). pp. 691-702. Sevilla.

LLORENTE G.A. , X. RUIZ & J. NADAL (1983). - Incidence des pesticides organochlorés sur trois espèces d'anatides du Delta de l'Ebre (Tarragona, Espagne). VIes Journées études sur les pollutions marines en Méditerranée. Cannes. pp. 479-486.

RUIZ X. (1982). - Contribucion al conocimiento de la biologia y ecologia de Bubulcus ibis ibis. (L) 1758 en el delta del Ebro . Tesis Doctoral. Barcelona.

RUIZ X. , G.A. LLORENTE & J. NADAL (1984). - Distribution pattern of Organochlorine Compounds in five tissues of Bubulcus ibis nestlings (Aves Ardeidae) from the Ebro Delta, North East Spain. Vie et Milieu., 34 (1) : 21-26.

RUIZ X., G.A. LLORENTE & J. NADAL (sous presse). - Dinamica de los compuestos organoclorados en cuatro tejidos de una poblacion de garcillas bueyeras durante un ciclo entre periodos de reproduccion. Actas II. Congreso Iberoamericano de Ornitologia. Xalapa, Veracruz, México.

SCHNEIDER R. (1982). - Polychlorinated biphenyls (PCBs) in cod tissues from the western Baltic : Significance of equilibrium partitioning and lipid composition in the bioaccumulation of lipophilic pollutants in gill-breathing animals. Meeresforsch, 29 : 69-79.

WATTS R. (1980). - (Editor) Analysis of pesticide residues in human and environmental samples. A compilation of methods selected for use in pesticide Monitoring programs. United States Environmental Protection Agency.

PCBs AND DDTs IN MARINE SEDIMENTS FROM THE NORTH-EASTERN COASTAL AREA OF SPAIN

by

F. BROTO*, M. GASSIOT*
X. TOMAS* and J. ROS **
* Instituto Quimico de Sarria
s/n. Barcelona 17 (Espana)
** C.E.O.T.M.A., Madrid (Espana)

Abstract

The results obtained in the analysis of samples of marine sediments, gathered in accordance with a systematic plan are reported. The work is directed towards the evaluation of the pollution level of the zone under study and in order to establish periodic control plans for the future. The samples were gathered during the months of April 1981 and May 1982 and cover the zone comprised between Barcelona and the Ebro River delta.

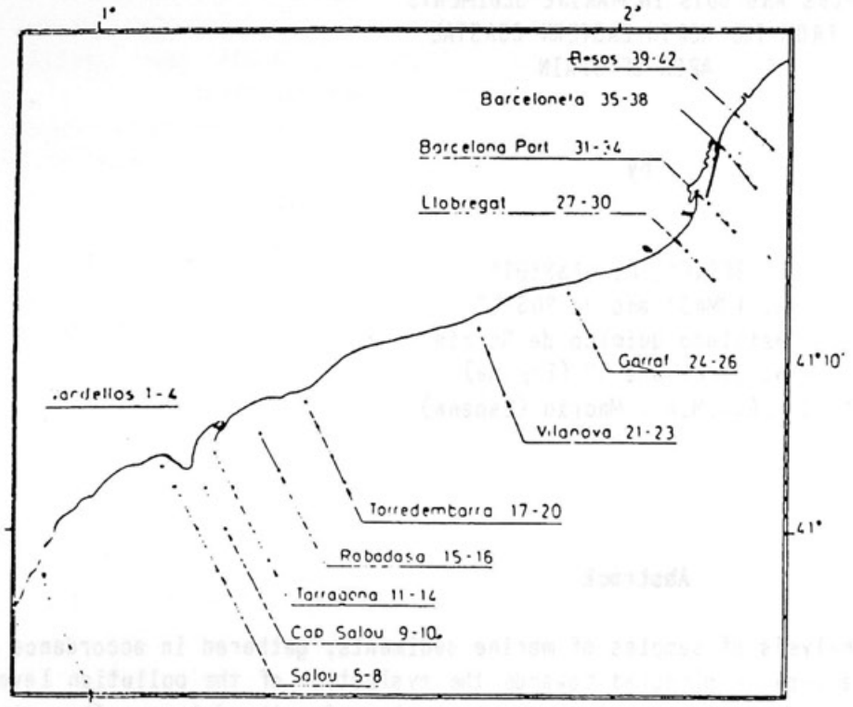
*
* *

Introduction

This paper is a part of a wider research scheme, called Catpol, by which it is intended to know the pollution level of the Catalonian coastal zone, and its evolution, and establish the bases for the development of opportune protection measures.

The zone which is the object of our study extends from the mouth of the Ebro River, the largest in Spain, to the mouth of River Besos, on the northern border of the Barcelona municipality, encompassing both and includes two important cities: Barcelona, with nearly two million inhabitants, and Tarragona, with over one hundred thousand ; on the other hand, it receives the effluents from a very densely populated countryside, with systems of depuration of residual waters only under construction. The presence of the most important harbour on the Spanish Mediterranean coast and of the largest industrial nucleus of Spain cause this coastal zone to have a special interest.

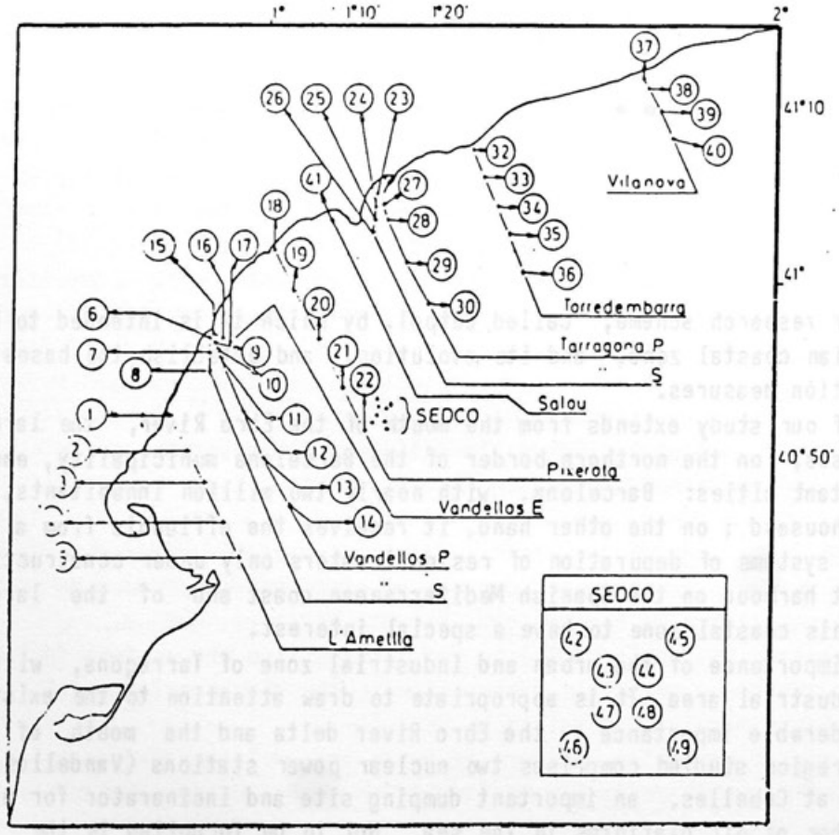
Also to be mentioned is the importance of the urban and industrial zone of Tarragona, with a large chemical and petrochemical industrial area. It is appropriate to draw attention to the existence of two agrarian zones of considerable importance : the Ebro River delta and the mouth of the Llobregat River. Furthermore, the region studied comprises two nuclear power stations (Vandellos and Asco), a thermal generating plant at Cubelles, an important dumping site and incinerator for urban residual waste (Garraf) and a number of oil platforms in the sea. Not to be forgotten is the fact that the effluents of the Ebro River come from a vast hydrographic basin.



40°50' N — 41°30' N (1° = 6mm)
0°50' E — 2°20' E (1° = 3mm)

CATPOL - 82

Figure 1. - Geographic distribution of sampling stations (april 1981).



40°30' N — 41°15' N (1° = 6mm)
0°30 E — 2° 0 E (1° = 3mm)

CATPOL-81

ESTACIONES

Figure 2. - Geographic distribution of sampling stations (may 1982)

The results presented here are based on two campaigns carried out in April 1981 and May 1982 (figs. 1, 2) in accordance with a systematic sampling scheme, modelled on some previous trial analyses and data of geographic, geo-economic and demographic types. The sampling stations for the 1982 campaign were established in accordance with the results obtained the previous year.

Experimental

The sediments samples were gathered by the oceanographic vessel Jafuda Cresques, from the Spanish Oceanographic Institute, a spoon dredger of the Shipek type being utilized. The samples were stored inside aluminium containers at 20 °C up to the moment they were analysed. The samples were dried by lyophilization. The extraction was made in a Soxhlet with hexane; the clean-up was done with concentrated sulphuric acid. Then the analysis was carried out by gas chromatography under the following conditions :

Perkin Elmer 990 Gas Chromatograph with ECD (Ni^{63}) detector, using double column systems: two identical columns were used with 1.5 % OV-17 and 1.95 % QF-1 on 80-100 mesh Supelcoport, the flow being 40 ml/min^{-1} Argon with 5 % methane. The oven temperature was 210 °C and that of the injector and detector 250 °C.

Column A is a usual one, where the first chromatogram of the sample is carried out. Column B is one acting in series with an alcaline micro-reactor placed in the glass liner and gives a chromatogram in which DDTs and DDDs have become changed into DDEs and DDMUs (1).

The pp'DDE concentration is estimated in the first chromatogram, that of PCBs in the second, and that of the DDTs and DDDs from the difference between both. The PCBs concentration is taken off in Aroclor 1254, 1260 and 1268 using an algorithm described previously (2) generating a standard chromatogram as similar as possible to that of the sample.

The results are presented in tables 1 and 2. In these samples, the main Aroclor is the 1260, a small proportion of 1254 also appearing.

Treatment of the results

So as to make a preliminary evaluation of the results, these are drawn on a map of the zone studied, with contour lines corresponding to diverse concentrations. Such contour lines have been determined by the adjustment of one surface which responds to experimental data (3).

From a first examination of these results it is inferred that the most polluted zones are to be found in front of the cities of Barcelona and, to a lesser degree, Tarragona, as well as the area under the influence of the Garraf cliffs. The levels of pollution decrease rapidly as one moves away from these localities. On the other hand, a high level of DDTs is detected at the stations situated around the delta of the Ebro River, fitting in with the eminently agricultural character of this region. Finally the pollution levels are notably low in the profiles of Vandellos, Vilanova and points situated around the oil-platform.

The results obtained may provide some additional information of great interest, if correlations can be established between the organochlorated compounds detected; and between these and the characteristics of the sampling stations. The technique chosen to that end is the analysis of the main components, satisfactorily applied to similar studies made on data from the bay of Cannes and the mouths of the Rhône River (4).

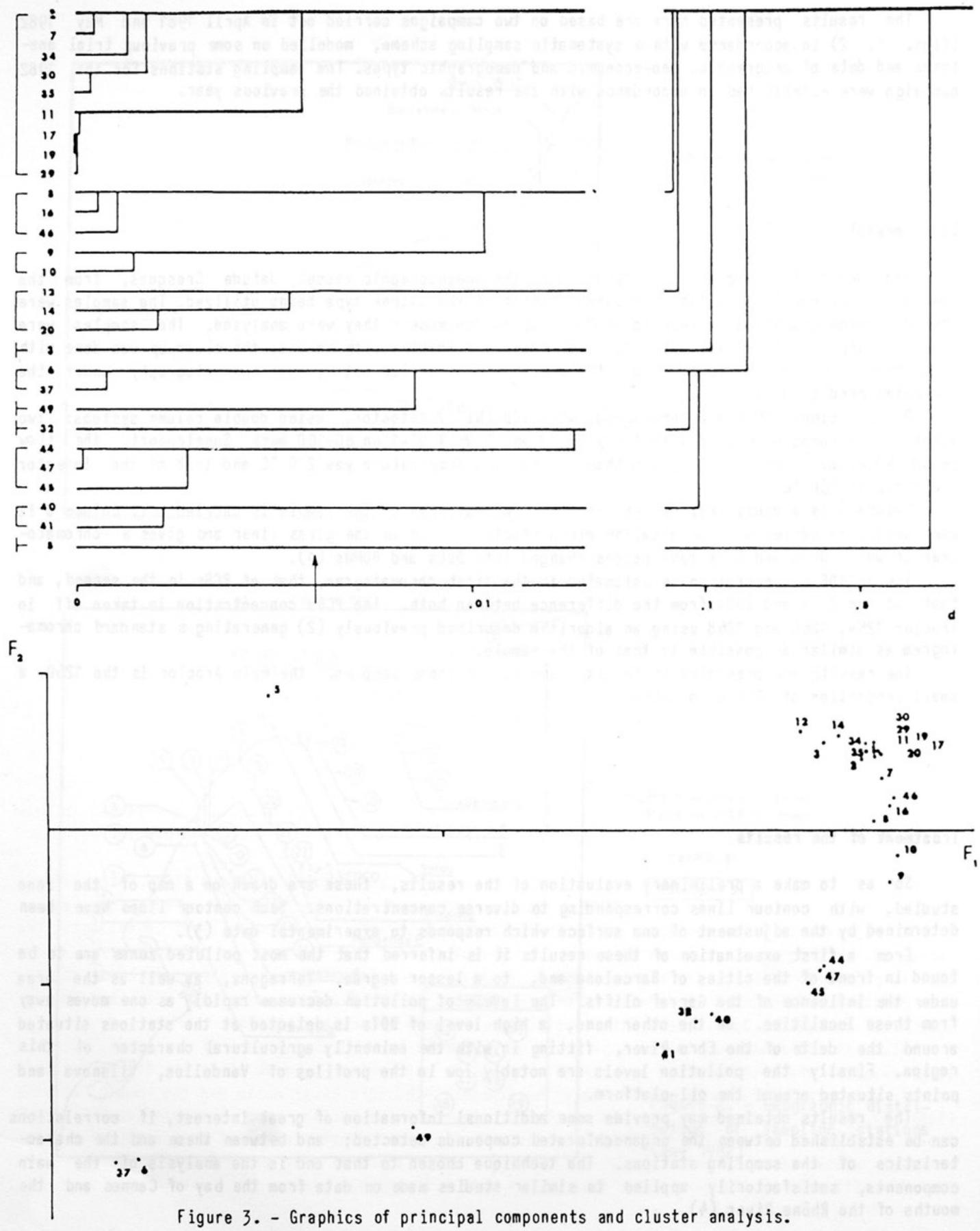


Figure 3. - Graphics of principal components and cluster analysis.

SAMPLE	PCBs* (ppb)	pp´DDE (ppb)	pp´DDD (ppb)	pp´DDT (ppb)
2	17.6	1.8	3.5	1.0
3	45.4	4.0	6.5	1.5
4	145.0	6.9	11.5	5.0
5	200.0	8.1	14.6	13.3
6	3.0	0.1	0.1	0.1
7	16.7	0.7	0.4	0.2
8	11.6	0.7	0.3	0.1
9	8.5	0.4	0.2	0.05
10	10.9	0.7	0.1	0.1
11	13.5	0.7	0.4	0.1
12	10.8	1.0	0.3	0.2
14	13.3	1.4	1.6	3.6
16	7.9	0.6	0.4	0.2
17	17.1	1.0	0.3	0.1
19	13.4	0.7	0.4	0.1
20	7.8	0.5	0.1	0.1
21	7.5	0.3	0.4	---
23	122.0	7.5	1.2	1.2
25	54.0	0.1	0.1	---
26	15.9	1.1	0.6	0.2
27	69.7	0.3	0.2	---
28	26.3	1.1	0.2	0.3
29	14.1	0.3	0.7	0.1
30	18.2	0.8	1.0	0.2
32	1.3	0.1	0.05	0.1
33	33.6	1.6	1.4	0.05
34	20.7	1.2	1.2	0.9
35	25.5	1.6	2.3	0.2
37	4.2	0.2	0.05	0.05
38	6.3	0.8	0.8	0.2
39	12.0	0.4	0.3	---
40	29.0	1.2	0.7	0.1
41	27.9	0.4	0.05	0.05
43	6.2	0.4	0.3	0.1
45	7.1	0.4	0.4	0.1
49	6.2	0.2	0.2	0.05
48	6.3	0.3	0.2	0.05
47	5.6	0.2	0.3	0.05
46	5.5	0.2	0.3	0.05

* referreds to Aroclor 1254

Table 1. - Results of sediment samples (april 1981) in ng. pollutant/gr. dry sample. Global precision of the analytical method 20 %.

SAMPLE	PCBs* (ppb)	pp´DDE (ppb)	pp´DDD (ppb)	pp´DDT (ppb)
1	8.7	1.1	0.4	---
2	12.0	0.6	0.5	0.2
3	8.7	1.0	0.7	0.2
4	8.3	0.6	0.7	0.4
5	7.0	0.2	0.6	2.3
6	15.0	0.3	0.3	0.5
7	5.6	0.6	0.5	---
8	4.1	0.4	0.2	0.2
9	16.0	0.9	0.7	0.2
10	11.0	1.1	0.8	0.4
11	18.0	1.3	0.6	0.6
12	18.0	1.1	---	---
13	9.2	0.8	0.6	0.2
14	6.2	0.9	0.5	---
15	14.0	1.2	0.9	0.4
16	19.0	2.0	1.3	0.3
17	12.0	0.6	0.2	0.3
18	7.8	1.1	0.5	0.3
19	7.9	0.7	1.0	0.3
20	5.4	0.6	0.8	1.1
21	2.4	0.6	0.1	0.4
22	8.7	0.7	0.3	---
23	18.0	0.2	1.9	1.0
24	2.8	0.3	0.05	0.05
25	41.0	1.6	1.1	1.6
26	25.0	1.1	0.9	0.9
27	80.0	12.2	13.7	4.3
28	39.0	2.5	1.6	1.3
29	23.0	1.1	2.0	0.3
30	6.8	---	0.8	---
31	201.	7.4	7.8	---
32	100.	12.2	20.8	5.2
33	138.	1.9	16.3	13.0
34	14.0	0.9	1.3	0.3
35	483.	45.4	88.0	35.2
36	350	47.8	54.4	40.0
37	72.0	0.3	5.1	1.7
38	21.0	1.0	1.1	1.7
39	384.	67.8	24.8	102.
40	88.0	6.7	14.2	3.4
41	14.0	1.1	1.3	0.7
42	6.3	---	0.6	---

* referreds to Aroclor 1254

Table 2. - Results of sediment samples (april 1982) in ng. pollutant/gr. dry sample. Global precision of the analytical method 20 %.

Total PCB and DDT data of 37 sampled stations shows a highly significative (P 0.1 %) correlation coefficient (r = 0.9529). Assuming linear relationship between both variables, a regression equation gives :

$$PCB = 1.41 + 10.74\ DDT$$

easily interpreted in a chemical sense.

Stations with an abnormal level in total PCB or DDT are detected, and their geographical location shows all of them to be in the Ebro delta (agronomic pollution).

Principal components analysis and clustering techniques have been applied in order to study the potential grouping of sampling stations with reference to a specified number of variates. In a such a way, 28 station data of PCBs, DDTs and sedimentological parameters were processed. Satisfactory P.C.A. results were obtained (two first components explain 74 per cent of the total variance) and Guttman's effect is manifested between stations as fig. 3 shows. It also shows station groups obtained by cluster analysis.

References

1) BROTO-PUIG F., M. GASSIOT-MATAS, R. MARTINEZ-FONRODONA (1982). - Application of an on-line KOH microreactor to the resolution of interferences between PCBs and DDTs in the G.C. of H.M.W. organochlorinated contaminants. VIes Journées Etud. Pollutions, Cannes C.I.E.S.M., 449-454.

2) GASSIOT M., X. TOMAS, F. BROTO, L.G. SABATE, G. CODINAS (1982). - Algorithms for the quantitative determination of PCBs in chromatograms of organochlorinated contaminants: Application to samples of sediments and marine organisms. In Analytical Techniques in Environmental Chemistry 2. Pergamon Press, Oxford-New York 249-258.

3) RINGOT J.L. (1980). - Utilisation de l'analyse factorielle des correspondances et de la classification automatique dans l'étude de la répartition de pollutants métalliques devant l'embouchure du Rhône. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M., 109-116.

4) RINGOT J.L. (1980). - Dynamique et répartition des substances nutritives et des métaux lourds dans les sédiments superficiels de la baie de Cannes et du delta du Rhône: utilisation de l'analyse des correspondances et de la classification ascendante hiérarchique. Thèse Doctorat. Université de Paris-Sud p. 282.

UPTAKE OF CONTAMINANTS BY BIRDS WINTERING IN AN ITALIAN LAGOON

by

FOCARDI S., C. FOSSI
C.LEONZIO and M.A. PALADINI
Dipartimento Biologia Ambientale
Via della Cerchia 3
53100 Siena (Italy)

Within the framework of the United Nations Environment Programme for the Mediterranean, after five years of monitoring water, sediments and representatives of marine fauna, we turned our attention to water birds (FOCARDI et al., 1980; RENZONI et al., 1982; COTTIGLIA et al., 1982). One of the reasons for that kind of project was the limited amount of information on this topic gathered over the last 20 years (RENZONI et al., 1973; VIVIANI et al., 1974; MENDOLA et al., 1977; VANNUCCHI et al., 1978; BIJLEVELD et al., 1979; BOURNE and BOGAN, 1980). (°)

Among water birds those species wintering in the Mediterranean area pose special problems because they alternately visit areas at different levels of contamination and/or with different forms of contamination.

We have analysed 19 specimens of the black-necked grebe (Podiceps nigricollis) collected in a brackish lagoon near Marano in north-eastern Italy. The birds were shot during the first week of October (shortly after their arrival in the lagoon) and during the first week of April (before leaving the lagoon). (°°)

The tissues and organs analysed were: uropygial gland, subcutaneous fat, muscle, liver, kidney and brain.

After the material was freeze-dried, trace elements (mercury, lead, cadmium and selenium) and chlorinated hydrocarbons (HCB, lindane, pp'DDE, PCB) were analysed. Analytical techniques are reported in a previous paper (FOCARDI et al., 1980). Accuracy of methodology was evaluated by the intercalibration exercises conducted by the International Laboratory of Marine Radioactivity of the I.A.E.A. (Monaco, Principality of Monaco).

Residues of the trace metals analysed are reported in table 1. The comparative analysis of the average values of residues in the birds arriving at the lagoon (September 1983) and about to leave it (April 1983 and 1984) clearly shows that during their wintering in the Italian lagoon the content of Se, Cd and Pb, does not present any particular trend. On the other hand, that of mercury clearly

---

(°) Research supported by F.A.O/U.N.E.P. (Med Trust Fund) and by a ECC Grant (ENV/512/I).
(°°) The birds were shot under a special permit issued to Prof. A. RENZONI by the Regional Government of Friuli - Venezia Giulia.

	Hg		Se		Cd	Pb	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$ SD	$\bar{x}$	SD
April 1983, n=5							
FAT	1.14	0.29	3.03	3.62	<0.02	0.53	0.55
UROPYGIAL G.	10.24	3.50	5.24	4.25	<0.02	0.69	0.68
MUSCLE	11.83	3.86	4.44	0.99	<0.02	0.83	0.31
LIVER	57.90	11.40	15.57	1.78	<0·02-0·19*	0.85	0.28
KIDNEY	35.04	6.89	10.75	3.55	0.61 0.22	0.80	0.14
BRAIN	14.91	3.71	5.51	3.06	<0.02	0.65	0.20
October 1983, n=8							
FAT	0.53	0.20	3.57	1.14	<0.02	0.41	0.22
UROPYGIAL G.	3.77	2.29	8.98	4.03	<0.02-0.15*	0.80	0.45
MUSCLE	3.70	1.53	6.73	1.76	<0.02-0·14*	0.44	0.22
LIVER	9.37	2.23	12.19	4.33	<0.02-0·40*	<0·10-0·37*	
KIDNEY	7.63	1.53	11.47	2.22	0.94 0.86	0.27	0.09
BRAIN	3.61	2.43	12.79	5.49	<0.02	<0·10-1.52*	
April 1984, n=6							
FAT	1.50	0.73	7.15	3.37	<0·02-0·17*	<0.10	
UROPYGIAL G.	9.86	3.12	13.83	2.57	<0·02-0·17*	<0.10	
MUSCLE	5.99	1.69	3.57	0.80	<0.02	<0.10	
LIVER	48.52	11.07	16.29	0.98	0.35 0.12	<0·10-0·21*	
KIDNEY	14.80	4.57	6.62	1.52	0.79 0.62	<0·10-0·41*	
BRAIN	14.75	3.89	12.03	2.32	<0·02-0·50*	<0·01-0·30*	

$\bar{x}$ = mean; SD = standard deviation * = range.

Table 1. - Trace elements (mg/kg dry weight) in tissues.

	E.O.M.%		HCB		γ-HCH		pp'DDE		PCB*		PCB/DDE	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
April 1983, n=5												
FAT	90.7	4.6	0·13	0·03	0·09	0·08	6.35	4·54	3.06	1.98	0.5	0·2
UROPYGIAL G.	64.6	9.3	0·11	0.06	0.16	0.09	3.28	2.11	1.67	0.77	0.5	0.2
MUSCLE	12.7	4.6	0.02	0·01	0.01	0.01	0.69	0.43	0.32	0·14	0·5	0·2
LIVER	9.2	2.4	0.02	0.01	0.01	0.01	0.43	0·16	0.29	0·09	0.7	0.3
KIDNEY	8.6	2.4	0.01	0.01	<0.001		0.45	0.24	0.29	0.15	0.7	0·4
BRAIN	25.3	9.9	0.04	0.03	0.08	0.04	0.47	0.33	1.29	0.62	3.1	1.1
October 1983, n=8												
FAT	73.6	9.9	0·06	0.03	0.10	0.23	1.80	1.87	0·27	0.19	0.3	0·3
UROPYGIAL G.	54.2	8.1	0.04	0·03	0.09	0·08	2.03	2.68	0.22	0.19	0.6	0·9
MUSCLE	9.4	0.7	0.01	0·01	0.01	0.02	0.19	0·18	0.06	0.06	0·3	0·1
LIVER	12.4	1.3	0.02	0.02	0·01	0.01	0.41	0.27	0.07	0·04	0.2	0·1
KIDNEY	7.6	3.0	0·02	0·01	0·01	0.01	0·17	0.16	0.05	0.04	0.3	0·1
BRAIN	16.2	5.2	0·08	0.08	0.13	0·19	0.72	0.69	0·19	0.10	0·5	0·4
April 1984, n=6												
FAT	86.2	4.6	1.60	3.12	0.09	0.10	6.15	3.01	5.85	5.37	0.9	0·9
UROPYGIAL G.	51.9	0.8	1.24	1·98	0.06	0.11	3.70	1·57	2.87	1.99	0·8	0.6
MUSCLE	8.3	2.2	0.18	0.33	<0.001		0.48	0.27	0.55	0.47	1.1	0·9
LIVER	13.8	1.9	0.09	0.08	<0.001		0.53	0.21	0.39	0.19	0·9	0·8
KIDNEY	10.3	2.3	0.12	0.17	<0.001		0.29	0.14	0.38	0.26	1·4	0·9
BRAIN	21.4	5·0	0.17	0.21	0.02	0.02	0.36	0.15	1.18	1·04	2.5	1·3

$\bar{x}$ = mean; SD = standard deviation; E.O.M. = Extracted Organic Matter;
PCB* = calculated as Aroclor 1260.

Table 2. - Chlorinated hydrocarbons (mg/kg dry weight) in tissues.

increases over the wintering period in all the six tissues analysed. In the liver (Fig. 1) the average spring level is six times higher than that at the time of the bird's arrival, in the brain five times, in the kidney almost four times, and between two and three times in the other three tissues.

Residues of the analysed chlorinated hydrocarbons are reported in table 2. Lindane and HCB are at very low levels both in the groups arriving at the lagoon and in the group leaving the lagoon, whereas the residues of DDE and PCB increase quite sharply during the wintering period. In the fat the DDE at the end of the wintering period is four times higher than that at the time of arrival (Fig. 1); double in the muscle and in the uropygial gland and without any trend in the other tissues analysed. At the end of the wintering period the PCB are an order of magnitude higher than at the time of the arrival in the lagoon in the fat (Fig. 1) and in the uropygial gland; between four and six times higher in the other four tissues.

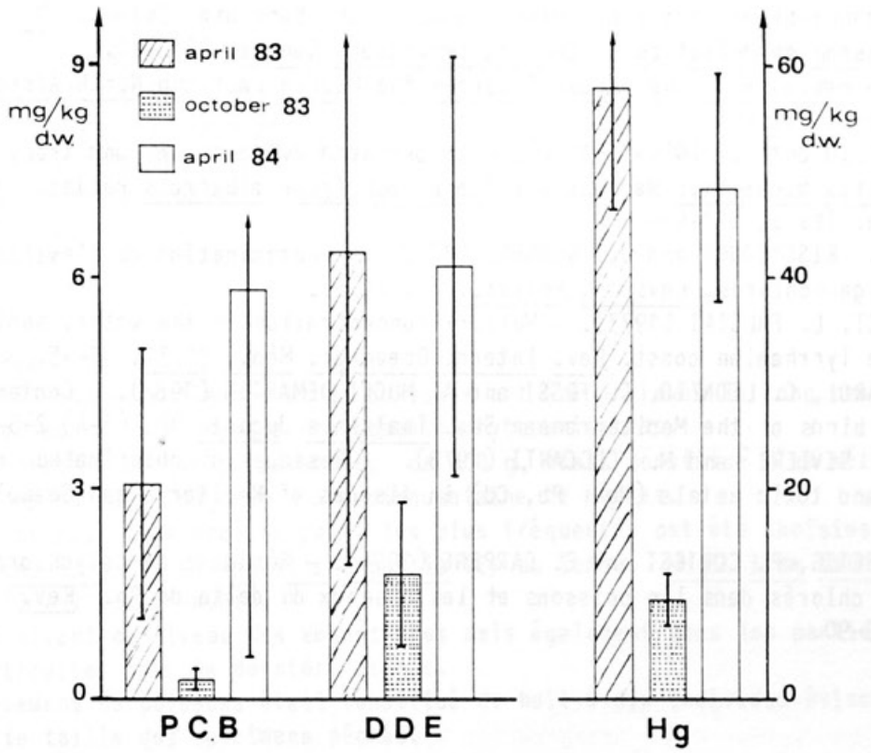


Figure 1. - Variations in the residues of chlorinated hydrocarbons in the fat and mercury in the liver

The body burden of these three contaminants (Hg, pp'DDE, PCB) at the end of the wintering period is much higher than at the beginning. The biological half-life of the three contaminants is quite long; however, the much lower levels at the time of their arrival than at the end of the wintering period would seem to demonstrate that during the breeding season in their summer quarters the birds eliminate most of the pollutants accumulated in the lagoon during the winter.

We know little about the movement of this bird; according to CRAMP (1980) the species is "migratory and dispersive" and "winter on lakes, reservoirs and coastal waters". In the west Palaearctic it winters chiefly along "coasts from the British Isles to Iberia, on ice-free lakes... and on and around the Mediterranean Sea". "The few recoveries of rings indicate south-west and south-east autumn movements; among the banded birds recovered in Italy, two were ringed in Denmark and two in Czechoslovakia".

Notwithstanding the scarcity of data on their migratory routes, it seems safe to say that the birds wintering in Italy have their breeding grounds in central and north European countries. In the latter countries the residues of Hg, pp'DDE and PCB in the grebe's food seem therefore lower than in the lagoon of Marano.

References

BIJLEVELD M.F.I.J., P. GOELDLIN and J. MAYOL (1979). - Persistent pollutants in Audouin's Gull (Larus audouinii) in the Western Mediterranean: a case-study with wide implications? Environ. Conserv., 6, (2), 139-142.

BOURNE W.R.P. and J.A. BOGAN (1980). - Organochlorines in Mediterranean sea-birds. Environ. Conserv., 7, (4), 277-278.

COTTIGLIA M., S. FOCARDI, C. FOSSI, C. LEONZIO, C. MASCIA and A. RENZONI (1982). - Contaminants in tissues of shore-birds from a polluted lagoon of the Sardinia Island. VI C.I.E.S.M./I.O.C.-/U.N.E.P. Workshop on Pollution of the Mediterranean, Cannes, 293-297.

CRAMP S. (1980). - Handbook of the Birds of Europe the Middle East and North Africa - Vol. I. Oxford University Press, Oxford.

FOCARDI S., C. LEONZIO and A. RENZONI (1980). - Chlorinated hydrocarbons and trace metals in eggs of Larus argentatus michaellis Naumann and Sterna albifrons albifrons Pallas. Atti I° Congresso S.I.T.E, Parma, Italy, 411-414.

MENDOLA J.T., R.W. RISEBROUGH and J. BLONDEL (1977). - Contamination de l'avifaune camarguaise par des résidus organochlorés. Environ. Pollut., 13, 21-31.

RENZONI A. E. BACCI, L. FALCIAI (1973). - Mercury concentration in the water, sediments and fauna of an area of the Tyrrhenian coast. Rev. Intern. Oceanogr. Méd., 31/32, 17-45.

RENZONI A., S. FOCARDI, C. LEONZIO, C. FOSSI and A. MOCCI DEMARTIS (1982). - Contaminants of resident and migratory birds of the Mediterranean Sea. Thalassia Jugosl. 18, (1-4) 245-252.

VANNUCCHI C., S. SIVIERI and M. CECCANTI (1978). - Residues of chlorinated naphtalenes, other hydrocarbons and toxic metals (Hg, Pb, Cd) in tissues of Mediterranean Seagull. Chemosphere, 6, 483-490.

VIVIANI R., G. CRISETIG, P. CORTESI and E. CARPENE (1974). - Résidues de polychlorobiphenils (PCB) et de pesticides chlorés dans les poissons et les oiseaux du delta de Po. Rev. Intern. Océanogr. Méd., 35/36 79-90.

RESIDUS D'ORGANOCHLORES DANS LES SEDIMENTS ET LES POISSONS DES PRINCIPAUX FLEUVES MAROCAINS

par

A. LAMRINI
Institut Agronomique et Vétérinaire Hassan II
Section Halieutique - BP 6202, Rabat-Instituts (Maroc)

Au Maroc, les principales régions d'utilisation des organochlorés tant en agriculture qu'en industrie sont le Gharb, le Tadla et les Doukkala. Elles sont traversées par deux fleuves : le Sebou pour la première et l'Oum-er-Rebia pour les deux autres. Ces fleuves se trouvent ainsi pollués par les produits employés, en particulier par le DDT, HCH, HCB, dieldrine et PCB.

Dans cette note, nous nous proposons d'apprécier le degré de contamination des sédiments et des poissons des deux fleuves.

Matériel et méthodes

Trente échantillons de sédiments et de poissons ont été prélevés le long des deux fleuves durant les mois de mars et avril, périodes de traitements phytosanitaires.

Pour les sédiments, chaque prélèvement, de 200 g, était constitué d'un mélange d'une vingtaine de prises effectuées sur une longueur d'une cinquantaine de mètres.

Pour les poissons, deux espèces parmi les plus fréquentes ont été choisies dans chaque fleuve. Ce sont Mugil cephalus (D) et Anguilla anguilla (E) au Sebou, Mugil lisa labrosus (A et B) et Cyprinus carpio (C) à l'Oum-er-Rebia.

Ces espèces vivent au niveau des embouchures mais également dans les parties les plus éloignées de la mer, en particulier pour la dernière espèce.

Chaque prélèvement de poissons était constitué de huit à dix individus éviscérés et mélangés en raison de la petite taille des spécimens pêchés.

Les lieux de prélèvement pour l'ensemble des échantillons (sédiments et poissons) figurent sur la carte.

La technique d'analyse utilisée est la chromatographie en phase gazeuse après avoir effectué une extraction avec une concentration de l'extracum, une purification de ses constituants et une séparation entre PCB et les autres organochlorés par une dégradation alcaline.

Résultats

Les résultats des analyses sont présentés dans les tableaux 1 et 2.

1. Degré de contamination des sédiments

Dans les deux fleuves, les teneurs en polluants détectés peuvent se répartir suivant leur concentration en deux groupes : l'un est représenté par les produits à faibles concentrations : HCB, HCH, HCH et dieldrine, l'autre à concentrations plus élevées : DDT et ses isomères et PCB.

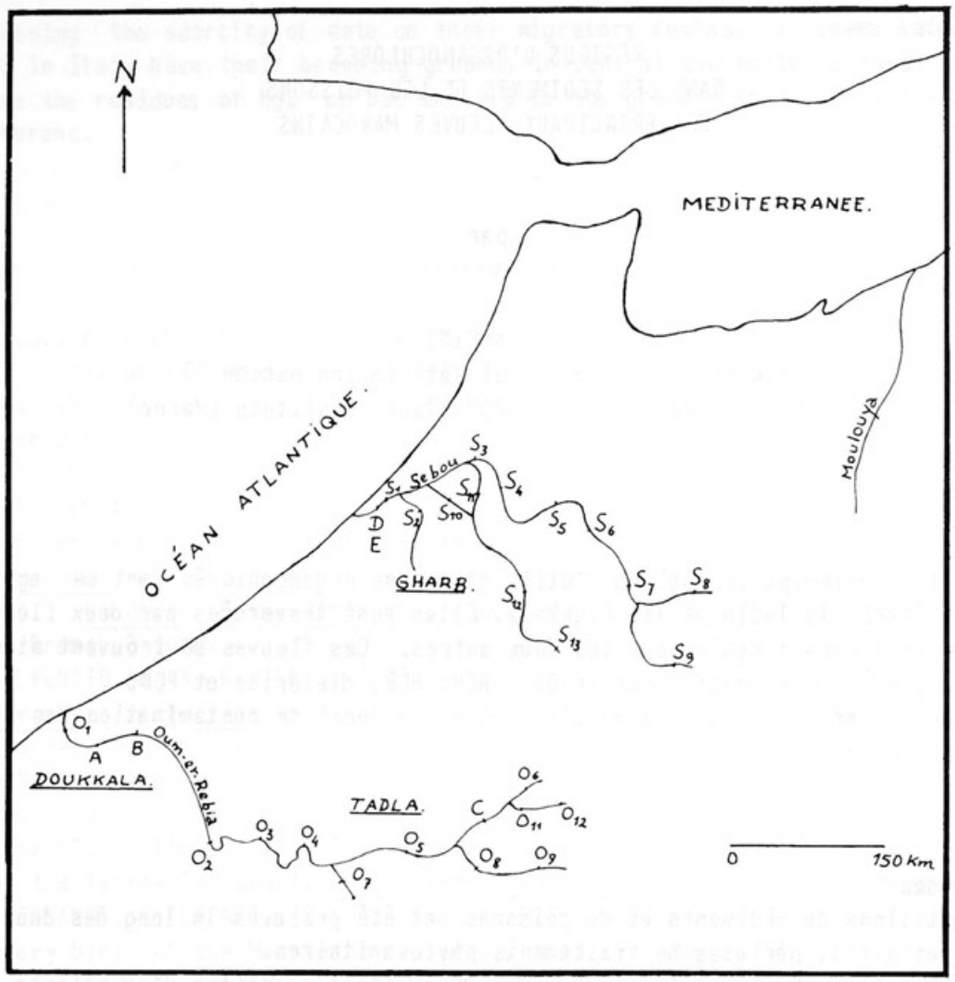


Figure 1. - Situation des prélèvements.

Dans le premier groupe, on constate que 54 % des prélèvements du Sebou et 70 % d'Oum-er-Rebia sont contaminés par les résidus d'HCB alors qu'ils sont contaminés presque en totalité par HCHα, HCHα et dieldrine dans les deux fleuves.

En fonction de leur taux résiduel moyen le classement dans l'ordre décroissant de ces produits diffère quelque peu d'un fleuve à l'autre :

Sebou : HCHα, dieldrine, HCHα et HCB

Oum-er-Rebia : HCHα, HCHα, HCB et dieldrine

quoiqu'il en soit pour les quatre produits, les sédiments de l'Oum-er-Rebia sont plus contaminés que ceux du Sebou.

Dans le deuxième groupe, la contamination globale des prélèvements par le DDT et ses isomères est assez élevée, elle décroît selon l'ordre suivant : pp'DDT, op' DDT, TDE et DDE. Néanmoins, les valeurs respectives enregistrées sont légèrement plus élevées au Sebou.

Quand aux PCB, donnés par le titre en phénoclor DP5 (ou Aroclor 1254), ils contaminent l'ensemble des échantillons, le titre moyen est identique dans les deux fleuves, les valeurs les plus élevées sont enregistrées au niveau des stations proches des usines.

Le rapport moyen Σ DDT/PCB dépasse largement l'unité, ce qui signifie que la contamination par le DDT est plus importante que celle due aux PCB.

Prélèvements		HCB	HCH α	HCH β	HCH γ	Dieldrine	pp'DDT	op'DDT	TDE	DDE	ΣDDT	PCB	ΣDDT/PCB
SEDIMENTS	S1	0,04	0,14	nd	0,37	0,22	13	3,70	0,78	0,50	18,00	10	1,80
	S2	nd	0,08	nd	0,19	0,14	12	8,00	0,19	0,58	20,80	3	6,93
	S3	0,36	0,23	nd	0,68	0,25	10	4,90	1,50	0,53	16,90	13	1,30
	S4	nd	0,12	nd	0,40	0,23	14	5,20	0,77	0,58	20,20	6	3,37
	S5	0,02	0,15	nd	0,27	0,19	16	4,00	1,00	0,48	21,50	20	1,07
	S6	0,20	0,50	nd	0,90	0,20	20	6,00	1,00	0,30	27,30	20	1,36
	S7	nd	0,17	nd	0,50	0,19	14	3,60	0,67	0,84	19,10	10	1,91
	S8	0,10	0,14	nd	0,49	0,85	10	2,60	0,32	0,68	13,70	5	2,74
	S9	nd	0,12	nd	0,48	0,17	7,60	2,60	0,49	0,18	10,90	25	0,43
	S10	0,04	0,14	nd	0,54	0,12	10	4,20	0,84	0,17	15,20	10	1,52
	S11	0,06	0,30	nd	0,40	0,20	15	3,70	0,90	0,80	20,40	5	4,08
	S12	nd	0,25	nd	0,40	0,30	20	4,50	1,00	0,40	25,90	10	2,59
	S13	nd	0,04	nd	0,10	0,10	8,8	nd	0,16	0,36	9,30	2	4,65
	Moyenne	0,06	0,18		0,50	0,24	13,10	4,07	0,74	0,49	18,40	10	1,84
	V%	176	65		46	79	40	46	51	43	28	71	144
Poissons	D	20	20	3,6	7	2,6	940	100	85	60	1130	1010	1,12
	E	15	7	16	14	10	1130	150	120	39	1640	1740	0,94

- Les teneurs sont exprimées en ppb/MF. nd : non détectable.
- V%: Coefficient de variation.

Tableau 1. - Teneurs en organochlorés dans l'Oued Sebou et ses affluents.

Prélèvements		HCB	HCH α	HCH β	HCH γ	Dieldrine	pp'DDT	op'DDT	TDE	DDE	ΣDDT	PCB	ΣDDT/PCB
SEDIMENTS	01	0,02	0,13	nd	0,34	0,23	15	0,70	0,59	0,49	19,80	10	1,98
	02	1,00	0,30	nd	0,25	0,10	4,8	1,00	0,25	1,00	7,00	9	0,78
	03	nd	0,10	nd	0,50	0,20	15	4,00	0,90	0,30	20,00	10	2,02
	04	nd	0,06	nd	0,20	0,20	3	0,50	0,17	0,42	4,10	10	0,41
	05	0,06	0,25	nd	0,77	0,17	12	4,60	0,50	0,26	17,30	20	0,86
	06	nd	1,00	nd	0,70	nd	18	2,10	2,80	0,90	21,80	16	1,36
	07	1,00	0,20	nd	0,70	0,17	17	4,60	1,00	0,16	15,80	16	0,98
	08	nd	0,16	nd	0,32	nd	9,3	1,40	0,90	0,60	17,60	3	5,86
	09	0,03	0,30	nd	0,35	0,20	5,7	0,90	0,18	0,50	7,20	6	1,20
	010	0,06	0,14	nd	0,32	0,31	13	3,80	0,87	0,55	18,20	10	1,82
	011	0,08	0,35	nd	0,65	0,30	24	7,20	1,20	0,72	33,10	9	3,68
	012	0,12	0,23	nd	0,44	0,35	13	3,70	1,00	0,43	18,10	10	1,81
	Moyenne	0,20	0,27		0,46	0,18	11,9	3,12	0,70	0,53	16,68	10	1,67
	V%	190	92		43	64	50	63	50	46	46	46	80
Poissons	A	20	3	80	7	5	1840	220	120	50	2230	1290	1,73
	B	2	2	1,5	8	2,6	560	70	30	16	680	460	1,48
	C	2	1	2,1	2	1	220	24	10	4	230	140	1,64

- Les teneurs sont exprimées en ppb/MF. nd ; non détectable.
- V%: Coefficient de variation.

Tableau 2. - Teneurs en organochlorés dans l'Oued-er-Rebia et ses affluents.

2. Degré de contamination des poissons

Le niveau de contamination est bien plus élevé pour l'ensemble des organochlorés recherchés surtout par le groupe du DDT et PCB. Au Sebou, l'Anguille présente une contamination plus importante que le Mulet, ce qui pourrait s'expliquer par le fait que la première espèce vit plus longtemps, qu'elle est plus riche en graisses qui accumulent les organochlorés et qu'elle peut remonter les cours d'eau sur de longues distances.

A l'Oum-er-Rebia, la contamination des Mulets est plus élevée qu'au Sebou où la Carpe est la moins touchée. De plus, d'après les valeurs détectées on constate une augmentation de concentration dans les poissons de la plaine du Tadla (C) vers l'embouchure du fleuve (B et A).

Le rapport Σ DDT/PCB est de l'ordre de l'unité au Sebou alors qu'il dépasse une fois et demi à l'Oum-er-Rebia qui est ainsi plus contaminé par le DDT que par les PCB.

Puisque la pêche de l'Anguille et du Mulet (Mugil cephalus), a été effectuée au même endroit qu'une station (S1) de prélèvement de sédiment sur le Sebou, et qu'une prise du Mulet (Mugil lisa labrosus) à été faite au même endroit qu'une station de prélèvement de sédiment (O1) sur l'Oum-er-Rebia, des coefficients de concentration ont été calculés dans chaque cas pour l'ensemble des produits recherchés (Tableau 3).

ORGANOCHLORES	S1	MUGIL CEPHALUS (D)		ARGUILLA ANGUILLA (E)		O1	MUGIL LISA LABROSUS (A)	
		TAUX	V	TAUX	V		TAUX	V
HCB	0,04	2	50	15	375	0,02	20	1000
HCHα	0,14	2	14	7	50	0,13	3	23
HCHγ	0,37	7	19	14	38	0,34	7	20
Dieldrine	0,22	2,6	12	10	45	0,23	5	22
pp' DDT	13	940	72	1130	102	15	1840	123
op' DDT	3,70	100	27	150	40	3,70	220	60
TDE	0,78	85	109	120	154	0,59	120	203
DDE	0,50	16	32	39	78	0,49	50	102
ΣDDT	18	1130	63	1640	91	19,80	2230	113
PCB	10	1010	101	1750	174	10	1290	129

Tableau 3. - Coefficients de concentration (V).

Mis à part Mugil cephalus (au Sebou) qui présente les coefficients les moins élevés et dont le plus important est relatif au TDE, les autres espèces concentrent énormément l'HCB, le TDE et les PCB; les autres produits sont beaucoup moins concentrés.

Discussion et conclusion

Les prélèvements analysés ont été effectués vers les premières semaines d'utilisation d'organochlorés, le niveau de pollution détecté résulterait alors surtout d'une accumulation des produits à travers les années antérieures. On peut penser que le degré de contamination serait beaucoup plus important vers les mois de juin, juillet et août.

En comparant nos résultats avec ceux obtenus en d'autres pays, on peut en déduire que le niveau de contamination des échantillons testés est intermédiaire entre celui des pays relativement exposés aux produits et celui des pays très exposés.

Cette situation n'est tout de même pas confortable car le Maroc fait appel jusqu'à présent à de faibles quantités de pesticides. Mais il sera contraint d'accroître leur usage dans un proche avenir, ce qui aggraverait le problème de la pollution surtout avec le système anarchique de la commerciali-

sation de tels produits, l'inconscience des usagers de ces toxiques et l'insuffisance de la règlementation marocaine en matière d'organochlorés.

Références

R.L. CARSON, (1963). - Le printemps silencieux. Edit. Plan.

A. GILBERT et al., (1976). - La chromatographie en phase gazeuse et ses applications, 35-48 pp, Lab, France édit. Paris.

A. LAMRINI, (1978). - Contribution à l'étude de la pollution aquatique par les pesticides organochlorés - Thèse. Doct. Vet. Inst. - Agro. et Vet. Hassan II, 104 p.

R. MITCHELL, (1972). - Water pollution microbiology: Wilemtercience, New York - London and Sydney.

R.C. Thomson MUIRHEAD (1971). - Pesticites and Frehswater Faune: Academic Press, London and New York.

OMS (1972). - Risques pour la santé du fait de l'environnement pp. 219 - 226, Genève.

YL. RICHOU BAC et al., (1972). - Contribution de l'environnement et de la faune par les pollutions industrielles : les diphényles polychlorés (PCB), Bull. Acad. Vet., Tome CXLVIII, 293-303 Vigots-Frères Editeurs.

RUKSTANDS et BERICHTE, (1971). - Residue Reviews (Residus of pesticides and other Foreign Chemicals in Food and Feeds), Vol. 35.

A.A. WALTER, (1975). - Detectors for Use in GC Analyses of pesticides: journal of chromatographie. Science, Special issue Analysis of pesticides part III, vol. 13, n° 7, pp 329-333, Niles U.S.A.

ON THE PRODUCTION OF TRIHALOMETHANES IN SEAWATER AND WASTE WATER SAMPLES DURING LABORATORY CHLORINATION PROCESS

by

V. HOCENSKI, M. PICER and N. PICER
Center for Marine Research Zagreb
The Rudjer Boskovic Institute
POB 1016 - 41001 Zagreb (Yugoslavia)

Abstract

Water chlorination leads to generation of various halogenated products of natural and waste water organic matter. In natural seawater humic matter usually makes the greatest part of the total organic matter. However, in the seawater polluted with other organic wastes the other organic matter is also present in significant concentrations. Coastal seawater samples collected from the Rijeka Bay heavily polluted with organic wastes and relatively clean seawater samples collected in the area outside the Rijeka Bay are laboratory chlorinated. The influence of organic matter in seawater samples and the production of volatile lipophilic organohalides during their chlorination by using relatively simple ECD fingerprint method are investigated.

*
* *

Introduction

In the past decade the reactions of chlorine in fresh water and seawater environment have been intensively studied (JOLLEY, 1976: JOLLEY et al.,1978; JOLLEY et al., 1980). In the marine environment these studies focus on the stress to aquatic environment produced by chlorinated effluents from sewage-treatment plants, swiming pools, chlorinated tap water and cooling water of various power plants and industrial installations, where chlorine is used as a desinfectant or antifouling agent (DUURSMA and PARSI, 1976). A very important question is: Do these halogenated organics, the product of chlorination process, accumulate in the aquatic life and reach back to man through the food chain? (GORCHEV, 1978). Therefore it is very important to obtain data on those lipophilic materials in the aquatic ecosystem which are produced during water clorination (PICER, 1982).

Methodology

Description of water samples

Seawater samples were collected from a ship at a depth of 1 m using a sampler, as recommended by the UNEP MED 1 Programme. Station 1 was located about 1 km off the coasts, relatively far away from the heavy ship traffic and the industrial wastewater discharge sites. The station was under a weak

influence of wastewaters discharged by a small town. The other seawater samples (Stations 2, 3, 4, 5 and 6) were collected at locations about 100 m off the Rijeka city wastewater discharge sites. Station 3 was situated in a heavily polluted harbour. This area receives also several smaller wastewater efffluents. Wastewater samples (Effluents 2, 5 and 6) were collected at sites just prior to the effluents points of entry into the sea. Effluent 4 samples were collected at the site where wastewater mixes with the estuarine water.

Chlorination and estimation of THM concentrations

Water samples were laboratory chlorinated adding sodium hypochlorite solution to 1 mg l-1 active chlorine. After the contact time of 18 hours at 283-285° K the residual oxidants were quenched with 10 % Na2S2O3 water solution.

The amounts of volatile lipophilic organohalides before and after chlorination of water samples were estimated by using the Norin and Renberg extraction method (NORIN and RENBERG, 1980). The water samples were transferred into volumetric flaks filled up to the mark. Two ml of mixture of hexane and diisopropyl ether (1+1) were added. The flasks were shaken by hand for 2 minutes. After the phases had been separated, a part of the organic phase was removed for gas chromatographic determination. The extracts were injected into a Hewlett Packard gas chromatograph with an electron capture detector. The carrier gas flow (N2) was 30 ml min-1, injector and detector were held at 423 and 473° K respectively.

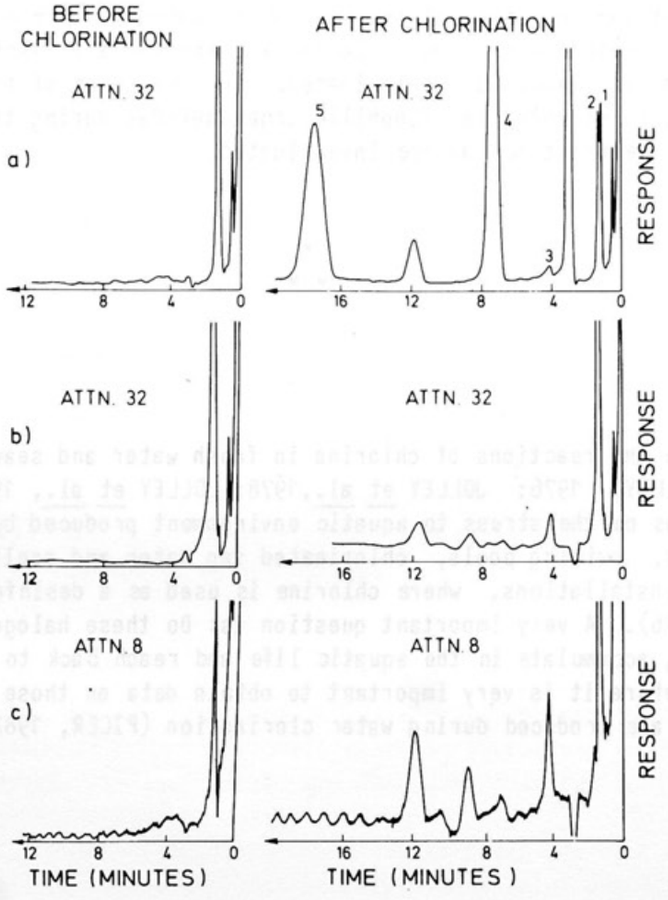


Fig. 1. - The EC chromatograms obtained before and after laboratory chlorination of the wastewater sample: a) Effluent IV; b) Effluent V; c) Effluent VI.
Identified peaks: 1 - CHCl3; 2 - C2Cl4; 3 - CHCl2Br; 4 - CHClBR2; 5 - CHBr3.

Glass column packed with 10 % SP 1000 on Chromosorb WHP 100/120 mesh was used isothermally at 328° K. Some organohalides ($CHCl_3$; $CHCl_2Br$: $CHClBr_2$; $CHBr_3$ and C_2Cl_4) were identified by comparison with the standards. The concentrations of these pollutants were calculated from the calibration curves obtained by additing standards into redistilled water samples.

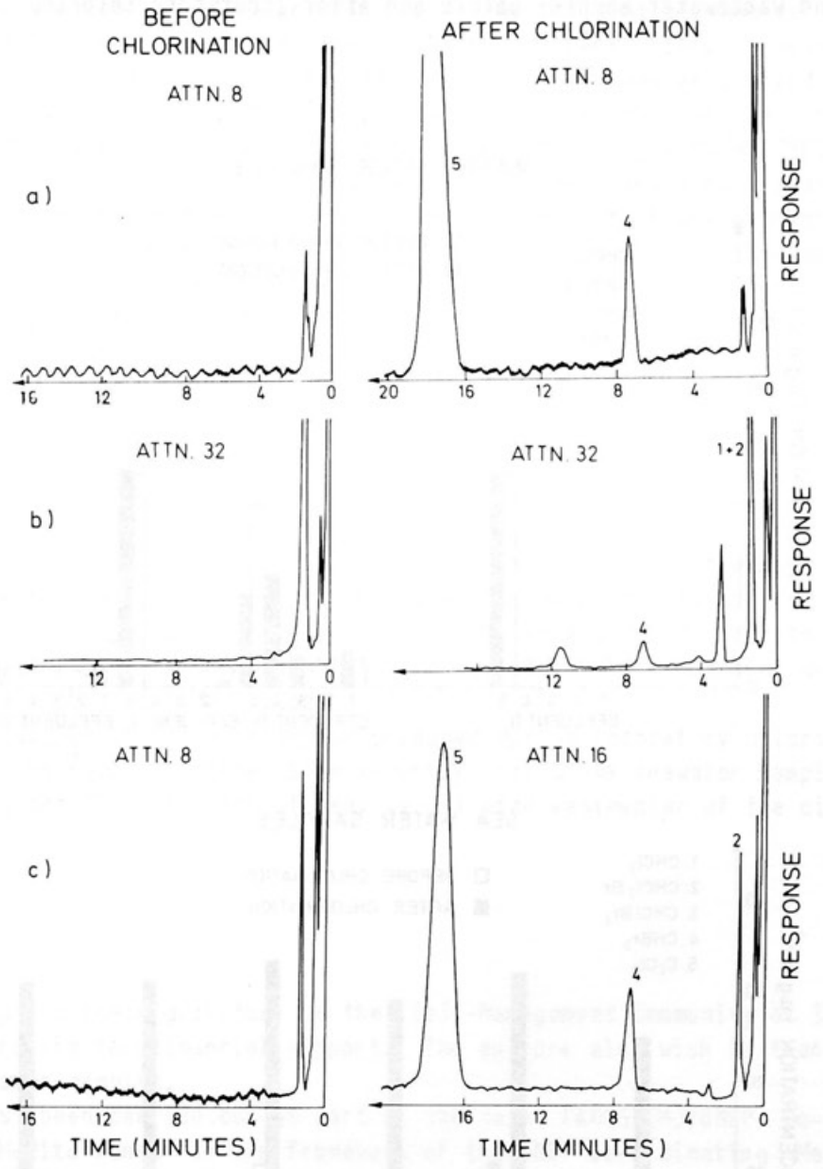


Fig. 2. - The EC chromatograms obtained before and after laboratory chlorination of the water samples: a) Station I; b) Effluent II; c) Station II.
Identified peaks: 1 - $CHCl_3$; 2 - C_2Cl_4; 3 - $CHCl_2Br$; 4 - $CHClBr_2$; $CHBr_3$.

Results and discussion

Figure 1 presents the EC chromatograms of the three wastewater effluents extracts obtained before and after chlorination. As seen, the effluent IV sample is greatly affected by seawater and therefore the production of brominated trihalometanes during the chlorination process is significantly higher than in the other samples presented. The EC chromatograms of the clean water sample (Station I), the wastewater sample (Effluent II) and the seawater sample (Station 2), located very

close to the wastewater discharge into the sea, obtained before and after chlorination process are presented in Fig. 2. Although the coastal waters at Station 2 are under a strong influence of wastewaters heavily polluted with organic materials, the pattern EC chromatograms does not significantly differ from EC chromatograms of the seawater sample collected in the relatively clean area.

Figure 3 shows the comparison of concentrations of identified light volatile halogenated hydrocarbons in seawater and wastewater samples before and after laboratory chlorination.

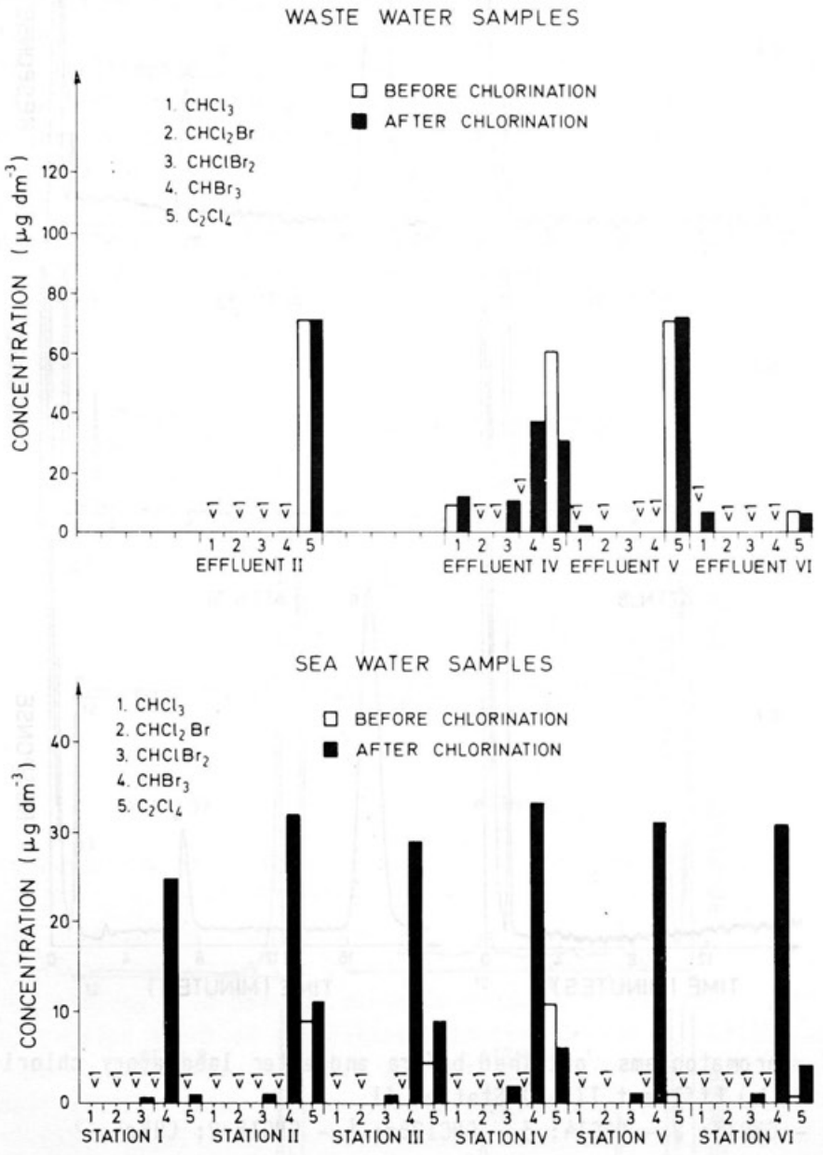


Fig. 3. - Concentrations of identified light volatile chlorinated hydrocarbons in wastewater and seawater samples before and after laboratory chlorination.

As Figure 3 shows tetrachlorethylen appeared in all wastewater samples in the concentration range of 60-70 ug dm-3 as well as in Effluent VI whose concentration of this pollutant was about 10 times lower. Concentrations of this pollutant after the discharge of wastewater into the sea are significantly lower in seawater samples collected at the above stations, obviously owing to dilution

processes. Some other identified light volatile organohalides are produced during the chlorination process, and as excepted the main chlorinated product in seawater is $CHBr_3$. In wastewater samples concentrations of trihalomethanes varied more than in seawater samples. The production of $CHCl_3$ is in the range of 1-13 $\mu g\ dm^{-3}$, depending on the amounts and the organic materials present. However, production of these halomethanes is influenced also by the concentration of bromide ions (MINEAR and BIRD, 1980). The production of $CHBr_3$ and $CHClBr_2$ in the Effluent IV samples is significantly higher compared to other wastewater samples. Relatively higher concentrations of brominated trihalomethanes are obviously produced during sampling procedure when wastewaters were mixed with estuarine waters containing significant amounts of bromide ions which during chlorination process formed more of brominated compounds. The presence of relatively high concentrations of $CHCl_3$ in these wastewater samples before chlorination could be explained wastewaters discharged by a small paper mill into the common wastewater channel marked as Effluent IV. The comparison of the production of identified trihalomethanes in clean seawater (Station 1) during laboratory chlorination and the other seawater samples which are under the influence of heavily polluted wastewaters, did not reveal any significant differences. The only significant difference observed was the concentration of tetrachlorethylen, which however is not produced during the chlorination process.

Conclusion

From the preliminary results obtained during our investigation of organohalogen materials that have formed during water chlorination we can make the following conclusions :

1. - Concentrations of organohalogenide volatile materials after laboratory chlorination process of wastewater and seawater samples are significantly higher in comparison with concentrations of these materials before chlorination process.

2. - The production of Trihalomethanes produced during laboratory chlorination of relatively clean seawater samples does not differ significantly from those seawater samples collected from the stations which are under the influence of heavily polluted wastewater of the city of Rijeka.

Acknowledgement

The authors express their gratitude to the Self-Management Community of Interest for Scientific Research of S.R. Croatia for financial support. The authors also wish to thank Mr. S. Mudri for his help in providing water samples.

This work has been carried out as part of the joint FAO(GFCM)/UNEP Co-ordinated Project on Pollution in the Mediterranean in the framework of the UNEP Co-ordinating Mediterranean Pollution Monitoring and Research Programmes.

References

DUURSMA E.K., P. PARSI (1976). - Persistence of Total and Combined Chlorine in Sea Water. Neth. J. of Sea Res., 10,192-214.

GORCHEV H. (1978). - Conference Adjournement: Closing Remarks, in: Water Chlorination, Environmental Impact and Health Effects, Vol. 2, R.L. Jolley, H. Gorchev and D.H. Hamilton, Jr. Eds. Ann Arbor Sci., Ann Arbor, Mich., pp 887-890.

JOLLEY R.L., (Ed.) (1976). - Water Chlorination, Environmental Impact and Health Effects, Vol. 1, Ann Arbor, Sci., Ann Arbor, Mich.

JOLLEY R.L., H. GORCHEV, D.H. Jr. HAMILTON (Eds.) (1978).- Water chlorination,Environmental Impact and Health Effect, Vol. 2, Ann Arbor Sci., Ann Arbor, Mich.

JOLLEY R.L., W.A. BRUNGS, R.B. CUMMING, V.A. JACOBS (Eds.) (1980). - Water chlorination, Environmental Impact and Health Effects, Vol. 3, Ann Arbor Sci., Ann Arbor, Mich.

MINEAR R.A. and J.C. BIRD, (1980). - Temperature, pH and Trihalomethanes: Impact of Bromide Ion Concentration on Yield, Species Distribution, Rate of Formation and Influence of Other Variables,in: Water Chlorination, Environmental Impact and Healths Effects, Vol. 3, R.L. Jolley, W.A. Brungs, R.B. Cumming, V.A. Jacobs (eds.) Ann Arbor Sci., Ann Arbor, Mich., pp 151-160.

NORIN N. and L. RENBERG. - (1980). Determination of trihalomethanes (THM) in water using efficiency solvent extraction, Water Res., 14, 1397-1402.

PICER M. (1982). - Methodological Problems and Actual Pollutant Levels C. Halogenated Hydrocarbons (Review paper), VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M., pp. 445-448.

CHLORINATED INSECTICIDES AND POLYCHLORINATED BIPHENYLS IN WATER, SEDIMENT AND MUSSEL OF THE ROVINJ COASTAL WATERS

by

N. PICER, M. PICER and N. MIKAC
Center for marine Research Zagreb
The Rudjer Boskovic Institute
POB 1016 - 41001 Zagreb (Yugoslavia)

Abstract

Persistent chlorinated insecticides and polychlorinated biphenyls were analysed over the period of several years in water, sediment and mussels (Mytilus galloprovincialis) from the Rovinj area. Analyses were performed on subsurface water samples collected at the 1 m depth. Sediment samples were collected by a grab sampler while mussels were manually collected in intertidal waters.

The results of monitoring chlorinated hydrocarbons in various parts of investigated marine ecosystem are discussed concerning the influence of local pollution sources.

*
* *

Introduction

Chlorinated insecticides and polychlorinated biphenyls belong to the most persistent organic pollutants in the human environment. Their toxicological and harmful effects on aquatic and terrestrial ecosystems are well documented (EDWARDS, 1975). Investigating the distribution of these organics in the delicate and endangered marine ecosystems, as the Rovinj area in the North Adriatic, is very important in order to better understand the mechanisms of their transport through the marine environment. Rovinj area coastal waters from the western coast of Istria peninsula have recently been polluted by chlorinated insecticides from a relatively close and very important agricultural area in the northern Italy. On the other hand, it is well known that significant amounts of persistent chlorinated hydrocarbons are polluting marine environment through various urban and industrial waste waters and solid discharges, marine trafic activities, and other various human activities (GOLDBERG, 1976; PICER et al., 1978; PICER, 1979).

The aim of this work is to investigate the fate of chlorinated insecticides and PCBs in relatively small marine ecosystem which is under a strong influence of open North Adriatic waters.

Methods

Water analysis

Concentrations of chlorinated hydrocarbons from seawater samples were determined by solvent extraction with petroleum ether and some samples were treated by using XAD-2 resin column (PICER and PICER, 1980). The extract was dried by passing it through a Na_2SO_4 anh. column, evaporated to 1 ml and cleaned up on an alumina column. PCBs were separated from organochlorine insectcides on a miniature silica gel column (PICER and AHEL, 1978). Special attention was paid to obtain blank values, as small as possible (PICER et al., 1982). Eluates concentration 0.1 ml, were analysed by gas chromatography.

Sediment analysis

Sediments were collected by means of a standard grab sampler. Representative aliquots of 10 g were extracted in a Soxhlet extractor during a four-hour period with 1:1 volume mixture of acetone and n-hexane. Extracts were evaporated to 1 ml and cleaned by passing them through the column of activated alumina and by means of the KCN solution in acetone. Separation and further treatment were carried out as described above.

Mussel analysis

Mussels (Mytilus galloprovincialis) were collected manually in intertidal or very shallow waters at various localities. The analytical procedure included homogenization and extraction with petroleum ether, filtration through a column of Na_2SO_4 anh. and cleaning by using alumina column. Separation and further treatment were carried out as described for water samples.

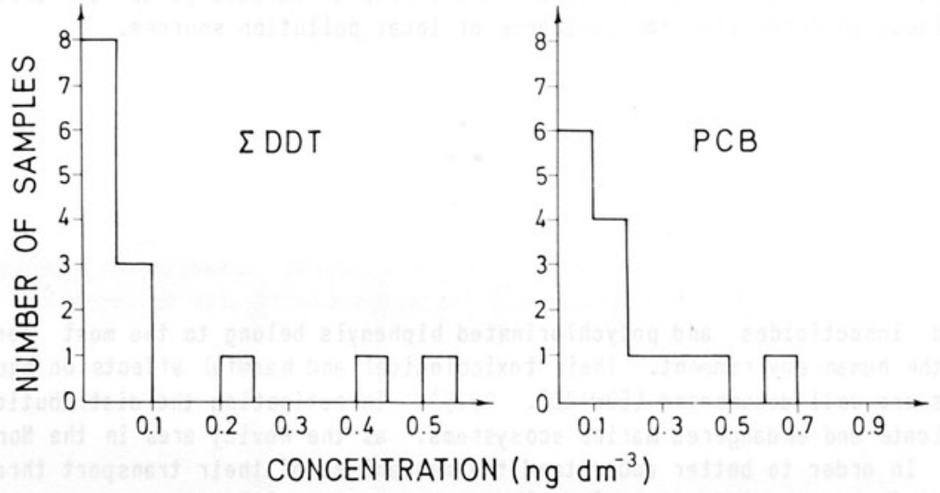


Fig. 1. - Distribution of frequencies of chlorinated hydrocarbons concentrations in seawater samples.

Results and discussion

The distribution of the results obtained from sea water samples collected in the Valalta Bay near Rovinj is shown in Fig. 1 (position of the Valalta Bay is presented in Fig. 2). As seen, most of the results lie beyond the analytical sensitivity of the method used. It has to be stressed that some

results presented in Fig. 1 were obtained by analysing only 10 litre samples, and as a matter of fact also some "higher" values are probably even higher because of a lower sensitivity limit and blank influence (PICER and PICER, 1978). All in all, it could be concluded that in outer waters of the Rovinj area concentrations of total DDT lie between 0.05 and 0.1 ng dm-3 and 0.1 and 0.2 ng dm-3 of PCBs.

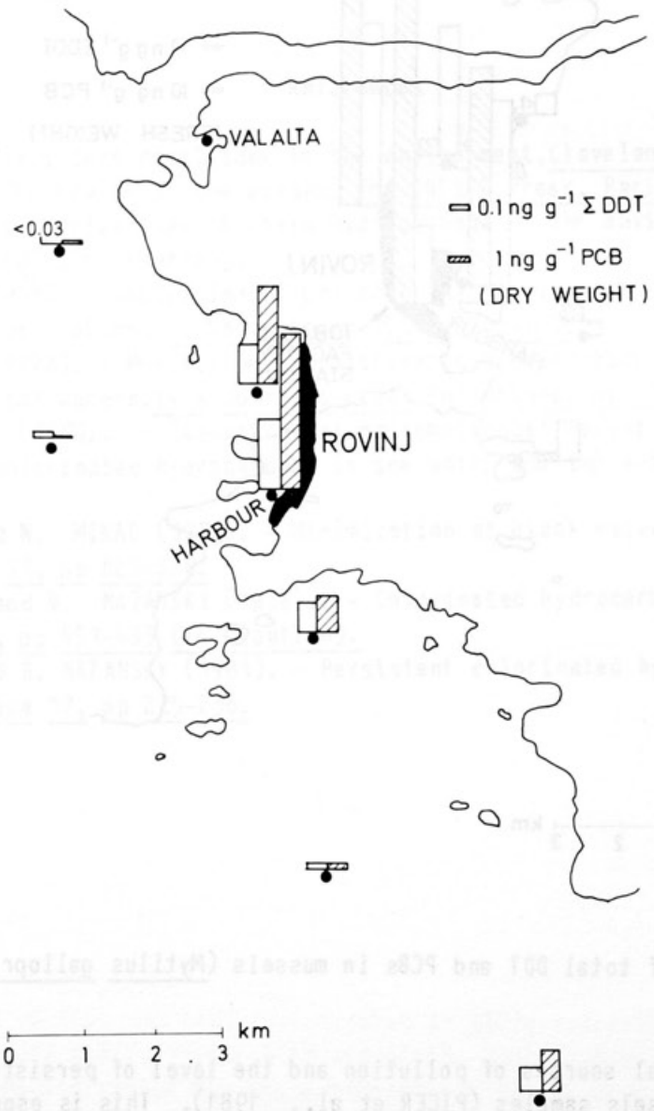


Fig. 2. - Concentrations of total DDT and PCBs in sediments (arithmetic mean).

Because sediments are less changeable in comparison with the water column, the sample collection at chosen stations was generally performed only once. The results obtained are presented in Fig. 2. When at the same station the sampling analyses were performed several times, the results are presented as arithmetic means. Concentrations of chlorinated hydrocarbons found in mussels (Mytilus galloprovincialis) are presented in Fig. 3. Collection of mussel samples was performed at least three times and the results presented are arithmetic means. From the figures given it can be concluded that the investigated area can be divided into two different zones. The first one is located near local sources of pollution (harbour, industrial and urban waste water discharges) and the second one is located only several kilometers from the town, where concentrations of investigated pollutants are about ten to twenty times lower in comparison with the first zone. Also, the results confirm the

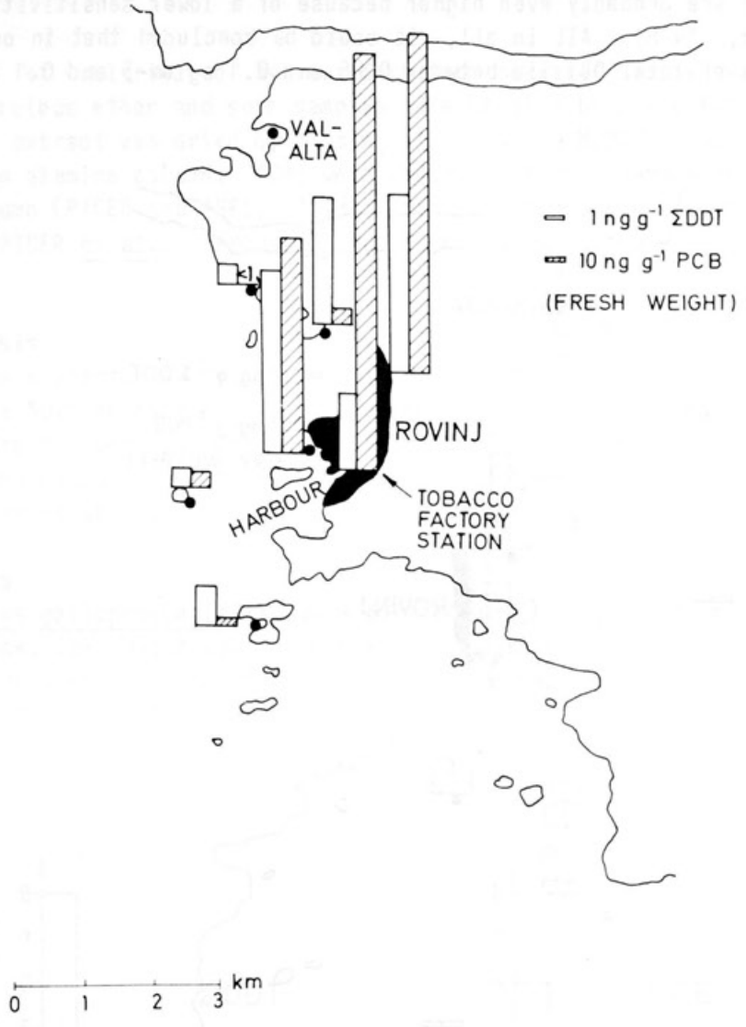


Fig. 3. - Concentrations of total DDT and PCBs in mussels (Mytilus galloprovincialis) as arithmetic mean.

relationship between the local sources of pollution and the level of persistent chlorinated hydrocarbons in the sediment and mussels samples (PICER et al., 1981). This is especially apparent for the PCBs concentrations. Namely, concentrations of PCBs are significantly higher in comparison with DDT and its analogues in the samples collected in the vicinity of the local pollution sources. In the samples collected far from the local sources of pollution, concentrations of total DDT were almost as those as PCBs and in some samples even higher.

Conclusion

From the presented results of concentrations obtained for investigated pollutants in sea water, sediments, and mussels samples, it is evident that hydrodnamic characteristics of the Rovinj area permit high dilutions of chlorinated hydrocarbons, especially in its northern part. Higher concentrations of pollutants are observed only in samples collected very close to local sources of pollution.

Acknowledgement

The authors express their gratitude to the Self-Management Community of Interest for Scientific Research of S.R. Croatia for financial support. This work has been carried out as a part of the joint FAO(GFCM)/UNEP Coordinated Project on Pollution in the Mediterranean in this framework of the UNEP Coordinating Mediterranean Pollution Monitoring and Research Programmes.

References

EDWARDS C.A. (1975). - Persistent pesticides in the environment,Cleveland, USA, CRC Press.

GOLDBERG E.D. (1976). - The health of the oceans. The UNESCO Press, Paris.

PICER M. (1979). - Halogen derivatives of chain hydrocarbons in the environment, (a review).Pomorski zbornik 17, pp 577-601 (in croatian).

PICER M. and M. AHEL (1978). - Separation of polychlorinated biphenyls from DDT and its analogues on a miniature silica gel column. J. Chromatogr. 150 pp 119-127.

PICER N. and M. PICER (1978). - Monitoring of chlorinated hydrocarbons in water and sediments of the North Adriatic coastal waters.IVes Journees Etud. Pollutions, pp 133-136, Anthalya, C.I.E.S.M.

PICER N. and M. PICER (1980). - Evaluation of macromolecular resins for the determination of low concentrations of chlorinated hydrocarbons in sea water and tap water. J. Chromatogr. 193 pp 357-369.

PICER N., M. PICER and N. MIKAC (1982). - Minimization of blank values of the miniature silica gel column.Chemosphere 11, pp 825-828.

PICER M., N. PICER and B. NAZANSKY (1978). - Chlorinated hydrocarbons in the sea, (a review). Pomorski zbornik 16, pp 451-483 (in croatian).

PICER M., N. PICER and B. NAZANSKY (1981). - Persistent chlorinated hydrocarbons in the Rijeka Bay. Thalassia Jugoslavica 17, pp 225-236.

POLYCHLORINATED BIPHENYLS IN NEAR SEA ATMOSPHERIC SAMPLES FROM THE MEDITERRANEAN IN 1975 TO 1977

by

Jean-Pierre VILLENEUVE
International Laboratory of Marine Radioactivity
Musée Océanographique, M.C. - 98000 Monaco (Principality of Monaco)

Abstract

Atmospheric polychlorinated biphenyls were monitored over a two-year period at a coastal sampling station in Monaco and from ships in the Mediterranean Sea. PCBs were adsorbed on to silicone-coated glass beads using a high volume air pump. Results from the open Mediterranean Sea ranged from 0.04 to 0.3 ng/m3. Although sample numbers were small and spatial coverage was wide, these preliminary data suggest lower atmospheric concentrations in 1977 compared to 1975 samples. Results from the coastal sampling station in Monaco ranged from 0.03 to 1.0 ng/m3. The high values seemed to be related to warm temperatures and wind directions from industrialized areas. These results are presented as background results for comparison with current and future monitoring efforts in the Mediterranean.

*
* *

Introduction

Atmospheric transport of PCBs has been investigated in different areas of the world (HARVEY and STEINHAUER, 1974; BIDLEMAN and OLNEY, 1974a; GIAM and ATLAS, 1982; TANABE et al., 1982) but there is a lack of data for the Mediterranean Sea where atmospheric inputs seem to play an important role (BURNS and VILLENEUVE, 1982; ELDER et al., 1976).

In 1975 a programme was initiated for measurements of the concentrations of PCBs in the near atmosphere in order to assess the possibility of a correlation between PCB concentration and wind direction. These data are presented as background measurements for current and planned monitoring programmes in the Mediterranean.

The atmospheric transport of PCBs can be achieved in vapour or particulate phases. By empirical definition the fraction which passes through a glass-fibre filter is called vapour phase, and that which is retained is defined as particulate. Determinations by various high volume methods have usually shown that more than 90 per cent of the airborne PCBs pass through a glass-fibre filter and are collected on appropriate adsorbant materials (HARVEY and STEINHAUER, 1974a, b).

Date	Temperature	Volume of Sample (m^3)	wind direction	PCB (Ar. 1254) ng/m^3
75-08-05	25.6	190	N/NW	0.5
-08-25	21.5	200	N/NW	0.5
-10-03	20.1	370	N/NW	1
-10-06	18.9	400	N/NW	0.4
-10-07	18.9	390	E/NE	0.4
-10-21	14.5	320	E	0.5
-10-28	16.6	400	N/NW	0.2
-12-01	11.9	410	N	0.3
-12-08	11.3	410	N	0.4
-12-09	11.2	410	N/NW	0.4
-12-10	11.5	430	E	0.3
76-01-14	10.4	360	N	0.3
-01-26	6.4	380	SW	0.1
-01-27	4.5	280	N/NW	0.04
-01-28	4.8	400	N	0.04
-01-29	6.1	390	N	0.05
-02-02	8.2	390	NW	0.08
-02-03	10.2	390	N/NW	0.07
-02-04	9.3	390	N/NW	0.07
-02-05	10.7	380	E/NE	0.04
-02-06	13	380	NE	0.03
-02-09	6.1	410	E	0.07
-02-10	8.2	410	N/NW	0.05
-02-11	9.1	410	N/NW	0.08
-02-12	9.3	410	NW	0.05
-11-09	12	340	N/NW	0.96
-11-15	11.5	320	N/NW	0.5
77-01-25	10.5	430	SW	0.47
-01-31	9.5	410	N/NW	0.48
-02-03	8	410	N/NW	0.47
-02-07	10	380	E/NE	0.91
-02-10	12	510	W/SW	0.66
-02-14	10	430	S/SW	0.38
-02-23	11	380	E/NE	0.63
-02-25	13	510	W/SW	0.45
-03-02	10	470	N/NW	0.41

Table 1. - PCB concentrations in the near ocean atmosphere (Musée Océanographique, Monaco).

	Sampling station		(PCB) ng/m^3
Western Basin			
September 1975	Cavalaire Harbour	OC	0.3
	42°30'N 6°30'E	OC/1	0.3
	40°30'N 6°30'E	OC/3	0.2
	37°30'N 6°30'E	OC/6	0.2
July 1977	41°12'N 8°41'E	CS 41	0.08
	40°00'N 7°30'E	CS 44	0.05
Tyrrhenian sea			
September 1975	40°00'N 11°40'E	OC/9	0.1
Ligurian sea			
September 1975	42°17'N 9°45'E	OC/13	0.3
Adriatic sea			
November 1977	Off Split	CAL	0.1
	Off Mljet	CAL	0.04
	40°20'N 18°54'E	CAL 1	0.08

Table 2. - Over ocean air concentration of PCBs in the Mediterranean Sea (Aroclor 1254 equivalents).

Method

For this work, a high volume air sampler (Bendix Corp.) was used. After thorough precleaning, a cast aluminium filter holder/adsorbant reservoir was filled with 330 grams of glass beads (4 mm diameter) coated with 0.25 % OV 17 silicone oil which traps PCBs from air. The adsorbant reservoir was attached to the pump which was run at a flow rate of 82-88 m3 per hour. The pump was run so that a volume of 200-400 m3 of air was sampled. Samples used for correlation experiments were collected on top of the Musée Océanographique in Monaco at about 100 m above sea-level. The pump was facing the sea.

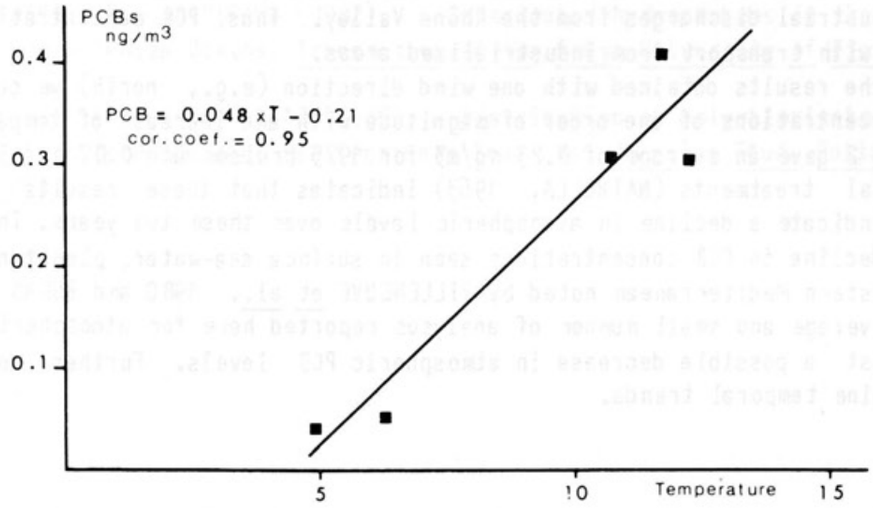


Fig.1a: PCB's concentrations versus temperature for winds blowing from North.

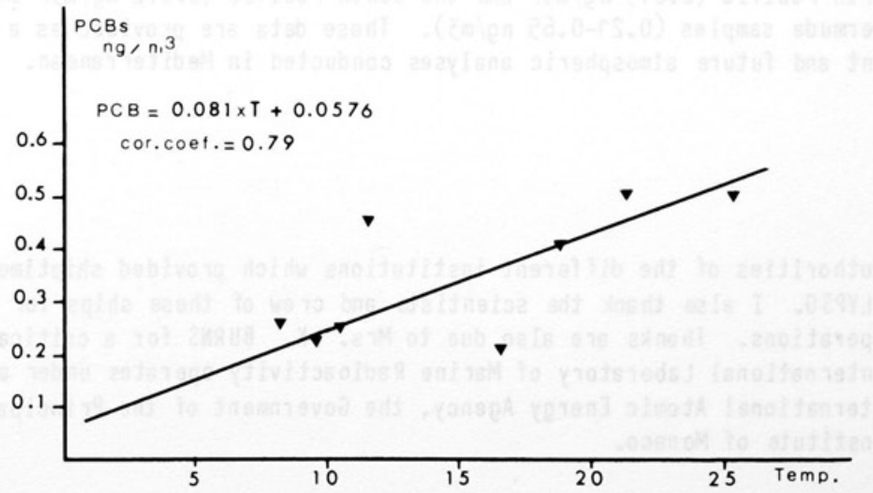


Fig.1b: PCB's concentrations versus temperature for winds blowing from N/NW.

Aboard ships, samples were collected at the bow and oriented into the wind. A retention efficiency experiment was realized with spike beads and was found to be 90 per cent (ELDER et al., 1976). Blanks were obtained by handling a batch of coated beads in an identical procedure but without running the pump. After sampling, the adsorbant holder was wrapped in foil and frozen for analysis in the laboratory. Results are expressed as Aroclor 1254 equivalent.

Results and discussion

Results of the analysis for the correlation experiments are reported in table 1. Table 2 gives results of samples taken during various cruises aboard research ships in the Mediterranean Sea: NO OCEONE in September 1975, BO CORNIDE DE SAAVEDRA in July 1977 and RV CALYPSO in November 1977. Most of the samples collected between August 1975 and March 1977 were taken under north and north/north-west wind directions. Plotting PCB concentrations versus temperatures (Fig. 1) gives a correlation coefficient of 0.95 for winds blowing from the north and 0.79 for winds blowing from north/north-west. This difference can be explained by the fact that wind coming from the north passes over the Alps where there are no particular industries. On the contrary, winds coming from north/north-west are loaded with industrial discharges from the Rhône Valley. Thus, PCB concentrations in atmospheric samples correlated with transport from industrialized areas.

Considering the results obtained with one wind direction (e.g., north) we could note and increase in PCB concentrations of one order of magnitude with and increase of temperature of 20 °C. Results from table 2 gave an average of 0.23 ng/m3 for 1975 cruises and 0.07 ng/m3 for 1977 cruises. Applying statistical treatments (NATRELLA, 1963) indicates that these results are statistically different and may indicate a decline in atmospheric levels over these two years. This would correlate with the apparent decline in PCB concentrations seen in surface sea-water, plankton and sediment trap material in this western Mediterranean noted by VILLENEUVE et al., 1980 and BURNS et al., 1984. But the wide spatial coverage and small number of analyses reported here for atmospheric samples can only be used to suggest a possible decrease in atmospheric PCB levels. Further monitoring must be conducted to determine temporal trends.

Conclusions

Concentrations reported here were of the same order as values reported by TANABE et al. (1982) for the Central Pacific (0.19-0.32 ng/m3) and Antarctic Ocean (0.11-0.23 ng/m3), by GIAM and ATLAS (1982) for the North Pacific (0.049 ng/m3) and the South Pacific (0.012 mg/m3) and by BIDLEMAN and OLNEY (1974a) for Bermuda samples (0.21-0.65 ng/m3). These data are provided as a background for the comparison of current and future atmospheric analyses conducted in Mediterranean.

Acknowledgements

I thank the authorities of the different institutions which provided shiptime on BO CORNIDE DE SAAVEDRA and RV CALYPSO. I also thank the scientists and crew of these ships for their assistance during sampling operations. Thanks are also due to Mrs. K. BURNS for a critical reading of the manuscript. The International Laboratory of Marine Radioactivity operates under a tripartite agreement between the International Atomic Energy Agency, the Government of the Principality of Monaco and the Oceanographic Institute of Monaco.

References

BIDLEMAN T.F., C.E. OLNEY (1974a). - Chlorinated hydrocarbons in the Sargasso sea atmosphere and surface water. Science 183, 516-518.

BIDLEMAN T.F., C.E. OLNEY (1974b). - Bull. Environm. Contam. Toxicol. 11, p. 442.

BURNS K.A., J.P. VILLENEUVE (1982). - Dissolved and particulate hydrocarbons in water from a spring

sampling of the Var river estuary (S. France). Toxicol. Environm. Chem. 5, 195-203.

BURNS K.A., J.P. VILLENEUVE, S.W. FOWLER (1984). - Fluxes and residence times of hydrocarbons in the coastal Mediterranean: how important are the biota? Estuar. Cstl. Shelf Sci. (in press).

ELDER D.L., J.P. VILLENEUVE, P. PARSI, G.R. HARVEY (1976). - Polychlorinated biphenyls in sea-water, sediment and over-ocean air of the Mediterranean. In: Activities of the International laboratory of Marine Radioactivity, 1976 Report. I.A.E.A., Vienna, 136-151.

HARVEY G.R., W.G. STEINHAUER (1974). - Atmospheric transport of poly-chlorinated biphenyls to the North Atlantic. Atmospheric Environm. 8, p. 777.

NATRELLA M.G. (1963). - Experimental Statistics Handbook 91. National Bureau of Standards, August 1963. Reprinted October 1966.

TANABE S., M. KAWANO, R. TATSUKAWA (1982). - Chlorinated hydrocarbons in the Antartic, Western Pacific and Eastern Indian Oceans. Transaction of the Tokyo University of Fisheries, n° 5, March 1982, 97-109.

VILLENEUVE J.P., D.L. ELDER, R. FUKAI (1980). - Distribution of polychlorinated biphenyls in sea-water and sediments from the open Mediterranean Sea. Ves Journées Etud. Pollutions, C.I.E.S.M., Cagliari, 251-256.

HYDROCARBURES NON VOLATILS A L'INTERFACE MER-AIR EN MEDITERRANEE OCCIDENTALE EN 1983

par

SICRE Marie-Alexandrine, HÔ Rozenn
MARTY Jean-Claude
SCRIBE Pierre et SALIOT Alain
Laboratoire de Physique et Chimie Marines, LA CNRS n° 353
Université Pierre et Marie Curie, tour 24
4, place Jussieu, 75230 PARIS Cedex 05 (France)

Résumé

Au cours de la campagne PHYCEMED II de septembre-octobre 1983, en Méditerranée occidentale, en période de faible productivité biologique, ont été échantillonnées à plusieurs stations hauturières des eaux prélevées en surface entre 35 m et 0,2 m, ainsi que la microcouche ultrasuperficielle. Les échantillons d'eau ont été filtrés afin de séparer les substances organiques en solution de celles associées aux particules en suspension (> 0,7 um). Les hydrocarbures extractibles, non volatils ont été extraits par le chloroforme et le dichlorométhane et séparés par chromatographie liquide. Les hydrocarbures non aromatiques ont été analysés par chromatographie en phase gazeuse sur colonne capillaire afin d'obtenir une distribution fine des n-alcanes par nombre d'atomes de carbone.

Les concentrations enregistrées sont faibles, variant pour les eaux superficielles de 0,3 à 5 ugl-1 pour les n-alcanes dissous (de 0,9 à 20,4 ug l-1 pour les hydrocarbures non aromatiques) et de 0,03 à 0,06 ug l-1 pour les n-alcanes particulaires (de 0,2 à 0,7 ug l-1 pour les hydrocarbures non aromatiques).

La comparaison de ces données avec celles obtenues en 1981 dans les mêmes zones, mais en période de forte activité biologique, montre une tendance à une augmentation des concentrations en hydrocarbures dissous et une diminution des concentrations en hydrocarbures particulaires.

Sur l'ensemble des stations, on ne constate pas d'accumulation des hydrocarbures dissous dans la microcouche de surface. On note même un appauvrissement d'un facteur 20 au large du delta du Rhône. A l'opposé, la microcouche de surface est très enrichie en hydrocarbures particulaires, mélange de produits pétroliers qui rendent compte des fortes valeurs du rapport composés non résolus/n-alcanes et de composés naturels d'origine continentale.

*
* *

Abstract

During the PHYCEMED II cruise in september-october 1983 in the Western Mediterranean Sea, during a period of low biological productivity, samples of surficial waters collected between 35 m and 0.2 m and surface microlayer samples have been obtained at several open sea stations. Water samples have

been filtered to obtain dissolved organic substances and organic compounds associated with particles (> 0.7 um). Extractable, non volatile hydrocarbons have been extracted using chloroform and dichloromethane and separated by liquid chromatography. Non aromatic hydrocarbons have been analysed by capillary gas liquid chromatography in order to provide a precise distribution of n-alkanes as a function of carbon number.

Concentrations observed for superficial waters are low, varying from 0.3 to 5 ug l-1 for dissolved n-alkanes (from 0.9 to 20.4 ug l-1 for non aromatic hydrocarbons), and from 0.03 to 0.06 ug l-1 for particulate n-alkanes (from 0.2 to 0.7 ug l-1 for non aromatic hydrocarbons).

A comparison of these values with those obtained in 1981, in the same areas but during a period of high biological productivity shows a general tendancy to an increase of the concentrations in particulate hydrocarbons.

Dissolved hydrocarbons are not accumulated in the surface microlayer. The depletion could reach high values, up to 20 for a station off the Rhône delta. But, the microlayer is very enriched in particulate hydrocarbons, consisting of a mixture of petroleum residues which lead to high values observed for the ratio non-resolved components/n-alkanes and of naturally continentally-derived compounds.

*
* *

Introduction

La mer Méditerranée est soumise à un problème grave de pollution, dû à l'accroissement des apports anthropogéniques côtiers de ses pays riverains en voie d'industrialisation, aux rejets au large liés au trafic maritime et aux apports éoliens de l'Europe industrielle (U.N.E.P., 1981). Pour ce qui concerne les hydrocarbures, les apports annuels de pétrole brut sont estimés à 0,5 à 1 million de tonnes par an (LE LOURD, 1977). Ce contexte pour une mer semi-fermée est particulièrement critique et explique le développement récent de programmes de surveillance de la "santé de la Méditerranée".

La campagne PHYCEMED du Suroît en Méditerranée occidentale en septembre-octobre 1983 a permis de dresser un constat de l'état de pollution des eaux superficielles en hydrocarbures, en période de faible production biologique. Ces données viennent compléter le constat effectué dans les mêmes zones au cours de la campagne PHYCEMED I en avril 1981, en période de forte activité planctonique (HO et al., 1982). Ceci complète également les données récentes obtenues ponctuellement dans les zones côtières par divers groupes de recherche : au large du delta de l'Ebre (ALBAIGES et al., 1984), en mer Ligure, au large de Monaco (BURNS & VILLENEUVE, 1983) et au large de Nice (GOUTX & SALIOT, 1980 ; SALIOT et al., 1983), au large de l'estuaire du Var (BURNS & VILLENEUVE, 1982), au large des côtes provençales (GUILIANO et al., 1981). Pour une revue, voir SALIOT & MARTY, 1984.

Pendant ces campagnes, ont été collectés dans des conditions ultra-propres des échantillons de microcouche de surface à l'aide d'un tamis métallique et d'eaux prélevées en surface.

Les analyses d'hydrocarbures ont été faites en séparant les composés dissous et associés aux particules en suspension. Après isolement des hydrocarbures non aromatiques, leurs concentrations et la distribution des n-alcanes par nombre d'atomes de carbone ont été déterminées par chromatographie en phase gazeuse sur colonne capillaire, afin de pouvoir utiliser des critères de différenciation entre hydrocarbures d'origine biologique et anthropique. Les résultats sont discutés en termes d'évaluation des états de pollution de la Méditerranée à différentes saisons et en termes d'accumulation des différentes contributions dans la microcouche de surface, frontière entre l'eau et l'air, zone privilégiée pour les échanges de matière entre l'océan et l'atmosphère.

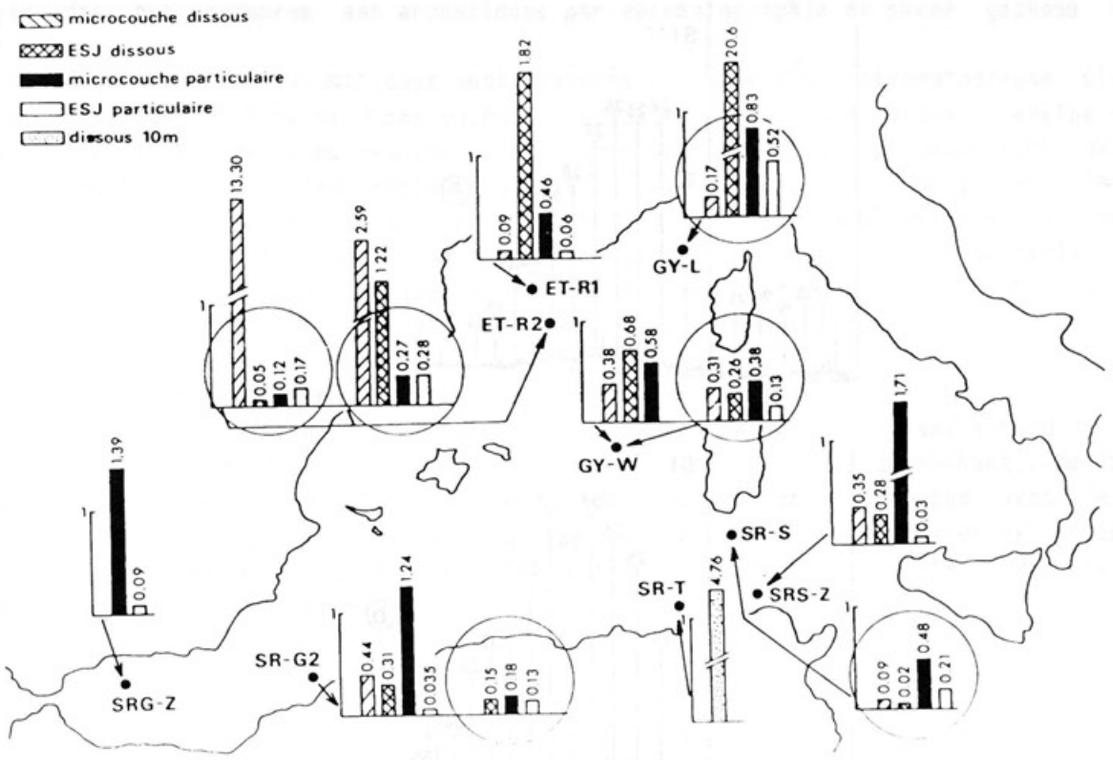


Fig. 1: Stations de prélèvement occupées pendant les campagnes PHYCEMED I (avril 1981) et PHYCEMED II (septembre-octobre 1983). Pour chaque station sont reportées les teneurs en n-alcanes dissous et particulaires (en µg/l) pour l'eau superficielle et la microcouche de surface. Les distributions entourées d'un cercle sont relatives à PHYCEMED I.

= n-alcanes dissous dans la microcouche

= n-alcanes particulaires dans la microcouche

= n-alcanes dissous dans l'eau superficielle

= n-alcanes particulaires dans l'eau superficielle

Matériel et méthodes

Sites de prélèvement (Fig. 1) et échantillonnage

Les échantillons ont été collectés en Méditerranée occidentale, au cours des campagnes PHYCEMED I et II du navire Le Suroît en avril 1981 et en septembre-octobre 1983.

Les échantillons d'eau (20 litres) ont été prélevés à partir d'un zodiac écarté hors de la zone de contamination du navire d'un mille minimum. Les échantillons de microcouche ont été obtenus à l'aide d'un tamis métallique en acier inoxydable qui prélève une couche d'eau de 0,44 mm d'épaisseur. Les échantillons d'eau sous-jacente ont été obtenus par immersion d'une bonbonne de verre à environ 0,2 m sous la surface. Les échantillons d'eaux surperficielles ont été collectés à l'aide d'une bouteille BRES, entièrement métallique. Ils ont été filtrés et immédiatement traités à bord.

Séparation des hydrocarbures dissous et particulaires

Les particules en suspension sont séparées de l'eau par filtration sous pression réduite sur des filtres en fibre de verre Whatman GF/F (porosité 0,7 um). Les filtres sont congelés immédiatement et conservés à -20° C jusqu'à l'analyse au laboratoire. Les lipides particulaires sont extraits des filtres par le dichlorométhane dans un appareil de Soxhlet pendant 30 heures. Les lipides dissous sont extraits dans un premier temps à pH 8 et ensuite à pH 2, après acidification par HCl, par le chloroforme.

Les hydrocarbures non aromatiques sont séparés par chromatographie d'adsorption sur microcolonne (HO et al., 1982).

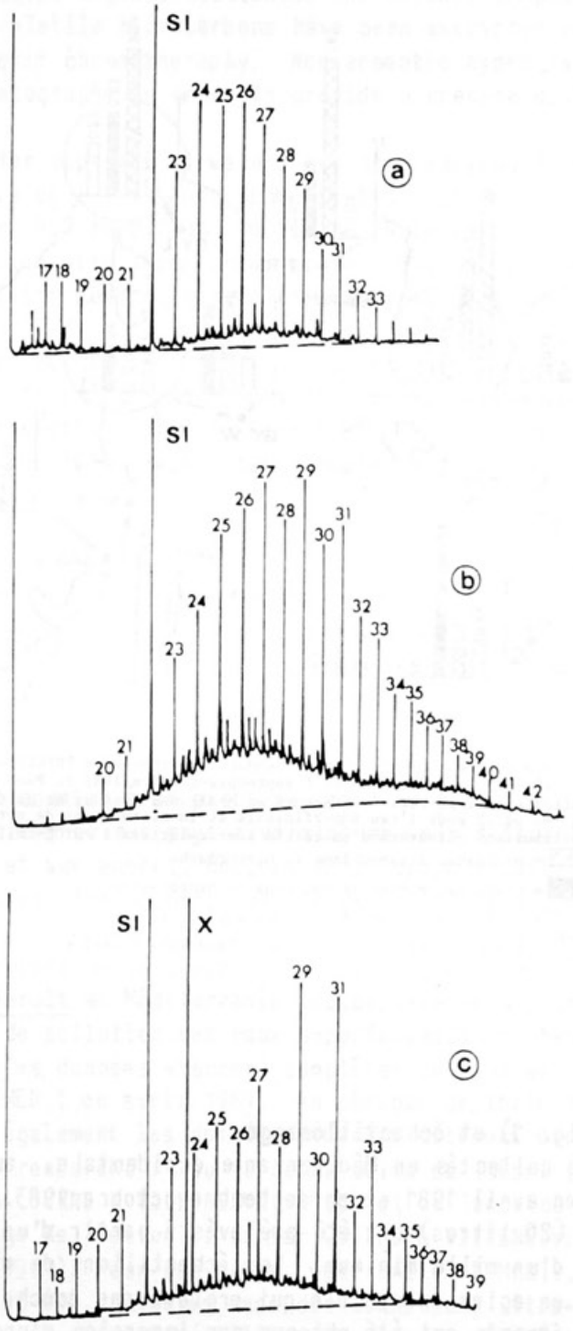


Fig.2[a]: Chromatogramme en phase gazeuse des hydrocarbures non aromatiques présents à l'état dissous dans la microcouche à la station SR-G-2. Pour les conditions expérimentales voir la section matériel et méthodes. Il s'agit ici de la fraction des hydrocarbures extraits à pH 8. Chaque n-alcane est repéré par son nombre d'atomes de carbone. S.I. = standard interne (n-C22).

[b]: Chromatogramme en phase gazeuse des hydrocarbures non aromatiques associés aux particules en suspension dans la microcouche à la station SR-G-2. S.I. = standard interne (n-C22).

[c]: Chromatogramme en phase gazeuse des hydrocarbures non aromatiques associés aux particules en suspension dans la microcouche à la station SR-G-Z. S.I. = standard interne (n-C22) X = composé non identifié

Analyse des hydrocarbures non aromatiques par chromatographie en phase gazeuse sur colonne capillaire

Les hydrocarbures non aromatiques sont analysés à l'aide d'un chromatographe Girdel 3.000, équipé d'un injecteur de Ross et d'une colonne capillaire, en silice fondue, revêtue de CP Sil 5 (Chrompack). Les aires des pics résolus et de l'enveloppe des chromatogrammes sont déterminées à l'aide d'un intégrateur calculateur Varian Vista 401. Les alcanes résolus sont identifiés par comparaison de leur temps de rétention par rapport à ceux de standards analysés dans les mêmes conditions. Ils sont quantifiés par rapport au standard interne, le n-C22, et le même facteur de calibration est utilisé pour l'enveloppe. Rappelons ici que les hydrocarbures volatils et de poids moléculaire inférieur à celui du n-C14 sont perdus au cours du protocole utilisé.

Blancs, limites de détection

Un soin tout particulier a été pris pour éviter toute contamination tant à bord du navire qu'au laboratoire : distillation des solvants, purification des filtres, des desséchants, de la silice,... Les blancs montrent de faibles traces de contaminants qui n'interfèrent pas avec les quantités d'hydrocarbures analysés. La précision obtenue sur les quantités d'hydrocarbures est estimée à approximativement 20 %, compte tenu de l'ensemble des étapes analytiques. La limite de détection est de l'ordre de 0,0015 $\mu g\ l^{-1}$ pour un n-alcane.

Résultats et discussion

En terme méthodologique d'approche de la pollution pétrolière, plusieurs critères existent : teneurs globales estimées par spectrofluorimétrie ou spectrophotométrie U.V. et I.R., teneurs en composés aromatiques, en hydrocarbures non aromatiques, en composés résolus, en n-alcanes, en isoprénoïdes (pristane, phytane), en oléfines,... par chromatographie en phase gazeuse (BLUMER et al., 1972; ALBAIGES et al., 1983 et références citées).

Nous avons choisi, préférentiellement à la mise en oeuvre de méthodes globales, le dosage de composés aux structures chimiques précises : les n-alcanes. Afin d'approcher la quantité globale d'hydrocarbures non aromatiques, nous avons calculé les aires des enveloppes des chromatogrammes (somme des n-alcanes, des pics résolus non-n-alcanes et des composés non résolus) Fig. 2 a, b , c. On peut aussi remarquer qu'actuellement les laboratoires travaillant sur la pollution de la Méditerranée par les hydrocarbures utilisent des méthodes d'extraction et de traitement variées : adsorption sur résine XAD 2 (BURNS & VILLENEUVE, 1983), adsorption sur mousse de polyuréthane (ALBAIGES et al., 1984) ou encore extraction liquide-liquide par le chloroforme (GOUTX & SALIOT, 1980; HO et al., 1982). A titre d'illustration l'extraction liquide-liquide menée à pH 8 deux fois, puis à pH 2 une troisième fois a mis en évidence un comportement non prévisible des hydrocarbures non aromatiques vis-à-vis de l'extraction. En effet, on récupère de 2 à 45 % des n-alcanes en acidifiant l'eau de mer avant la troisième extraction, alors qu'une troisième extraction à pH 8 ne devrait permettre de recouvrer que de 1 à 5 % des n-alcanes (ILIFFE & CALDER, 1974). On peut émettre l'hypothèse d'un piégeage des hydrocarbures par les micelles, libérés dans la phase aqueuse après acidification. En effet, le pourcentage de récupération après acidification est plus fort pour les échantillons de la microcouche de surface (de 22 à 75 %) riche en surfactants, et les distributions des n-alcanes par nombre d'atomes de carbone sont identiques pour les extraits à pH 8 et pH 2.

Nous avons choisi de reporter en terme de concentration les données relatives aux n-alcanes (de n-C14 à n-C37) et aux hydrocarbures non aromatiques non résolus qui constituent l'enveloppe des chromatogrammes (Fig. 2, a, b. c) par exemple. Ces composés constituent la majorité des hydrocarbures pétroliers (plus de 75 %) devant les composés aromatiques.

Tableau 1 : Campagne PHYCEMED II du Suroit en Méditerranée occidentale, septembre-octobre 1983. Concentrations en hydrocarbures dissous et particulaires dans les eaux superficielles prélevées entre 35 m et 0,2 m.

Station et profondeur du prélèvement	HYDROCARBURES DISSOUS				HYDROCARBURES PARTICULAIRES			
	n-alcanes µg/l	HNA totaux[a] µg/l	ENV[b] µg/l	ENV/n-alcanes	n-alcanes µg/l	HNA totaux[a] µg/l	ENV[b] µg/l	ENV/n-alcanes
Bassin nord occidental								
ETR1 , 0,2 m	1,82	2,72	0,71	0,4	0,06	0,68	0,60	10
Bassin central occidental								
GY-W , 0,2 m	0,68	2,08	1,37	2,0	n.a.[c]			
GY-W , 35 m	0,29	0,90	0,60	2,1	n.a.[c]			
Mer d'Alboran								
SRG-Z , 0,2 m	n.a.[c]				0,09	0,68	0,58	6,4
SRG-2 , 0,2 m	0,31[d]	1,62[d]	1,31[d]	4,1	0,03	0,55	0,51	14,6
Zone Sud Sardaigne								
SR-T , 10 m	4,76[d]	20,40[d]	12,88[d]	2,7	n.a.[c]			
SRS-Z , 0,2 m	0,28	1,37	0,97	3,5	0,03	0,22	0,18	5,6

(a) HNA : hydrocarbures non aromatiques

(b) ENV : enveloppe = ensemble des composés non résolus par chromatographie en phase gazeuse

(c) n.a. : non analysé

(d) La troisième extraction à pH 2 n'a pas été effectuée.

Concentrations des eaux superficielles en hydrocarbures

Les concentrations des eaux prélevées en surface (0,2 m, 10 m et 35 m) en hydrocarbures dissous et associés aux particules en suspension sont données dans le tableau 1. Les concentrations en n-alcanes dissous varient de 0,3 à environ 5 ug l-1, ce qui correspond à une variation de la concentration en hydrocarbures non aromatiques de 0,9 à 20,4 ug l-1.

Les concentrations en n-alcanes particulaires sont plus faibles et varient de 0,03 à 0,06 ug l-1, ce qui correspond à une variation de 0,2 à 0,7 ug l-1 pour les hydrocarbures non aromatiques.

A titre comparatif, nous avons reporté sur la figure 1 les concentrations en n-alcanes obtenues en Méditerranée au cours de la campagne PHYCEMED II et de la campagne PHYCEMED I qui s'est déroulée en avril 1981 (HO et al., 1982). Il faut noter que ces deux campagnes se sont déroulées respectivement en période de faible et de forte productivité biologique, comme l'attestent les concentrations et les distributions des acides gras saturés et insaturés dans les eaux de surface (HO, 1982 : SICRE et al., en préparation). D'une manière générale, on remarque que les concentrations en n-alcanes sont faibles, inférieures à 2 ug l-1 pour les deux campagnes. Il faut cependant souligner deux exceptions pour lesquelles les concentrations en n-alcanes dissous sont fortes et indiquent une pollution pétrolière : GY-L en mer Ligure (20,6 ug l-1, en 1981) et SR-T au sud de la Sardaigne (4,98 ug l-1, en 1983).

Cependant ces observations restent ponctuelles et difficiles à interpréter dans le temps en terme d'évolution d'autant que les prélèvements ont été effectués à des profondeurs différentes. Par exemple, la pollution observée à la station SR-T à 10 mètres peut concerner un volume d'eau plus conséquent que ceux des stations échantillonnées très près de la surface. La comparaison des données obtenues en 1981 et en 1983 montre une tendance à une augmentation des concentrations en hydrocarbures dissous et une diminution en hydrocarbures particulaires.

Si l'on considère les n-alcanes, nos résultats sont directement comparables avec ceux de l'équipe de J. ALBAIGES. Les teneurs trouvées en Méditerranée hauturière sont comparables pour les n-alcanes particulaires à celles déterminées à différentes saisons au large du delta de l'Ebre (0,01 - 0,06 ug l-1, ALBAIGES et al., 1984). Par contre les teneurs en n-alcanes dissous sont supérieures à celles d'ALBAIGES et al., qui se situent dans la gamme 0,02-0,05 ug l-1. Cette différence peut ici s'expliquer en partie par la mise en oeuvre de procédés d'extraction des hydrocarbures différents.

Nature des hydrocarbures présents dans les eaux superficielles

D'après la distribution régulière des n-alcanes sans prédominance des composés à nombre impair ou pair d'atomes de carbone, commune à tous les échantillons d'hydrocarbures extraits des eaux superficielles, (Fig. 2a) on peut penser à une origine mixte pour ces composés : des résidus de type pétrolier et une source marine sommant les contributions végétales, animales et bactériennes (SALIOT, 1981). Cependant en utilisant la valeur du rapport enveloppe/n-alcanes comme critère de pollution adapté d'après MAZUREK & SIMONEIT (1984) on constate que les hydrocarbures particulaires sont nettement de type pétrolier (les valeurs du rapport enveloppe/n-alcanes sont supérieures à 5,voir tableau 1), alors que les hydrocarbures en solution portent moins une empreinte de pollution (les valeurs du rapport enveloppe/n-alcanes sont inférieures à 4). La forte contamination des hydrocarbures particulaires par des produits non résolus du type naphtènes est bien visible sur le chromatogramme de la figure 2b où une forte enveloppe s'identifie, avec deux maxima correspondant aux n-C26 et n-C36.

Etude de la microcouche de surface

Sur l'ensemble des stations, on ne constate pas d'accumulation des hydrocarbures dissous dans la microcouche de surface par rapport à l'eau prélevée à 0,2 m. On peut même noter un réel appauvrissement d'un facteur 20 de la microcouche à la station ET-R1. Cette situation d'appauvrissement de la microcouche par rapport à l'eau sous-jacente est inhabituelle (BOEHM, 1980 ; MARTY & SALIOT, 1976 ; MARTY et al., 1979). On peut penser ici qu'à cette période de faible productivité biologique, les conditions ne sont pas favorables à la libération de quantités importantes d'acides gras et de polysaccharides et donc à l'établissement d'une microcouche stable et par suite à l'accumulation des hydrocarbures biogéniques et anthropogéniques. On ne constate pas de différence notable dans les

distributions des n-alcanes de la microcouche et de l'eau sous-jacente qui sont du type de celle montrée sur la figure 2a.

A l'opposé, la microcouche de surface est très enrichie en hydrocarbures particulaires. Les facteurs d'enrichissement en n-alcanes sont de 7,7 à la station ET-R1, de 15,4 à la station SR-G-Z, de 35,4 à la stations SR-G-2 et de 53,5 à la station SR-S-Z.

Quelle est l'origine de ces hydrocarbures ? On trouve indubitablement des produits de type pétrolier non résolus qui rendent compte des fortes valeurs du rapport enveloppe/n-alcanes trouvées aussi bien dans les particules de l'eau sous-jacente (tableau 1) que dans celles accumulées dans la microcouche. Plus intéressante est la mise en évidence de composés d'origine continentale naturelle, bien identifiée au niveau des n-alcanes à nombre impair d'atomes de carbone dans la gamme C23-C31. Ces produits sont en effet des composés constitutifs des cires cuticulaires des végétaux supérieurs (KOLATTUKUDY & WALTON, 1972 ; CALDICOTT & EGLINTON, 1973). A titre d'exemple, le chromatogramme des hydrocarbures non aromatiques particulaires accumulés dans la microcouche de la station SR-G-Z est montré sur la figure 2c. Les caractéristiques de ce mélange d'hydrocarbures sont i) la grande importance des composés non résolus ou enveloppe, ii) la non prédominance dans les hydrocarbures < C20 des n-C15, n-C17 ou pristane. composés biogéniques dont la faible concentration s'explique par la faible productivité biologique des eaux (SALIOT, 1981) et iii) la prédominance nette dans les hydrocarbures > C20 des n-C25, n-C27, n-C29, n-C31 et n-C33. On peut penser à une origine éolienne pour ces composés, qui ont été trouvés dans les aérosols et notamment sur les particules de grande taille dans diverses provinces océaniques : en océan Atlantique (MARTY & SALIOT, 1982) au large des côtes du Pérou (SCHNEIDER et al., 1983) et en Méditerranée (MARTY et al., soumis).

Les analyses en cours actuellement sur les aérosols collectés au cours de la campagne PHYCEMED II devraient permettre de vérifier et de quantifier l'importance de la contribution éolienne à la pollution hauturière de la Méditerranée précédemment mise en évidence en 1981 (HO et al., 1983), pour le bassin nord-occidental.

Remerciements

Nous remercions vivement le Dr. J.C. BRUN-COTTAN, Chef de mission de la campagne PHYCEMED II.

Références

ALBAIGES J., R.W. FREI et E. MERIAN, Eds, (1983): - Chemistry and analysis of hydrocarbons in the environment. Gordon and Breach Science Pub., New York, 314 p.

ALBAIGES J., J. GRIMALT, J.M. BAYONA, R. RISEBROUGH, B. DE LAPPE et W. WALKER. II (1984). - Dissolved, particulate and sedimentary hydrocarbons in a deltaic environment. Org. Geochem., 6, 237-248.

BLUMER M., P.C. BLOKKER, E.B. COWELL et D.F. DUCKWORTH (1972). - Petroleum. In: A guide to marine pollution. E.D. GOLDBERG, Ed. Gordon and Breach Science Pub., New York, pp. 19-40.

BOEHM P.D. (1980). - Evidence for the decoupling of dissolved, particulate and surface microlayer hydrocarbons in Northwestern Atlantic continental shelf waters. Mar. Chem., 9, 255-281.

BURNS K.A. et J.P. VILLENEUVE (1982). - Dissolved and particulate hydrocarbons in water from a spring of sampling the Var River estuary (S. France). Toxicological and Environmental Chemistry., 5, 195-203.

BURNS K.A. et J.P. VILLENEUVE (1983). - Biogeochemical processes affecting the distribution and vertical transport of hydrocarbon residues in the coastal Mediterranean. Geochim. Cosmochim. Acta, 47 995-1006.

CALDICOTT A.B. et G. EGLINTON (1973). - Surface waxes. In: Phytochemistry, III Inorganic elements and special groups of chemicals. MILLER L.P., Ed. Van Nostrand Reinhold, New York, pp. 162-194.

GOUTX M. et A. SALIOT (1980). - Relationship between dissolved and particulate fatty acids and hydrocarbons, chlorophyll a and zooplankton biomass in Villefranche Bay, Mediterranean Sea. Mar. Chem., 8, 299-318.

GUILIANO M., A. ARLHAC, G. MILLE et J. CHOUTEAU (1981). Variations saisonnières de la teneur en hydrocarbures de l'eau de mer d'une frange littorale méditerranéenne : région de Carry-le-Rouet/Sausset-les-Pins. Tethys, 10, 53-58.

HO R. (1982). - La matière organique de l'air, de l'eau et du sédiment en Méditerranée occidentale : inventaire, processus d'échange, flux. Thèse 3e cycle. Université P. et M. Curie, Paris, 186 p.

HO R., J.C. MARTY et A. SALIOT (1982). - Hydrocarbons in the Western Mediterranean Sea, 1981. Intern. J. Environ. Anal. Chem., 12, 81-98.

HO R., J.C. MARTY et A. SALIOT (1983). - Les hydrocarbures à l'interface air-mer en Méditerranée occidentale, 1981. VIes Journées Etud. Pollutions, C.I.E.S.M., Cannes, 1982 : pp. 39-45.

ILIFFE T.M. et J.A. CALDER (1974). - Dissolved hydrocarbons in the Eastern Gulf of Mexico Loop current and the Caribbean Sea. Deep-Sea Res., 21, 481-488.

KOLATTUKUDY P.E. et T.J. WALTON (1972). - The biochemistry of plant cuticular lipids. In: Progress in the chemistry of fats and lipids. HOLMAN R.T., Ed. Pergamon Press, Oxford, pp. 121-175.

LE LOURD P. (1977). - Oil pollution in the Mediterranean. Ambio, 6, 317-320.

MARTY J.C., R. HO et A. SALIOT N-alkanes, PAH. fatty acid salts and esters as a function of particle size in aerosols collected over the Mediterranean. Soumis à J. Geophys. Res.

MARTY J.C et A. SALIOT (1976). - Hydrocarbons (normal alkanes) in the surface microlayer of sea-water. Deep-Sea Res., 23, 863-873.

MARTY J.C. et A. SALIOT (1982). - Aerosols in equatorial Atlantic air: n-alkanes as a function of particle size. Nature,298, 144-147.

MARTY J.C., A. SALIOT, P. BUAT-MENARD, R. CHESSELET et K.A. HUNTER (1979). - Relationship between the lipid compositions of marine aerosols, the sea surface microlayer, and subsurface water. J. Geophys. Res., 84, 5,707-5,716.

MAZUREK M.A. et B.R.T. SIMONEIT (1984). - Characterization of biogenic and petroleum-derived organic matter in aerosols over remote, rural and urban areas. In: Identification and analysis of organic pollutants in air. KEITH L.H., Ed, Ann Arbor Science/Butterworth Pub. Boston, pp. 353-370.

SALIOT A. (1981). - Natural hydrocarbons in sea-water. In: Marine organic chemistry., DUURSMA E.K. et DAWSON R., Eds, Elsevier, Amsterdam., pp. 327-374.

SALIOT A., C. ANDRIE, A. FEVRIER, M. GOUTX et M.J. TISSIER (1983). - Analysis and budget of biogeochemical markers in dissolved, small and large size suspended matter in the ocean. In: Advances in organic geochemistry 1981., BJORØY et al., Eds, John Wiley and Sons Limited, Chichester, pp. 251-258.

SALIOT A. et J.C. MARTY (1984). - Strategies of sampling and analysis for studying the hydrocarbon pollution at the water-atmosphere interface. NATO ASI, Beaulieu-sur-mer, Octobre 1984.

SCHNEIDER J.K., R.B. GAGOSIAN, J.K. COCHRAN et T.W. TRULL (1983). - Particle size distributions of n-alkanes and ^{210}Pb in aerosols off the coast of Peru. Nature, 304, 429-432.

SICRE M.A., J.C. MARTY et A. SALIOT. - Relationship between the lipid compositions of marine aerosols, the sea surface microlayer, and subsurface water from different mediterranean areas., en préparation.

U.N.E.P. (1981). - Selected bibliography on the pollution of the Mediterranean Sea 106 p.

DISSOLVED/DISPERSED PETROLEUM HYDROCARBONS SUSPENDED SEDIMENT, PLASTIC PELAGIC TAR AND OTHER LITTER IN THE NORTH-EASTERN MEDITERRANEAN

by

Cemal SAYDAM, Ilkay SALIHOGLU
Murside SAKARYA and Aysen YILMAZ
Middle East Technical University
Institute of Marine Sciences
P.K.28, Erdemli-Icel (Turkey)

Abstract

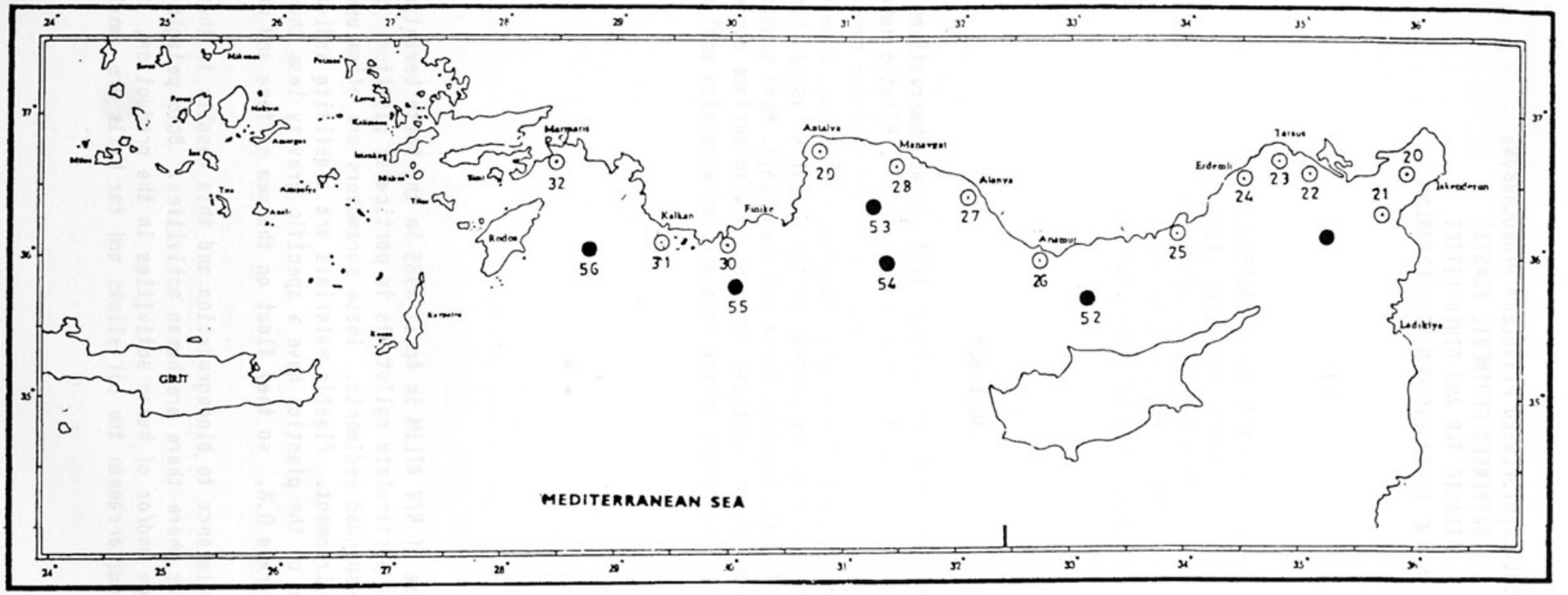
During the scientific cruise of R/V BILIM in April 1983 visual observations and sampling of litter and the sea surface covering about 50,000 km^2 were carried out. Plastic material, tar, vegetable matter and various artificial materials comprised the litter observed and sampled. The origin of the tar balls collected is not known. Among the plastic material especially, some of the plastic bags used for commercial purposes had the name and address of the companies using them. Some of these addresses belong to companies in Israel, Lebanon, Syria and even Libya. Most probably these materials were disposed of in the above mentioned countries and carried with surface currents to the areas observed. Besides litter the surface waters of the area studied were also analysed for suspended sediment content.

*
* *

Introduction

During the scientific cruise of R/V BILIM in April 1983 in the North Levantine Basin an area of about 50,000 km^2 was scanned for particulate pollutants in particular plastics, petroleum residues (oil slicks and tar balls) and suspended sediments. These parameters are often useful indicators of man's input into the marine environment. Plastic materials are a definite indicator of man's pollution of the environment. Some of the plastics have a specific gravity less than sea-water, e.g. polyethylene 0.91-0.96, polystyrene 0.8, so they float on the sea surface and accumulate on seashores.

Tar balls have a high resistance to biodegradation and this results in their ubiquitous and persistent distribution in regions where there are human activities. Both pelagic and beach tar can be the result of natural seepage and/or of human activities in the production, transportation and usage of petroleum. In the Mediterranean the oil slicks and tar balls are mostly due to human activities.



⊙ Coastal stations
● Off shore stations

Figure 1. - Sampling stations.

In April 1983 at twelve coastal and six offshore stations (See figure 1) sea-water samples were collected for dissolved/dispersed petroleum hydrocarbons and total suspended sediment analysis. At the above-mentioned locations pelagic tar was collected with the aid of the neuston net. Plastic materials were also collected either by hand or by nets. For comparison some of the stations were visited in June 1983, October 1983 and April 1984 and samples collected and analysed. In order to assess the suspended material input from land based sources and major rivers, samples of streams and sewage were also taken and the total suspended material contents determined.

Experimental

The total suspended sediments (TSS) of the north-eastern Mediterranean Sea between the gulf of Iskenderun and Rhodes (Fig. 1) was sampled during the April , June and October 1983 and April 1984 R/V BILIM cruises.

Sea-water samples collected by bucket were immediately filtered on board, dried and weighed at the laboratory on shore. The Total Suspended Sediment and Dissolved Oxygen concentrations measured for coastal and offshore stations for each cruise are listed in table I. The position of the stations are shown in figure 1.

Sea-water samples were collected 1 m below the surface with the aid of a 2.5 l volume weighted Amberlite bottle and they were treated and analysed for their DDPH content by following the method described by the I.O.C./W.M.O. Manual and Guide n° 7 (I.O.C. 1976). During the spectrofluorimetric measurements chrysene was used as a calibration material. The results are given in table II.

Pelagic tar, plastics and other litter from 0-40 cm depth were collected using a neuston net. To prevent the net from sampling in the ship's wake or bow wave it was mounted on athwartship ahead of the bow wave. Towing was done either in a circle about 1 km in diameter or in a linear tow of about 2 km (30 minutes at a speed of 3 miles per hour).

The results are given in table II and figure 2 for tar balls, pieces of nylon; opaque or clear spherules, and miscellaneous pieces of plastic and wood including cigarette filters.

Discussion

Total Suspended Sediment

From the results given in table I it is rather interesting that for the offshore station (stations 51 to 56) both during the April'83 and April'84 cruises roughly the same overall average TSS load was found. For these stations the settling velocities of particles would suggest that particles would sink rapidly to reach such distances (DRAKE, 1976). Thus the only possibility remains the primary biogenic production. The dissolved oxygen (DO) concentrations measured showed a marked increase which were again very similar both in the April'83 and April'84 cruises. The same picture is also true of the coastal stations. For the April'83 and April'84 cruises the average TSS loads were 2.55 and 2.74 mg/l respectively, whereas for the June'83 and Oct'83 cruises the TSS loads were 1.28 and 1.19 mg/l respectively. The DO concentrations in April were high enough to support the existence of primary biogenic activity.

As mentioned before, the locations of offshore stations cancels out the effect of land-originated particulate matter since such material would be lost from the surface waters. Therefore it is not possible to expect such high loads for these stations. The average for April'83 and '84 offshore TSS load would give 2.21 mg/l. The averages of June'83 and Oct'84 TSS load should give the background load since the input of suspended load should be at a minimum during these periods and it is 0.78 mg/l. This value is in good agreement with the rather sparse data existing for the north-eastern Mediterranean Sea. Concentrations between 0.5-1.0 mg/l have been measured by EMALYENOUS and SHIMKUS

Table I. - TSS and DO Concentrations in coastal and off Shore stations.

Stations	Cruise Dates							
	4/83		6/83		10/83		4/84	
Coastal	TSS	DO	TSS	DO	TSS	DO	TSS	DO
20	3.69	7.6	2.14	6.6	1.43	5.3	2.49	7.3
21	1.83	7.9	0.50	6.8	1.18	5.3	4.96	7.4
22	0.78	7.9	0.37	7.8	2.33	5.3	1.26	7.3
23	1.48	7.0	2.31	6.9	1.57	5.4	1.16	7.3
24	1.54	7.3	0.55	-	2.71	5.4	7.44	8.1
25	0.82	7.0	0.37	-	0.55	-	1.86	7.0
26	2.50	7.4	0.44	6.3	1.55	5.6	2.48	6.9
27	1.10	6.5	1.46	5.9	0.52	5.7	1.00	6.6
28	1.80	7.8	1.50	5.7	-	5.7	1.23	8.2
29	1.44	6.7	-	5.9	1.45	5.8	1.63	9.1
30	2.81	8.4	0.11	5.9	0.39	5.6	0.93	7.1
31	0.63	8.0	3.78	6.1	0.50	5.6	5.82	7.5
32	12.75	8.3	1.87	5.9	0.17	6.1	2.38	7.1
Average	2.55	7.54	1.28	6.34	1.19	5.56	3.56	7.12
Off Shore								
51	2.53	7.4	0.43	7.1	4.83	5.9	1.00	7.5
52	1.20	6.7	0.68	5.8	4.43	5.6	2.79	7.4
53	1.21	7.2	0.06	5.9	-	-	1.96	8.1
54	4.07	8.0	0.41	5.9	0.63	5.6	1.87	7.2
55	5.41	7.9	0.41	5.9	0.43	5.5	2.30	7.3
56	2.03	8.4	0.41	6.0	0.81	5.6	1.75	7.5
Average	2.47	7.60	0.40	6.10	1.39	5.64	1.95	7.50

TSS : in mg/l
DO : in mg/l
N.D : Not Detected

Table II. - Tar Balls and Litter Collected from the Surface Waters of Coastal and Off Shore Stations (mg/m2) and DDPH (µg/l).

Coastal Stations

Date: 22-27/4/1983

Station	Tar ball	Nylon Pieces	Spherules	Miscell*	DDPH
20	33.38	4.7	1.27	1.21	5.6
21	0.20	0.2	N.D	0.05	1.3
22	0.06	N.D	N.D	N.D	0.6
26	N.D	12	"	"	1.5
27	1.53	N.D	"	"	2.0
28	0.01	N.D	"	"	0.1
29	N.D	N.D	"	"	1.5
30	N.D	N.D	"	0.45	0.7
31	N.D	N.D	"	N.D	-
32	N.D	N.D	"	"	0.4

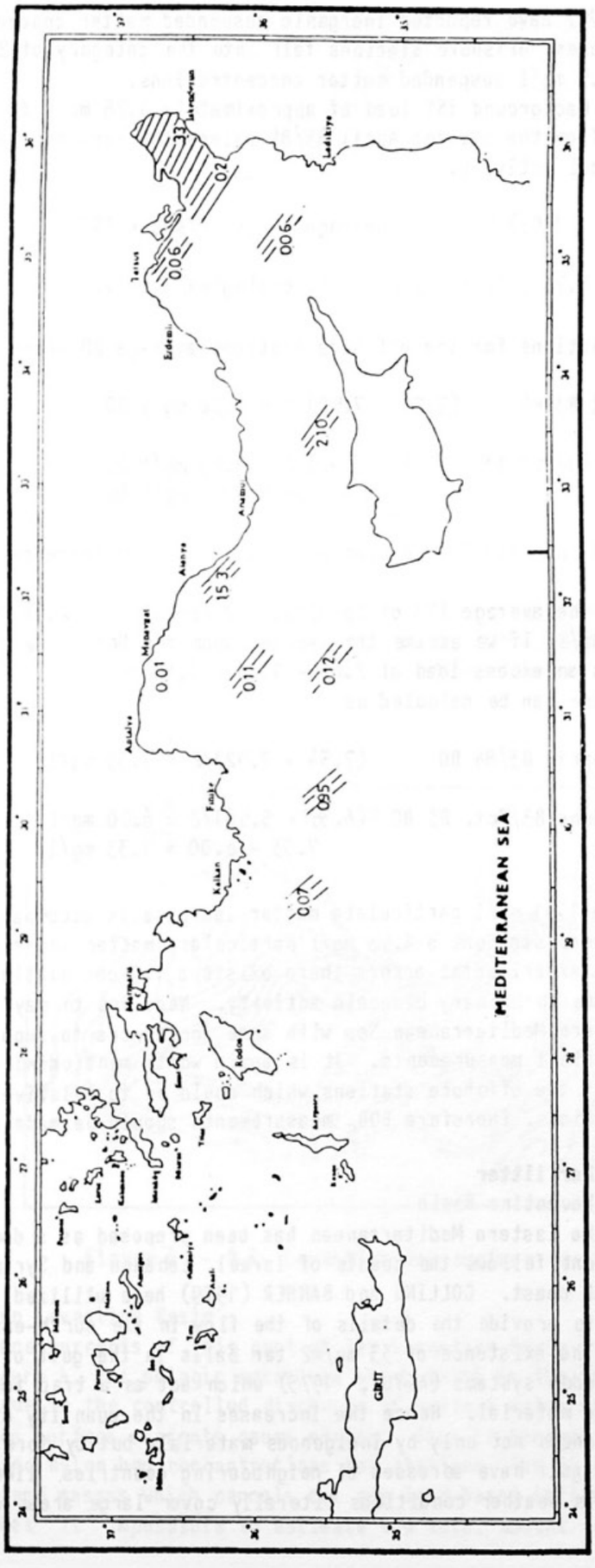
Off-Shore Stations

Date 22-25/83

51	0.06	0.40	N.D	0.06	0.8
52	2.10	N.D	"	0.08	3.8
53	0.11	N.D	0.10	0.72	1.8
54	0.12	3.15	N.D	0.03	2.1
55	0.95	0.20	"	0.03	1.0
56	0.07	N.D	"	N.D	2.2

N.D: Not Detected

* *Miscellenaeus.*



/// Pelagic tar observation areas

Figure 2. - Tar ball sampling areas and quantities (mg/m^2).

(1972). COLLINS and BANNER (1979) have reported inorganic suspended matter concentrations of 0.6-7.0 mg/l. The locations of the present offshore stations fall into the category of 20-30 m Secchi disc depths which should be around 0.5 mg/l suspended matter concentrations.

Therefore if we accept a background TSS load of approximately 0.78 mg/l for the offshore stations and subtract this value from the average April 83/84 values the resultant value should be the TSS load due to primary biological activity.

$$(2.47 + 1.95)/2 = 2.11 \text{ Average 83/84 offshore TSS}$$

$$2.21 - 0.78 = 1.43 \text{ mg/l, TSS to biological activity}$$

If one does the same calculations for the offshore stations average DO concentrations, then

$$\text{April 83/84} \quad (7.54 + 7.50)/2 = 7.52 \text{ mg/l DO}$$

$$\text{June 83/Oct 83} \quad (6.10 + 5.60)/2 = 5.85 \text{ mg/l DO}$$
$$7.52 - 5.85 = 1.67 \text{ mg/l DO}$$

Thus for 1.43 mg/l, TSS increase is accompanied by 1.67 mg/l DO increase for the offshore stations.

For the coastal stations the average TSS of April'83 and April'84 is 2.64 mg/l. The average June'83 and Oct'83 TSS is 1.23 mg/l. If we assume the average June and Oct. as a baseline coastal TSS load, then in April there exists an excess load of 2.64 - 1.23 = 1.41 mg/l.

The corresponding DO increase can be calculed as

$$\text{Average April 83/84 DO} \quad (7.54 + 7.12)/2 = 7.33 \text{ mg/l}$$

$$\text{Average June 83/Oct. 83 DO} \quad (6.35 + 5.56)/2 = 6.00 \text{ mg/l}$$
$$7.33 - 6.00 = 1.33 \text{ mg/l}$$

Thus for coastal stations a 1.41 mg/l particulate matter increase is accompanied by a 1.33 mg/l DO increase, whereas for offshore stations a 1.43 mg/l particulate matter increase accompanied by a 1.67 mg/l DO increase. Within experimental errors there exists a 1:1 correlation between the DO increase and TSS load increase due to primary biogenic activity. Needless to say, this needs further investigations in the north-eastern Mediterranean Sea with some incidences to support the correlation such as chlorophyll-a and nutrient measurements. It is again worth mentioning that there exist relatively high DO increases at the offshore stations which could be to relatively high biological oxygen demand of the coastal stations. Therefore BOD_5 measurements should be made as well.

Plastic, pelagic tar and other litter

a) North-eastern Corner of Levantine Basin

A cyclonic circulation in the Eastern Mediterranean has been proposed as a dominant mean current system. The steady surface current follows the coasts of Israel, Lebanon and Syria and turns west to flow along the southern Turkish coast. COLLINS and BANNER (1979) have utilized ERTS imagery and Secchi disc depth measurements to provide the details of the flow in the north-eastern corner of the Levantine basin in (figure 3). The existence of 33 mg/m2 tar balls in the gulf of Iskenderun is made possible by the presence of two eddy systems (AKYUZ, 1975) which act as a trap for the water masses and consequently for the pelagic material. Hence the increases in the quantity of pelagic material. The gulf of Iskenderun is influenced not only by indigenous materials but by foreign ones as well. Nylon bags collected from the gulf have adresses of neighbouring countries like Syria, Lebanon, Israel and even Egypt, and in calm weather conditions literally cover large areas of the sea surface.



Figure 3. - Iskenderun Bay Surface Currents.

The area of the Iskenderun gulf is about 2,000 km^2 and with a 33 mg/m^2 tar ball load it acts as a trap for about 66 tonnes of the tar balls, 10 tonnes of nylon bags, 2.5 tonnes of spherules and 2.5 tonnes of other pelagic materials.

The existence of low tar ball concentrations but relatively high nylon pieces for station 51 is in good agreement with the mean average surface current of this region. The load introduced to this part of the Levantine basin can be accepted as a baseline input of pelagic material to the study area.

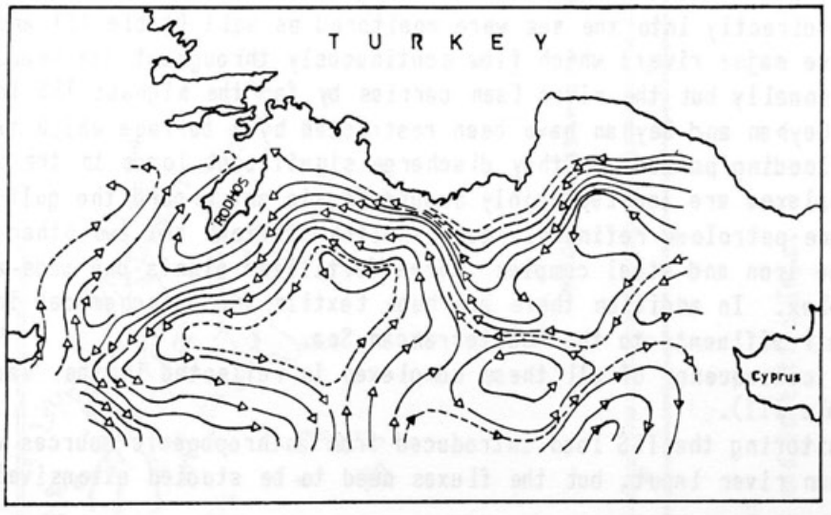


Figure 4. - N.E. Levantine Sea surface currents.

b) North-western Levantine Basin

The mean surface currents of this part of the Levantine Basin are proposed by OZTURGUT (1976) and are given in figure 4. The pelagic materials which exist in the surface waters and are introduced by tanker traffic due to the controlled discharge of ballast water (LE LOURD , 1977) accumulate in these regions where surface currents cause eddies. Thus it becomes possible to explain such high loads of tarball and nylon bag concentrations for stations like 54 and 55. These stations are far away from the mainland masses which cancels out any land-based influences. The patchiness of these stagnant areas makes it impossible to estimate the total amount of tarball and other pelagic materials.

Dissolved/dispersed petroleum hydrocarbons (DDPM)

From table II it clear that DDPH shows the same trend as tar balls and litter, i.e. the highest DDPH concentrations were measured in Iskenderun Bay (station 20) and offshore at station 52. This trend does not in itself connote a correlation between tar ball quantities and DDPH.

Relatively high DDPH concentrations in Iskenderun Bay can be attributed to the existence of two pipeline terminals within the bay and extensive tanker and ship traffic.

Table III. - TSS loads of Industrial Complexes Domestic Sources and Rivers (mg/l).

Rivers	Average	Range
Esen	1777	(81-4398)
Göksu	849	(41-1193)
Ceyhan	777	(57-2157)
Seyhan	73	(30-160)
Manavgat	48	(44-52)

Industrial and Domestic

	Average	Range
Gulf of Iskenderun :	7440	(1651-20428)
Mersin Bay :	49	(12-118)
Antalya :	21	(17-25)

Land-based sources

During the course of the study period the major rivers and those industrial and domestic effluents which flow directly into the sea were monitored as well (table III and figure 5).

There exist five major rivers which flow continuously throughout the year. All their suspended loads fluctuate seasonally but the river Esen carries by far the highest TSS load into the Mediterranean. The rivers Ceyhan and Seyhan have been restricted by a barrage which traks their suspension loads. Only during flooding periods do they discharge significant loads in the sea.

Industrial complexes are located mainly around Mersin and around the gulf of Iskenderun. Among others there exist one petroleum refinery, two pipeline stations and two other petroleum storage and filling depots, one iron and steel complex ,three fertilizer plants one soda-ash and one chromnium ore enrichment complex. In addition there are huge textile and agrochemical industries which indirectly discharge their effluents to the Mediterranean Sea.

The immediate consequence of all these complexes is reflected in the variations observed in their TSS loads (table III).

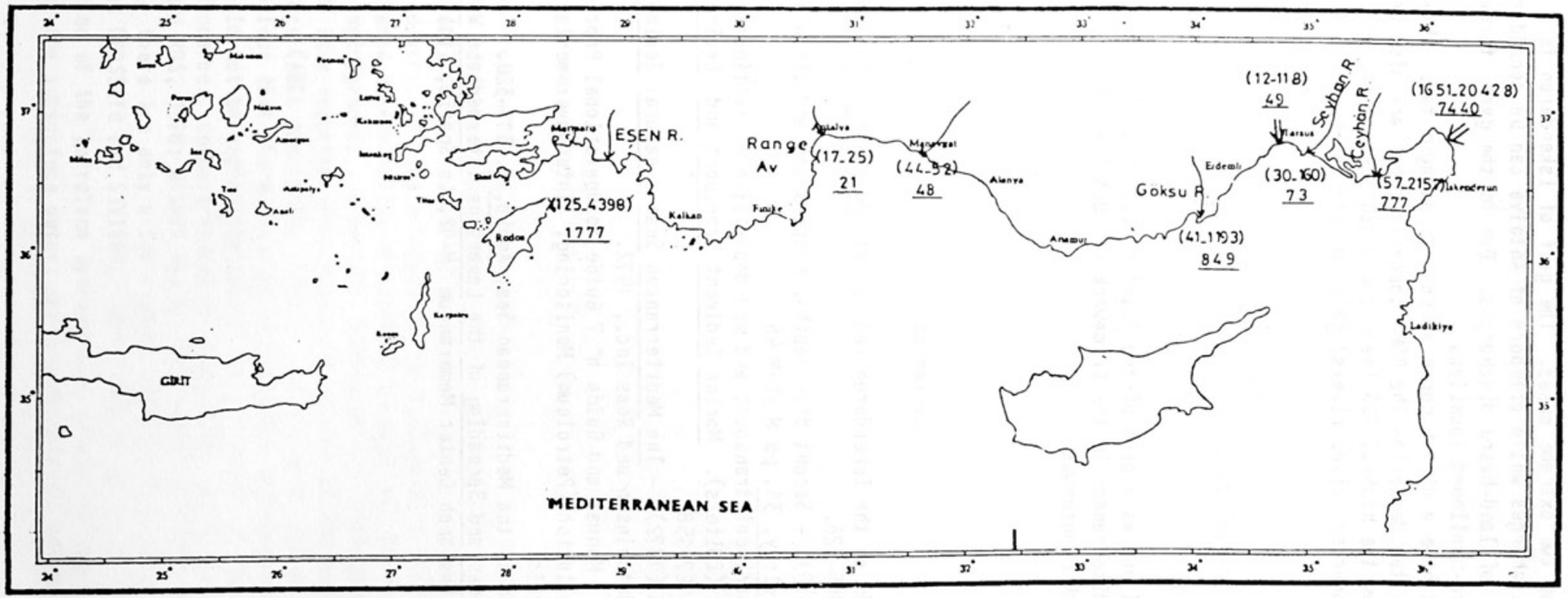
During the monitoring the TSS load introduced from anthropogenic sources was nearly an order of magnitude greater than river input, but the fluxes need to be studied extensively.

Conclusions

From the data and discussions presented in this work it is possible to draw the following conclusions.

1. It seems that there is a close correlation between dissolved oxygen concentrations and total suspended sediment. Within experimental error limits this correlation is 1/1, but this needs further confirmation.

2. Plastic, pelagic tar and other litter accumulates, mostly, in the regions where surface currents form gyres. The best example of this is the gulf of Iskenderun and offshore of Antalya,



--> River Input
==> Industrial and Domesttic Input

Figure 5. - Suspended sediment quantities from land sources (The values in paranthesis are the range, the underlined values are the average quantities in mg/l).

where the two locations represent two extreme cases. The gulf of Iskenderun is a semi-enclosed area which is open to land-based discharges while offshore of Antalya can be considered open sea and it escapes' the direct influence of land-based discharges. Due to the gyre formation the floating material accumulates in the above-mentioned locations.

3. Although DDPH does not have a direct correlation with pelagic tar, they both have similar trends. In the regions with high tar densities the DDPH concentrations are also relatively high.

4. Among land-based sources the highest TSS loads are discharged to the sea by human activities but due to high fluxes natural sources (i.e. rivers) gain importance. However, the fluxes have to be studied in more detail.

Acknowledgement

This work has been carried out as a part of the joint F.A.O. (G.F.C.M./U.N.E.P.) co-ordinated Project on Pollution in the Mediterranean in the framework of U.N.E.P. Co-ordinated Mediterranean Pollution Monitoring and Research Programmes.

References

AKYUZ E.F. (1957). - Observations on the Iskenderun red mullet (Mullus barbatus) and its environment, Proc. Gen. Counc. Med. 4, 305-326.

COLLINS M.B. and F.T. BANNER (1979). - Secchi disk depths, suspensions and circulation, north-eastern Mediterranean Sea Marine Geology, 31, pp M 39-M 46

DRAKE D.E. (1976). - Suspended sediment transport and mud deposition on continental shelves. In: D.J. Stanley and D.J.P. Swift (Editors), Marine Sediment Transport and Environmental Managament. Wiley, New York, N.Y., pp. 127-158.

EMELYANOV E.M. and K.M. SHIMKUS (1972). - The Mediterranean Sea: a natural Sedimentation Laboratory, Strondsburg, Pa., Dowden, Hutchinson and Ross Inc., 1972.

I.O.C. (1976). - I.O.C./W.M.O. Manual and Guide n° 7 Guide to Operational Procedures for the IGOSS Pilot Project on Marine Pollution (Petroleum) Monitoring, Intergovermmental Oceanographic Commission of U.N.E.S.C.O., Paris.

LE LOURD D. (1977). Oil pollution in the Mediterranean Sea, Ambio, 6, 317-320.

OZTURGUT E. (1976). - The sources and Spreading of the Levantine Intermediate Water in the Eastern Mediterranean, SACLANT ASW Research Center Memorandum SM-92, La Spezia, Italy 145 pp.

PETROLEUM HYDROCARBON POLLUTION IN THE RIJEKA BAY DETERMINED BY FLUORESCENCE SPECTROSCOPY AND GAS CHROMATOGRAPHY

by

M. AHEL
Center for Marine Research Zagreb
Rudjer Boskovic Institute
Zagreb (Yugoslavia)

Abstract

Applying spectrofluorometric and gas chromatographic methods, the distribution of petroleum hydrocarbons in the Rijeka Bay was investigated. Concentrations of petroleum hydrocarbons in the water column are rather low: mean values for polluted and unpolluted areas of the Rijeka Bay were from 1 to 7 ug/l and from 0.2 to 0.5 ug/l, respectively. Comparing the estimated input with the standing stock value obtained by the measurements, it can be concluded that the autopurification capacity of the water masses is very high. However, a significant accumulation of petroleum hydrocarbons in sediments (2-559 ug/g) and in organisms (plankton 60-1,000 ug/g; mussels 2-112 ug/g) was observed.

*
* *

Introduction

Oil pollution represents a considerable share of the Rijeka Bay load of organic materials. Estimated amounts of petroleum hydrocarbons discharged into the Rijeka Bay vary between 940-1,600 t/-year (SEKULIC, 1982; PICER, 1980) mostly from the land-based sources of the city of Rijeka, the refineries and industry. A complex study on the ecological situation in the Rijeka Bay has been conducted. It included determination of about 40 parameters. As the result of this ecological study several reports on hydrographic, chemical, biological and geological characteristics were published (JEFTIC, 1981). We have reported the preliminary results of the monitoring of petroleum hydrocarbons in subsurface waters (AHEL and PICER, 1978), and sediments and organisms (AHEL and PICER, 1979). However, it was obvious that the monitoring of petroleum hydrocarbons, which was limited only to sea-water samples, could not be representative of the assessment of the present state of pollution. Data on petroleum hydrocarbon concentrations in subsurface waters of the Mediterranean are relatively numerous (FARACO and ROS, 1978; WASHBY and EL DEEB, 1980; MIMICOS, 1980).

In contrast, there are only a few reports on the petroleum hydrocarbon concentration in sediments and organisms (FOSSATO and SIVIERO, 1974; MILLIE et al., 1982). The aim of this paper was to show the distribution of the petroleum hydrocarbons in several characteristic parts of the Rijeka Bay ecosystem including the subsurface water, surface microlayer, sediments, net zooplankton, mussels and fish.

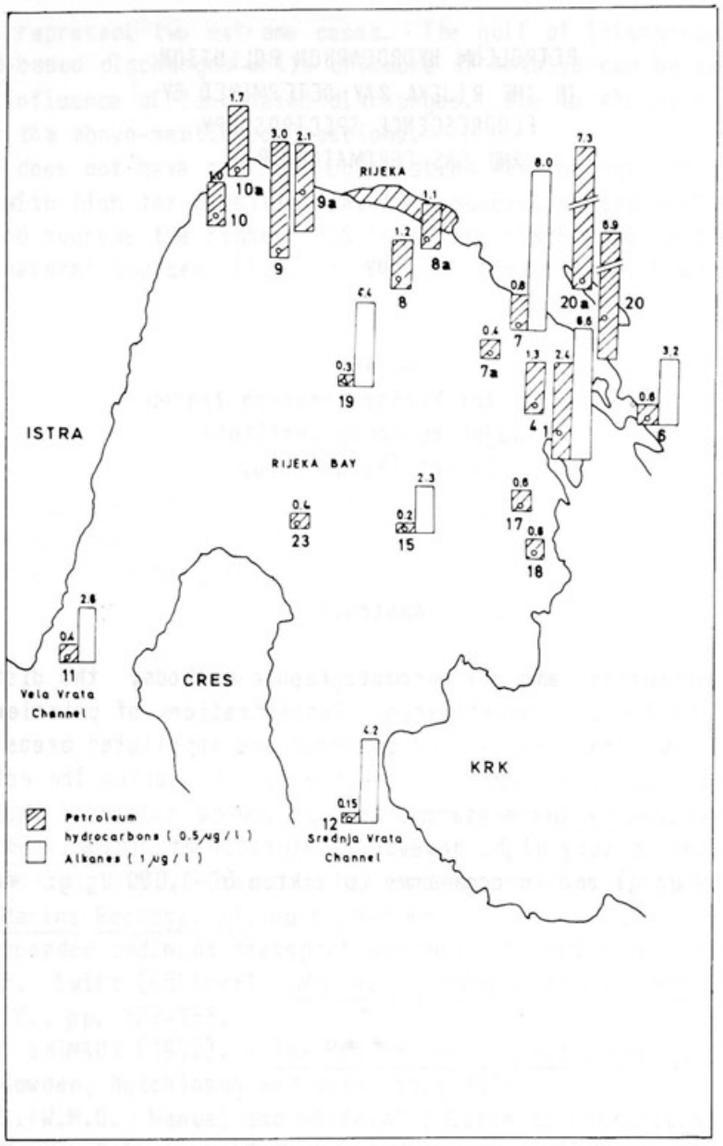


Figure 1. -Distribution of petroleum hydrocarbons in the water masses of the Rijeka Bay.

Materials and methods

Sampling of sea-water, sediments and organisms was performed in the period between 1976-1978 at the stations shown in figs. 1-3. Sea-water samples were collected at 1 m depth using either the glass bottle technique as recommended by U.N.E.P. (I.O.C./W.H.O./U.N.E.P., 1977) (analysed by fluorescence spectroscopy) or by 6 l-Van Dorn sampler (25 l sample, analysed by GC). Surface microlayer samples were collected using the Garrett technique (GARRETT, 1965). The sediments were sampled by a Van Veen grad sampler. Molluscs, predominantly mussels (Mytilus galloprovincialis) were collected manually from the intertidal zone. Plankton samples were collected using a neuston net (250 um, 1.3 m^2). Sediments and organism samples were wrapped in aluminmium foil and kept deep-frozen until analysis. Sea-water samples were extracted within 24 hours of collection.

Analytical procedures

Spectrofluorometric determination of petroleum hydrocarbons in sea-water samples followed the procedure recommended by U.N.E.P. (I.O.C./W.H.O./U.N.E.P., 1977).

Spectrofluorometric determination of petroleum hydrocarbons in sediments and organisms was based on the procedures proposed by HARGRAVE and PHILIPS (1975) and ZITKO (1971). An additional cleaning step using either deactivated alumina or silica gel was involved in the procedures mentioned. Quantification of the measurements was performed on the basis of the evaluation of the spectrofluorometric determination using the standard additions of crude oil to the sea-water (AHEL, 1983).

For gas chromatographic determination of hydrocarbons in sea-water a double extraction with n-pentane was performed.

Sediment samples were extracted by direct alkaline hydrolysis with 0.5 N KOH following the method by FARRINGTON and TRIPP (1975).

For plankton and mussel samples an alternative method using Virtis homogenization (FARRINGTON and MEDEIROS, 1975) was applied.

Cleaning and separation of the aliphatic and aromatic fraction was performed on the column (10mm x 20 cm) containing alumina (5 % water) packed over silica gel (activated at 120° C, 5 % water) in the ratio of 1:2. Eluation was carried out consecutively with 35 ml of n-pentane (non-aromatic hydrocarbons) and 30 ml of 20 % of benzene in n-pentane (aromatic hydrocarbons). Each fraction was reduced to a small volume (0.1 ml) and an aliquot of 10 ul was analyzed by gas chromatography.

Instruments

Fluorescence measurements were performed on the Farrand MK-1 and Turner 430 spectrofluorometers. Square quartz cells with 1 cm path length were applied. The measurement was carried out at the excitation/emission wave length ratio 290 nm/380-390 nm characteritic of the crude oil (Yugoslav crude oil, type Zutica) used as the reference. The fluorescence ratio of this crude oil relative to chrisene was about 5.

Gas chromatography was carried out on the Hewlett Packard 7,620 A instrument equipped with a flame ionization detector. A stainless steel column, 1.8 m long , 2 mm i.d. packed either with 3 % of OV-101 on GHP or with 3 % Apiezon L on GCQ, and a longer column (3;6 m) packed with 3 % OV-101 on GCQ were also applied. The temperature programming involved in the analyses was 70-270° C at 10 ° C/min and 100-300° C at 10° C/min for Apiezon L and OV-101 stationary phases, respectively. Qualitative and quantitative determinations were performed relative to the standard mixture of n-alkanes.

Results and discussion

Concentrations of petroleum hydrocarbons in subsurface waters of the Rijeka Bay determined by fluorescence spectroscopy were generally very low, being in the range of 0.1-38.6 ug/l with most frequent values between 0.1 and 2 ug/l. The distribution of petroleum hydrocarbons in subsurface waters of the Rijeka Bay is shown in figure 1.

Each value represents the arithmetic mean of eight determinations. The distribution shown reflects the position of the sampling sites relative to the main land-based pollution sources located on the northern shore. Thus, several zones can be pointed out with different levels of petroleum hydrocarbon pollution in subsurface water. In the first zone there were localities situated closest to the northern shore characterized by mean concentrations greater than 1 ug/l. The coastal zones far from the main pollution sources were characterized by concentrations of 0.5-1 ug/l. The concentrations gradually decreased towards the sea and the open waters of the Rijeka Bay showed concentrations even lower than 0.5 ug/l. Concentrations in the Vela Vrata Channel were higher than in the Srednja Vrata Channel, which is in good agreement with the dominant current system of the Rijeka Bay (LEGOVIC and VUCAK, 1981; SMIRCIC and ILIC, 1981).

It should be mentioned that the petroleum hydrocarbons load of the Rijeka Bay is not uniform in time and space. This indicates the influence of various distinct sources located on the northern shore. The influence of meteorological conditions at the moment of sampling also played an important role. Thus, the dominant wind in this area (NE) brought about a rapid dispersion of pollutants

through the Rijeka Bay water body and the differences between polluted and unpolluted areas were minimized.

Concentrations of non-aromatic hydrocarbons in subsurface waters (determined by gas chromatography) were almost in the range of 1-5 ug/l, whereas hydrocarbons in the aromatic fraction could not be detected. Concentrations of non-aromatic hydrocarbons, mostly n-alkanes, are shown in fig. 1, parallel to concentrations determined by fluorescence spectroscopy. However, it is suspected that the sea-water samples were contamined during the sampling procedure using a plastic Van Dorn sampler (ZSOLNAY, 1979).

Concentrations of petroleum hydrocarbons determined in surface microlayer samples, were significantly higher than those in subsurface waters as seen in table 1. Although no visible oil slicks were present at the time of sampling, rather high enrichment factor of 60-165 were obtained. This fact indicated that the neuston were exposed to significantly higher concentrations of petroleum hydrocarbons.

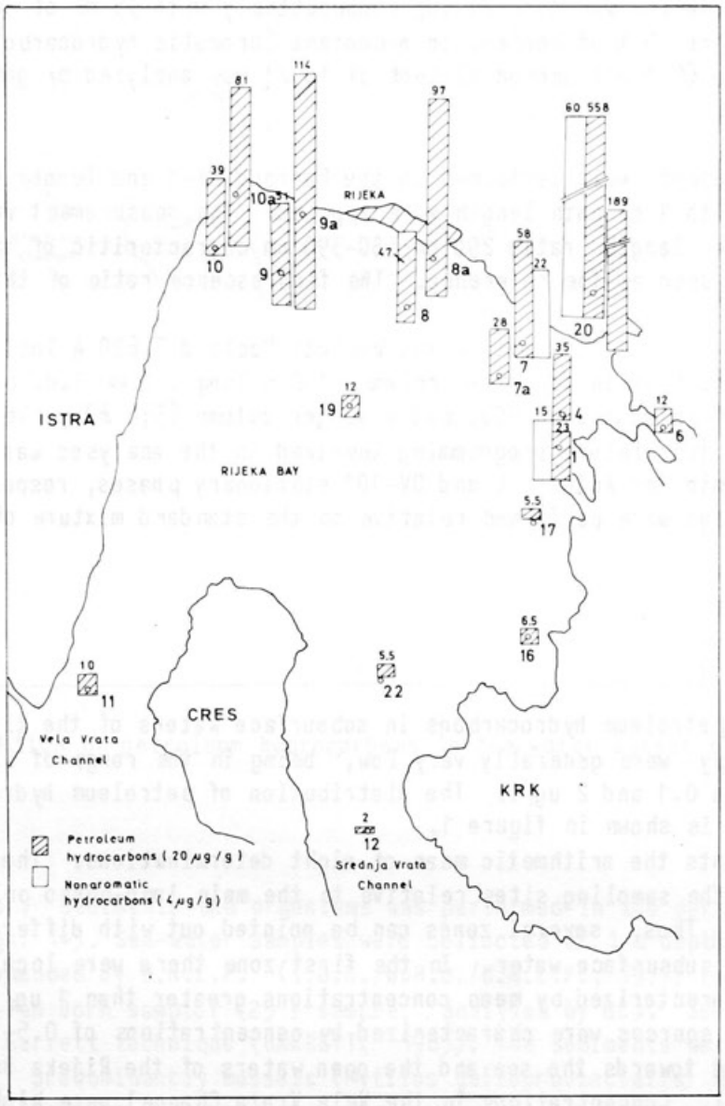


Figure 2. - Distribution of petroleum hydrocarbons in bottom sediments of the Rijeka Bay.

The concentrations of petroleum hydrocarbons in the Rijeka Bay sediments varied in a very wide range of 1.5-558 ug/g of the wet sediment. Sediment samples showed up the pollution topography of the Rijeka Bay in a very clear way. The distribution of petroleum hydrocarbon contamination in the sediments of the Rijeka Bay is depicted in fig. 2. As expected, significant differences between the

Table 1. - Concentrations of petroleum hydrocarbons in the surface microlayer and the subsurface (1 m) samples from the Rijeka Bay.

Station	Surface microlayer Concentration (µg/l)	Subsurface (1 m)	Enrichment factor
7	24	0.2	120
8a	120	2.0	60
10	20	0.3	67
10a	132	0.8	165
17	36	0.4	90

polluted coastal zones and unpolluted distant parts of the Bay were observed, indicating the dominant influence of land-based inputs. The concentrations of petroleum hydrocarbons in sediments as high as 40 ug/g were determined only in the samples collected at the stations in the northern coastal belt (10, 10a, 9, 9a, 9, 8a, 7, 20, 20a). The maximum concentration reaching 558 ug/g was observed in the sample from the Bakar Bay. The concentrations of petroleum hydrocarbons in the zone of the open waters of the Rijeka Bay were even lower than 10 ug/g, as determined by fluorescence spectroscopy, with the minimum concentration for station 12 being 2 ug/g. Thus, the concentration ratio at the most polluted (20a) and the cleanest (12) station in the Rijeka Bay was about 300 for the sediments and about 50 for water. In the same way as for the subsurface waters the concentration in the Vela Vrata Channel (12 ug/g) was several times higher than the concentration in the Srednja Vrata Channel (2 ug/g).

The concentrations of non-aromatic hydrocarbons in sediments determined by gas chromatography (15-60 ug/g) were rather lower than the concentrations estimated by fluorescence spectroscopy. This very probable that fluorescence spectroscopy gives an overestimation of the petroleum hydrocarbon concentrations in sediments. This can be explained by the fact that higher polyclic aromatic hydrocarbons are preferentially associated with suspended particles and therefore they are present in the sediments in a higher proportion than in the parent petroleum hydrocarbon mixture polluting the sea.

The analysis of a few net plankton samples from the polluted northern part of the Rijeka Bay indicate a relatively high contamination with petroleum hydrocarbons. Concentrations determined by fluorescence spectroscopy were 60 and 58 ug/g on the wet weight basis, whereas the concentrations determined by gas chromatography were as high as 1,000 ug/g. Such high concentrations could be explained by the contact of the plankton with the surface oil slicks which occur relatively frequently in the Rijeka Bay, as well as by a possible contribution of tar specks collected in the net together with the plankton.

The concentrations of petroleum hydrocarbons in mollusc samples (mostly mussels) determined by fluorescence spectroscopy were in the range of 1.5 to 19 ug/g on a fresh weight basis and the concentrations determined by gas chromatography were rather higher, reaching 32 to 112 ug/g. The distribution of petroleum hydrocarbon contamination in the Rijeka Bay indicated by both methods is shown in fig. 3. As can be seen, the pollution topography was almost the same regardless of the method applied for the monitoring, showing-as expected-the highest concentrations in the northern coastal zone and the lowest in localities in the Srednja Vrata Channel. The higher concentrations of petroleum hydrocarbons in organisms determined by gas chromatography can be explained by a more efficient extraction (Virtis homogenization) applied in these analyses, compared to relatively ineffective extraction used for spectrofluorometric analysis (shaking for 30 min).

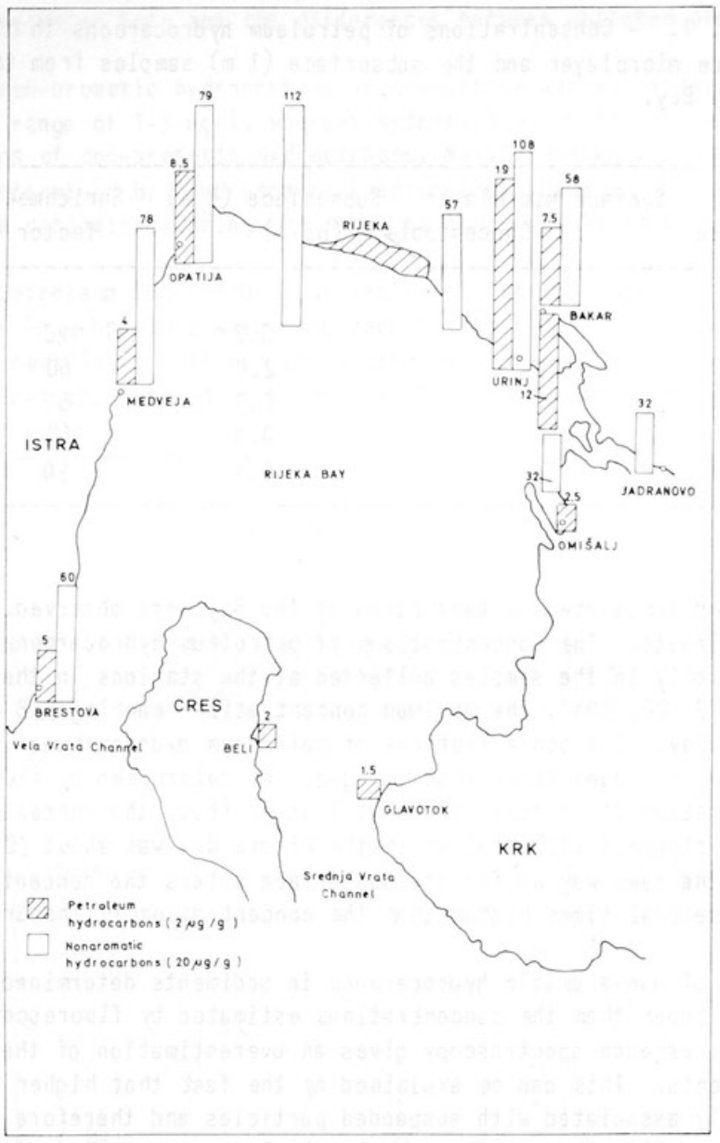


Figure 3. - Distribution of petroleum hydrocarbons in mussels of the Rijeka Bay.

Conclusions

In spite of a significant input of petroleum hydrocarbons in the Rijeka Bay, particularly by the land-based sources located on the northern shore, their concentrations in subsurface waters at 1 m depth are generally very low (0.1 to 38.6 ug/l). The pollution topography obtained from the analysis of the sediments closely follows the pollution level of the water body, but the differences between the directly polluted and the distant areas are more clearly pronounced, especially when the aromatic fraction of the hydrocarbon pollution (fluorescence spectroscopy) was monitored. The analyses of mussels pointed very precisely to the location of the main land-based sources of petroleum hydrocarbons pollution.

The only part of the Rijeka Bay little influenced by petroleum hydrocarbon pollution was the south-eastern part (the Srednja Vrata Channel).

Acknowledgement

This work was partly supported by the Authority for Scientific Research of S.R. Croatia. The comments, advice and criticism of Dr. Z. Pucar and Dr. M. Picer are acknowledged.

References

AHEL M. (1983). - Investigation of the pollution of Rijeka Bay by petroleum hydrocarbons, M. Sc. thesis, University of Zagreb (in Croat.).

AHEL M. and M. PICER (1978). - Monitoring of dissolved and dispersed petroleum hydrocarbons in Rijeka Bay, IVes Journées Etud. Pollutions, Antalya, C.I.E.S.M. 99-101.

AHEL M. and M. PICER (1979). - Oil and its derivatives in the North Adriatic, Proceedings of the Conference on the Protection of the Adriatic Sea, Hvar 1979, Yugoslavia, Part II, 397-404 (in Croat.).

FARACO F. and J. ROS (1978). - Pollution par les hydrocarbures des eaux superficielles de la Méditerranée occidentale, II Hydrocarbures dissous, IVes Journées Etud. Pollutions, Antalya, C.I.E.S.M., 117-121.

FARRINGTON J.W. and G.C. MEDEIROS (1975). - Evaluation of some methods of analysis for petroleum hydrocarbons in marine organisms, Conference on Prevention and Control of Oil Pollution, American Petroleum Institute, Washington D.C., 115-121.

FARRINGTON J.W. and B.W. TRIPP (1975). - A comparison of the analysis methods for hydrocarbons in surface sediments, ACS Symposium Series 18, 267-284.

FOSSATO V.U. and E. SIVIERO (1974). Oil pollution monitoring in the lagoon of Venice using the mussel Mytilus galloprovincialis, Mar. Biol. 25, 1-6.

GARRETT W.D. (1965). - Collection of slick-forming materials from the sea surface, Limnol. Oceanogr. 10, 602-605.

HARGRAVE B.T. and G.A. PHILLIPS (1975). - Estimates of oil in aquatic sediments by fluorescence spectroscopy, Environ. Pollution 8, 193-215.

I.O.C./W.H.O./U.N.E.P. (1977). - Manual for monitoring of oil and petroleum hydrocarbons in marine water and on beaches, Suppl. 7, Paris.

JEFTIC L.J. (ed.) (1981). - Collected papers of the ecological study of the Rijeka Bay 1976-1978, Thalassia Jugosl. 17, 141-380.

LEGOVIC T. and Z. VUCAK (1981). - Kinematics of water exchange in the Rijeka Bay, Thalassia Jugosl. 17, 155-176.

MILLE G., Y.Y. CHEN and H. DOU (1983). - Hydrocarbons présents dans des sédiments superficiels méditerranéens (Zone côtière Fos/mer-Monaco), IVes Journées Etud. Pollutions, Cannes, C.I.E.S.M., 1982, 191-198.

MIMICOS N. (1980). - Pollution by petroleum hydrocarbons along several Greek island coasts and harbours, Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M., 489-492.

PICER M. (1980). - Estimation of the mineral oil input in the Rijeka Bay, Ekologija 20, 5-16 (in Croat.).

SEKULIC B. (1982). - Impact of pollution on the Rijeka Bay ecosystem - present state and trends. Ph.D. Thesis, University of Ljubljana.

SMIRCIC T. and D. ILIC (1981). - Some results of the current investigations in the Rijeka Bay by drift card experiments, Thalassia Jugosl. 17, 177-198.

WASHBY S.D. and K.Z. EL DEEB (1980). - A study of the state of pollution by petroleum hydrocarbons along Alexandria coast, Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M., 257-262.

ZITKO V. (1971). Determination of the residual fuel oil contamination of aquatic animals, Bull. Environ. Contam. Toxicol., 5, 559-564.

ZSOLNAY A. (1979). - Caution in the use of Niskin bottles for hydrocarbon samples, Mar. Pollut. Bull. 9, 23-24.

PETROLEUM AROMATIC HYDROCARBONS IN THE PATRAIKOS GULF AND THE ESTUARY OF THE ACHELOOS RIVER, GREECE

by

N. MIMIKOS*, E. STAYRIANOUDAKIS**
and M. SCOULLOS ***
* Analytical Lab., N.R.C. "Demokritos
Aghia Paraskevi Attikis (Greece)
** Agricultural Bank of Greece
Hania Branch
*** Dept. of Chemistry, Univ. of Athens
13A Navarinou str., Athens 106 80 (Greece)

Abstract

The present paper summarizes the results of determination of petroleum aromatic hydrocarbons in an important coastal region of the Ionian Sea. The data derive from thirteen cruises carried out in the 1977-1982 period in the Patraikos Gulf and two cruises in 1982 and 1983 in the Estuary of the Acheloos River, directly associated with it.

In certain cases, throughout this period, high concentrations of dissolved and dispersed PAHs have been found, most probably due to pollution instances.

Regardless of seasonal peaks, the most alarming feature is the general increase in the background levels.

*
* *

Introduction

A considerable number of studies have been carried out on the levels of petroleum hydrocarbons in the Mediterranean Sea. However, relatively little is known about general trends in the various parts of it, because of the effects of local pollution incidents of various extent and lack of suitable data over relatively long periods.

In the present contribution an attempt is made to assess not only the level of polycyclic aromatic hydrocarbons (PAH) but also to identify any general trends in a very important coastal area of Greece, the Patraikos Gulf. the Patraikos Gulf (figure I) is open to the Ionian Sea and receives in its "outer" part the waters of the Acheloos River and in its "inner" part effluents from the city and port of Patras and its industries (SOULLOS, 1982). The area is also crossed by several shipping routes. Further description of the area is given in SCOULLOS et al., 1984.

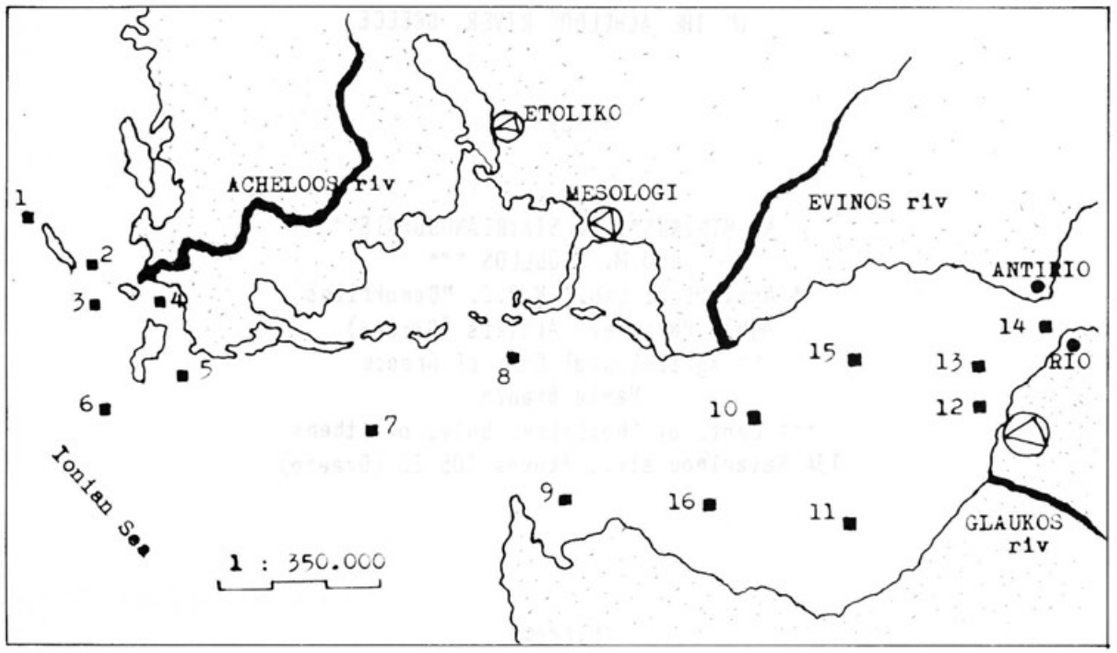


Figure I

The area studied and the stations sampled. Stations 1-8 correspond to the Estuary of Acheloos River and stations 9 to 14 to the "main" Patraikos Gulf.

Materials and Methods

The sampling for dissolved and dispersed PAHs was started in September 1977 and still continues. It was originally focused on the main Patraikos Gulf (see figure I) which was sampled twelve times. Since 1982 the Estuary of the Acheloos River, an area of great ecological and economic importance, has been included (sampled twice) and PAHs were determined in suspended particles and also in samples taken at 10 m depths. Samples (for determining dissolved PAHs) were collected by submerging carefully cleaned amber-glass bottles containing 50 ml fluorescence grade CCl_4 to depth of 1 and 10 m with the aid of a weighted stainless steel frame. Each bottle was sealed with a Teflon film which was punctured at the desired depth by using a special device. The samples were transported to the laboratory where they were extracted twice with CCl_4 (2x50 ml). The organic phase was evaporated in a rota - vapour apparatus and the residue was taken up with fluorescence grade n-hexane. The n-hexane aliquot was passed through a silica gel column for chromatographic separation of polar compounds (I.O.C. 1976 a, b).

Suspended particles were collected by filtration through a 045 u millipore filter from separate samples taken as previously described from the same stations and depths. The filters were soxhlet extracted with fluorescence grade n-hexane for 4h. The clean-up procedure was the same as for the water samples.

In most cases three separate replicate samples were taken and analysed from each station and depth, and the reported values are the mean values of three determinations. The fluorescence of PAHs was measured (KEIZER and GORDON, 1973) employing a Perkin-Elmer Mod. 203 fluorometer.

Stations	Depth (m)	1977	1978				1979			1980	1981		1982		
		5/9	20/2	6/6	31/7	27/10	17/3	5/5	31/8	18/4	25/7	12/10	11/8	11/10	
		d	d	d	d	d	d	d	d	d	d	d	d	d	p
9	1	14.0	1.1	7.8	3.3	3.6	20.8	3.8	3.8	1.3	2.0	3.4	1.2	2.0	3.0
10	1	8.6	2.3	1.7	-	3.4	28.2	3.5	2.6		2.3	3.5	6.2	2.9	2.4
10	10	-	-	-	-	-	-	-	-	-	-	-	-	4.2	2.8
11	1	-	-	-	-	-	-	-	-	-	-	-	-	4.7	1.4
11	10	-	-	-	-	-	-	-	-	-	-	-	-	2.5	2.0
12	1	9.5	1.4	3.7	1.8	1.8	14.9	9.8	4.6	1.3	3.5	2.3	12.7	7.8	2.1
13	1	9.6	1.3	3.1	1.4	1.3	15.9	12.5	3.9	7.0	2.2	2.9	3.0	3.0	-
14	1	10.3	1.3	2.3	1.8	4.9	7.9	7.2	3.6	1.3	3.1	2.3	3.1	4.2	-
15	1	14.5	3.8	3.6	1.2	2.1	6.6	7.4	3.7	-	3.0	3.0	8.3	4.2	-
16	1	15.3	2.1	3.4	3.1	4.0	11.7	6.7	3.5	-	2.7	3.4	5.2	3.9	-
mean		11.8	1.9	3.7	2.1	3.0	15.1	7.3	3.7	2.6	2.7	3.0	5.7	4.0	2.3

d = dissolved and dispersed p = particulate

Table 1
Concentration of PAHs in the Patraikos Gulf (in ug.L^{-1} chrysene equivalents).

Stations	Depth (m)	DATE			
		11.10.82		22.5.83	
		d	p	d	p
1	1			4.3	1.4
1	10			3.0	(6.0)*
2	1			2.8	2.4
2	10			3.7	0.9
3	1	3.9	2.3	2.8	0.8
3	10	3.2	1.9	-	
4	0			4.6	
4	1	3.2	1.9	4.0	2.7
4	10	3.9	2.3	4.4	0.6
5	1			4.2	0.8
5	10			4.5	3.0
6	1	2.2	1.3	3.8	(4.1)*
6	10	2.4	1.4		
7	1	4.4	2.8	3.7	2.1
7	10	2.9	1.7		
8	1	4.3	2.5		
8	10	4.3	2.5		
Mean 1		3.6	2.2	3.8	1.7 (2.0)**
Mean 10		3.3	2.0	3.9	1.5 (2.6)**
Mean 1 - 10		3.5	2.1	3.8	1.6 (2.3)**

d = dissolved and dispersed p = particulate

* values are excluded
** mean when all values are included

Table 2
Concentrations of PAHs in the Acheloos River Estuary (in ug.L^{-1} chrysene equivalents).

Results and discussion

The results of this study are summarized in tables 1 and 2. From table 1 it is apparent that the mean areal values for the Patraikos Gulf were in the range of 1.9-15.1 ug.L^{-1}. However, the majority of the samples fluctuates between 2 and 5 ug.L^{-1}

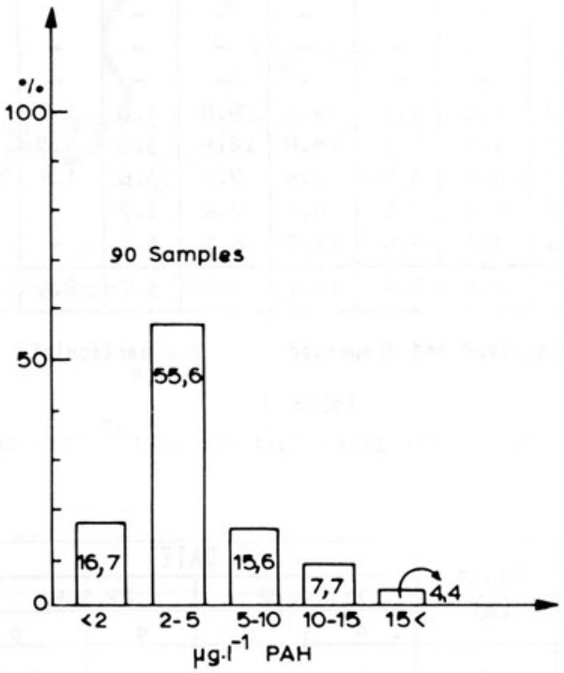


Figure II

Percentage distribution of water samples collected from the Patraikos Gulf (1977-1982) in ranges of defined concentrations.

Figure II shows that for the Patraikos Gulf more than 72 per cent of the analysed samples contained less than 5 ug.L^{-1} PAHs. Of the remaining 28 per cent, only a very small percentage (4.4) contained aromatic hydrocarbons in concentrations exceeding 15 ug.L^{-1}. On the other hand, the few samples analysed, in late 1982, for suspended particles show a concentration ranging between 1.4-3.0 ug.L^{-1} (mean: 2.3 ug.L^{-1}).

The figure is similar to that found in the Acheloos River Estuary during the same cruise (range 1.3-2.8 ug.L^{-1}, mean: 2.1) (sea table 2) and also in 1983.

From table 2 is also apparent that all (22) samples of the Acheloos Estuary had dissolved and dispersed PAH concentrations ranging between 2.2 and 4.6 ug.L^{-1}. In general their levels were slightly lower than those of the "main" Patraikos levels, which are relatively higher than those reported for some other areas of Greece (MIMIKOS, 1980, a, b; SCOULLOS et al., 1982).

Although it is known that present methods do not allow precise determination of the phase in which the hydrocarbons are presented, a rough indication is deduced that hydrocarbons in suspended particles represent 40-60 per cent of the total concentration.

From table 2 it is also clear that the concentrations of both dissolved and suspended PAHs at 1 m and 10 m depths have a rather random distribution and it is therefore, difficult to consider a concentration-depth relationship, although such a trend has been noted by several other authors (CORNER, 1975; MONOGAM et al., 1973).

Figure III gives an overall picture of the evolution of the oil pollution of the Patraikos Gulf. The September 1977 and March 1979 peaks might be attributed to pollution incidents.

The most interesting and alarming feature, however,is that ignoring all peaks, the background mean areal concentrations show a very stable gradual increase.

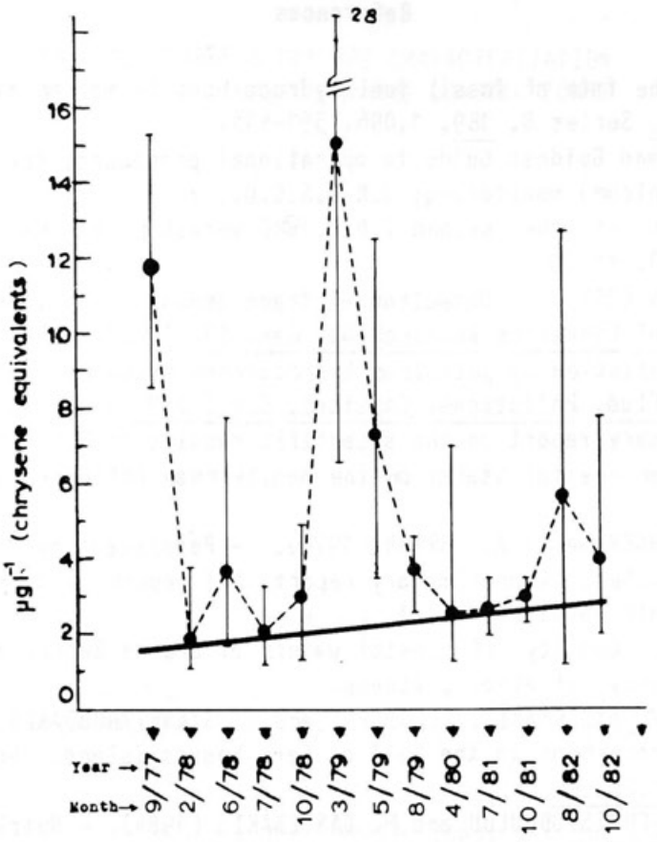


Figure III

Fluctuation of the mean areal concentration of PAHs in the Patraikos Gulf throughout the period 1977-82. The vertical lines show the range of the values. The line joining each mean value has been inserted to make the PAHs distribution easier to follow visually. The thick line joins the lowest mean values recorded.

Conclusions

The results of a five year study of PAHs in the Patraikos Gulf and the Estuary of the Acheloos River indicate that this area usually has total dissolved and dispersed concentrations ranging between 2-5 ug.L^{-1}. The suspended PAHs are present in concentrations of approximately 2 ug.L^{-1}. Probably the most interesting result is the general upward trend of the background concentrations observed throughout the period studied.

Acknowledgments

Several phases of the work have been supported partly by U.N.E.P./MED-POL phases I; I.O.C.-U.N.E.P./MED-POL II (GRE-04) and the Commission of the European Communities (ENV-560, GR-B).

References

CORNER E.D.S. (1975). - The fate of fossil fuel hydrocarbons in marine animals. Proceedings of the Royal Society (London), Series B, 189, 1,096, 391-413.

I.O.C. (1976 a). - Manuals and Guides: Guide to operational procedures for the IGOSS Pilot Project on marine pollution (petroleum) monitoring; U.N.E.S.C.O., n° 7.

I.O.C. (1976 b). - Report of the second I.O.C./WHO workshop on Marine Pollution (Petroleum) Monitoring; U.N.E.S.C.O. n° 10.

KEIZER P.D. and D.C. GORDON (1973). - Detection of trace amounts of oil in sea-water by fluorescence spectroscopy, Journal of Fisheries Research Bd. Can. 30, 1,039-1,046.

MIMICOS N. (1980, a). - Pollution by petroleum hydrocarbons along several Greek islands, coasts and harbors. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. 489-492.

MIMICOS N. (1980, b). - Summary report on the scientific results of MED-POL. Intergovernmental Review Meeting on Mediterranean coastal States on the Mediterrean Action Plan, U.N.E.P., Barcelona 11-13 Feb. 1980.

MONAGHAN P.H., J.H. SEELINGER and R.A. BROWN (1973). - Persistent hydrocarbons content of the sea along certain tanker routes. A preliminary report, API report of 18th Annual Tanker Conference, Hilton Head Island, South Carolina, U.S.A.

SCOULLOS M. (1982). - The Quality of coastal waters of Greece Report for the Commission of the European Communities, Univ. of Athens, Athens.

SCOULLOS M., N. MIMICOS, M. DASSENAKIS, L. BACHAS and E. STAVRIANOUDAKIS (1982). - Trace metals and petroleum aromatic hydrocarbons in the Gulf of Gera,Lesvos Island, Greece, VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M.

SCOULOS M., M. MAROULAKOU-CHRISTODOULOU and M. DASSENAKIS (1984). - Nutrients in the Patraikos Gulf and the Estuary of Acheloos.

CHEMICAL MARKERS FOR THE CHARACTERIZATION OF POLLUTANT INPUTS IN THE COASTAL ZONES

by

J. GRIMALT, J.M. BAYONA
J. ALBAIGES
Instituto de Quimica Bio-Organica (CSIC)
Jorge Girona Salgado, 18-26.
08034-Barcelona (Spain)

Abstract

A chemical marker is any compound or class od compounds in the environment of which the elemental or molecular characteristics suggest an unambigous link with a known natural or anthropogenic source.

To illustrate the significance of marker compounds for marine pollution studies in the Mediterranean, sediment samples were collected along the Catalan coast and analysed in detail by gas chromatography (FID and FPD) and by computerized GC-MS. Special emphasis was given to the aromatic fraction.

Long chain alkyl benzenes indicating domestic wastes were identified for the first time in Mediterranean samples. Organosulphur compounds, probably from petroleum used oils and coal tars,have also been identified. PAHs distributions, including alkyl homologue series (phenanthrenes, fluoranthenes + pyrenes and dibenzothiophenes) parent compounds distributions and certain individual PAH ratios (phenanthrene/anthracene: benzo(e)/benzo(a)pyrene have provided evidence of urban run-off as the most important PAH input to coastal sediments.

Levels of pyrolytic-like PAH in Catalan coast sediments range from 310-2,313 ng/g dry wt and benzo(a)pyrene from 15-70 ng/g. These values are similar to others found in other moderately polluted coastal marine areas.

*
* *

Introduction

Sediments have been repeatedly used for the study of the contamination of coastal zones by conservative organic pollutants, namely hydrocarbons (GEARING et al., 1976: FARRINGTON and TRIPP, 1977: KEIZER et al., 1978, VENKATESSAN et al., 1980) and the advantages and drawbacks ofthis use have also been discussed (PHILLIPS, 1983). However, this approach has not been attempted much in the Mediterranean, despite the interest in recognizing land-based pollutant sources in coastal areas. Only very recently, studies have been conducted in the Western Mediterranean, involving the Spanish (ALBAIGES, et al., 1982: GRIMALT, 1983) and French coasts (ARNOUX et al., 1980: MILLE et al., 1982a and b: BURNS and VILLENEUVE, 1983) as well as the deep sea basin between the Balearic Islands and those of Corsica and Sardinia (ARNOUX et al., 1982).

After a preliminary survey of hydrocarbons in sediment samples collected along the Spanish continental shelf (ALBAIGES et al., 1982), in the present paper we focus our attention on the composition of the aromatic fractions to illustrate the possibility of using certain components as markers of hydrocarbon pollutant sources in the area.

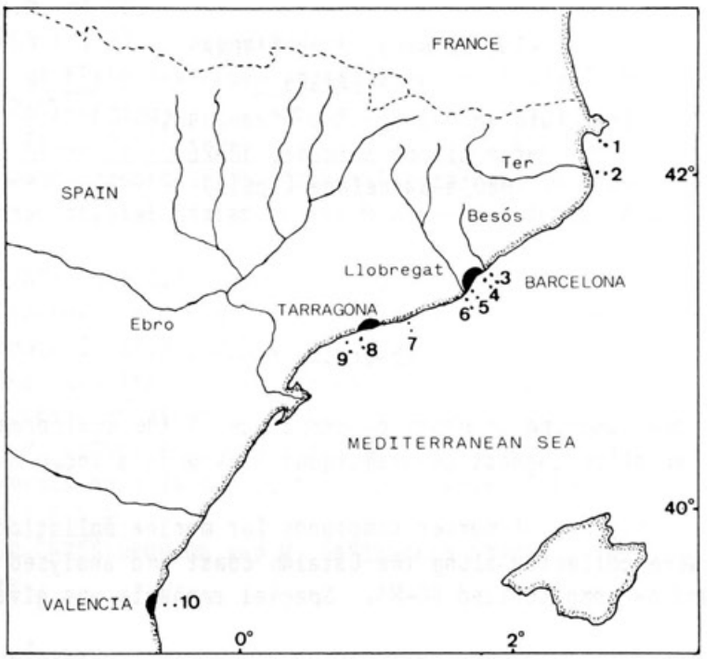


Figure 1. - Sample locations.

Material and methods

Sediments were collected with a previously cleaned grab sampler, wrapped in alumunium foil and stored at -20 °C until analysis. Before this, the samples were freeze-dried and sieved through 250 µm in order to remove coarse sands and obtain more reproducible and intercomparable results. Then, 20 g of sediment were extracted by sonication with methylene chloride-methanol (2/1) (4x30 ml) which provide an extraction efficiency better than 90 per cent (GRIMALT et al., 1984). The extracts were vacuum-evaporated near to dryness and saponified with 10 ml of 6 % methanolic KOH overnight. Neutrals were recovered with hexane (3x15 ml), desulphurized with activated copper and fractionated by column chromatography into saturates and aromatics (CUBERES et al., 1984).

The saturated fractions were analysed by HRGC on a 20 mx0.25 mm ID SE-52 column fitted in a Carlo Erba 4,160 model. The aromatic fractions were analysed by HRGC on a 30 m DB-5 column (J & W Scientific) placed in a PE 990 GC, equipped with a moving needle injector and conveniently modified for permitting the simultaneous detection of the column effluent by FI and FP detectors.

Identification of individual hydrocarbons was carried out by COM-GC-MS using a Hewlett-Packard 5,995 system. Series of alkyl aromatic hydrocarbons were monitored and quantified using the mass fragmentographic profiles.

Results and discussion

The sample sites are indicated in figure 1. They have been located in the vicinities of the large urban areas of Barcelona (3-6), Tarragona (8-9) and Valencia (10). In addition, three stations have been selected for obtaining baseline data (1, 2 and 7).

Table I. - Aliphatic Hydrocarbons in sediments (µg/g dry wt.).

Transect / Water depth(m)		n-alkanes total	n-alkanes >24/<24	n-alkanes CPI	pristane/phytane	UCM
1. Roses	225	1.5	7.8	4.1	2.2	4.6
2. Ter	62	1.9	12.6	3.0	2.5	9.8
	450	0.9	21.4	3.9	2.2	6.2
3. Besòs	25	17.0	7.1	1.5	0.8	460.0
	70	3.2	2.6	1.8	1.9	36.3
4. Barcelona	50	11.4	5.9	2.4	2.6	528.4
	80	3.4	11.5	2.1	1.8	27.9
5. Barceloneta	25	11.1	7.1	1.4	0.7	452.9
	80	3.9	2.6	2.0	2.1	63.7
6. Llobregat	25	3.3	4.1	1.6	2.3	73.4
	120	2.9	0.6	1.1	0.6	119.4
7. Torredembarra	10	3.2	1.8	1.5	0.6	57.9
	90	2.3	1.6	1.9	0.5	28.8
8. Tarragona	17	1.4	3.4	1.6	0.9	61.9
	125	2.5	2.7	2.6	0.9	36.7
9. Salou	15	0.9	3.9	1.6	1.4	21.4
	65	5.8	2.3	1.8	0.6	77.1
10. Valencia	10	1.0	4.8	3.5	1.2	12.3

The saturated fractions (Table I) exhibit similar patterns to those previously found for a larger set of sediment samples (ALBAIGES et al., 1982), although in the present case we have selected stations closer to polluted zones and particularly to the area of Barcelona.

In addition to these parameters we have stressed in previous papers the importance of using molecular markers for the identification of specific pollutant inputs (ALBAIGES, 1980: ALBAIGES et al., 1982: RISEBROUGH et al., 1983) and we have discussed the analytical methodology for fingerprinting series of saturated hydrocarbons, such as hopanes, steranes and rearranged steranes, of diagnostic value (ALBAIGES and ALBRECHT, 1979).

The application of the same approach to the present samples has enabled the identification for the first time in the Mediterranean of series of long chain alkybenzenes (LABs). These compounds usually appear in our fractionation procedure in the saturated fraction, in the elution range of C15-C20 n-alkanes and can be easily monitored with the ion m/e 91 as shown in figure 2. They have been found particularly abundant in sediments near Barcelona.

Several reports have recently shown that LABs occur in domestic wastes and in aquatic sediments adjacent to urban centres (EGANHOUSE et al., 1983: ISHIWATARI et al., 1983) and obtained evidence that they arise from contamination by anthropogenic detergent-bearing wastes. Until now LABs carried as residues with the sulphonated alkylbenzenes into detergents were considered to be of no environmental consequence, but it seems that they persist in the marine environment and then can be exploited as molecular markers for domestic wastes.

Aromatic hydrocarbons are usually minor components in contemporary aquatic sediments (WAKEHAM et al., 1980; LAFLAMME and HITES, 1978) and mainly consist of a few compounds arising from the early aromatization of biogenic molecules (e.g. steroids and triterpenoids). However, in the proximities of urban and industrial centres they occur in higher amounts and as complex assemblages of hydrocarbon species (PAH), reflecting different anthropogenic inputs.

In figures 3-5 are shown three chromatographic profiles representative of the analysed samples. In addition to the FID profile we have simultaneoustly recorded the FPD chromatogram which shows the organosulphur compounds (OSC) present in the samples. OSC have been shown to exist in airborne

Transect	1 Roses		2 Ter		3 Besòs		4 Barcelona Harbour		5 Barceloneta		6 Llobregat		7 Torredembarra		8 Tarragona		9 Salou		10 Valencia
Water depth(m)	225		62-450		25-70		50-80		25-80		25-120		10-90		17-125		15-65		10
phenanthrene (1)	18	20	13	171	11	33	26	307	48	170	95	23	31	95	8	17	28	4	
anthracene	0.9	3	0.5	39	1.3	5	4	65	4	42	10	1.8	1.4	0.9	0.7	1.7	3.2	3	
fluoranthene	34	58	44	332	30	121	63	538	113	318	135	71	104	73	40	40	66	265	
pyrene	34	40	31	333	27	107	59	527	95	290	182	58	107	69	36	42	58	84	
benz(a)anthracene	18	26	18	82	11	54	28	180	44	135	54	54	68	35	30	22	39	156	
chrysene+triphenylene	37	29	41	105	14	56	32	221	50	148	67	53	86	37	37	26	51	174	
benzofluoranthenes(b+j)	15	36	62	104	21	63	41	161	64	148	91	54	109	29	56	27	67	177	
benzo(k)fluoranthenes	35	21	38	59	12	39	26	103	39	105	48	48	89	11	42	19	51	2	
benzo(e)pyrene	--	22	--	88	15	54	33	123	58	139	67	71	108	18	54	46	61	101	
benzo(a)pyrene	7	24	23	71	13	59	29	134	40	168	59	50	116	14	44	24	57	148	
perylene	24	13	33	25	5	19	14	25	14	104	18	34	47	3	20	7	27	88	
indeno(1,2,3-cd)pyrene	30	29	55	47	16	54	30	74	53	112	42	49	84	8	46	16	47	78	
benzo(ghi)perylene	35	21	49	55	15	46	27	172	51	128	53	82	133	16	54	14	75	90	
coronene	-	11	23	40	4	21	6	55	15	28	12	15	12	8	17	5	14	30	
phenanthrene/anthracene	21	6.9	23	4.4	8.8	6.1	5.9	4.7	12.6	4	9.4	13.1	21.1	105	11.4	10.3	8.9	1.2	
fluoranthene/pyrene	1	1.5	1.4	1.0	1.1	1.1	1.1	1.0	1.2	1.1	0.7	1.2	1.0	1.1	1.1	0.9	1.1	3.1	
benz(a)anthracene/crysene	0.5	0.9	0.5	0.8	0.8	1.0	0.9	0.8	0.9	0.9	0.8	0.8	0.8	0.9	0.8	0.9	0.8	0.9	
benzo(e)pyrene/benzo(a)pyrene	--	0.9	--	1.2	1.2	0.9	1.1	0.9	1.4	0.8	1.1	1.4	0.9	1.3	1.2	1.9	1.1	0.6	
UCM (ug/g)	2.9	4.7	1.9	49.9	2.9	6.7	8.8	64.5	7.5	24.9	11.1	13.5	6.5	7.5	7.1	3.4	20.6	4.7	
total pyrolytic aromatic hydrocarbons (ng/g) (2)	345	429	467	1342	183	693	390	2313	637	1824	990	629	1064	310	475	288	615	1396	

(1) All concentrations of individual compounds are expressed as ng/g of dry sediment

(2) Phenanthrene, anthracene and perylene are excluded

Table II. - PAHs in sediments.

particulates and in petroleum, exhibiting particular distributions, so that they can be used as a complementary tool for oil spill and tar ball idenfications (ALBAIGES, 1980). However this approach has rarely been attempted for the characterization of chronic inputs in sediments (BATES and CARPENTER, 1979).

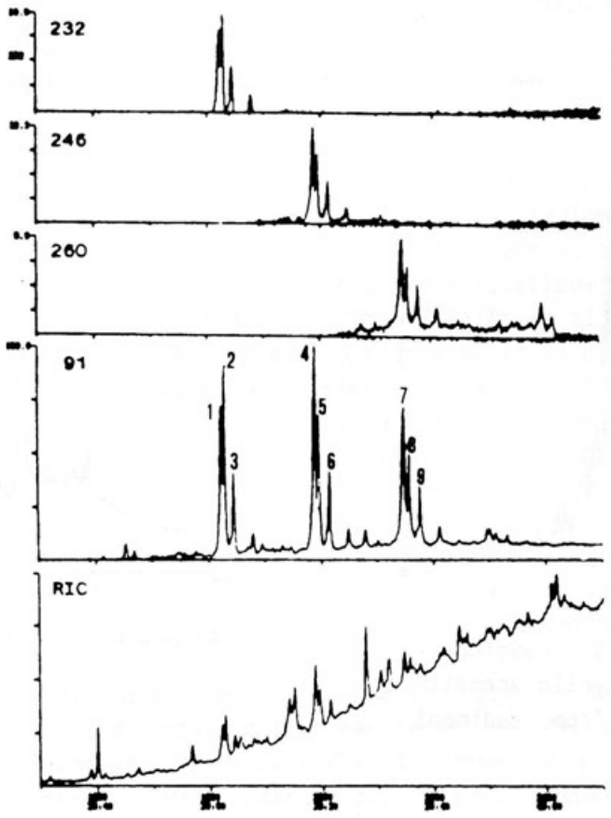


Figure 2. - Mass fragmentographic profiles of long chain alkylbenzenes (m/e 91) in the hydrocarbon extracts of sediments from station 6. Phenylundecanes (1-3), phenyldodecanes (4-6) and phenyltridecanes (7-9).

The chromatograms of figure 3 correpond to station 4, at the entrance to Barcelona harbour. A common feature of both profiles is that they show a broad molecular weight range of partially resolved compounds corresponding to homologous series of alkyl substituted aromatics. The main resolved peaks correspond to alkylphenanthrenes and dibenzothiophenes, clearly suggesting direct deposition of petroleum (crude oil or fuel oil).

Figure 4 shows the ditribution of PAHs which is generally found in surface sediments of the Mediterranean coast (ALBAIGES et al., 1982; MILLE et al., 1982: BURNS and VILLENEUVE, 1983) as well as in other coastal areas (LAFLAMME and HITES, 1978; LAKE et al., 1979: OVERTON and LASETER, 1980; VENKATESSAN et al., 1980; SPORSTOL et al., 1983, PRAHL and CARPENTER, 1983). It encompasses a series of unsubstituted aromatic hydrocarbons, characteristic of pyrolytic sources (automobile exhausts, coal and petroleum combustion, etc. and unresolved complex mixture (UCM) indicate of certain contributions of petroleum derived products.

Consistent with this contribution is the FPD profile which desserves some similarity with that shown in figure 3, although the alkyldibenzothiophenes are less abundant with respect to the UCM. On the other hand another series of OSC appears, that of the benzo(b)naphtothiophenes. The presence of

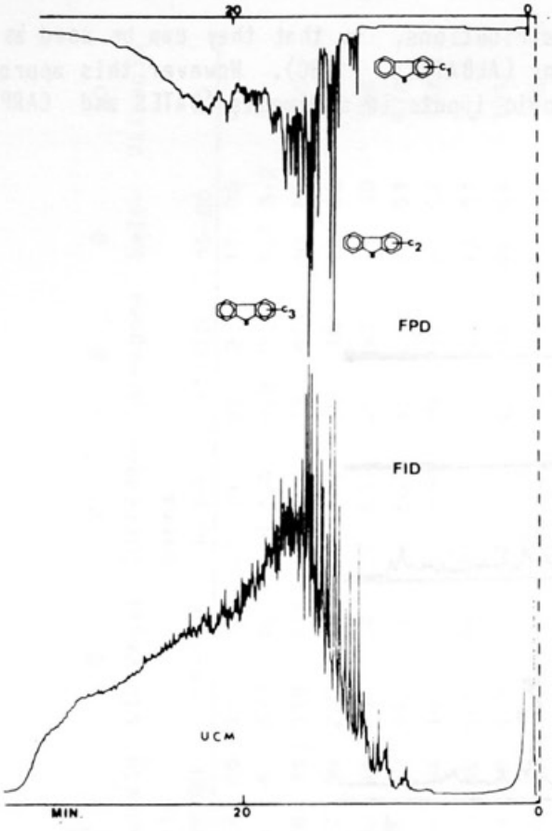


Figure 3. - FID and FPD chromatographic profiles of polycyclic aromatic hydrocarbons isolated from sediment of station 4 (50 m.).

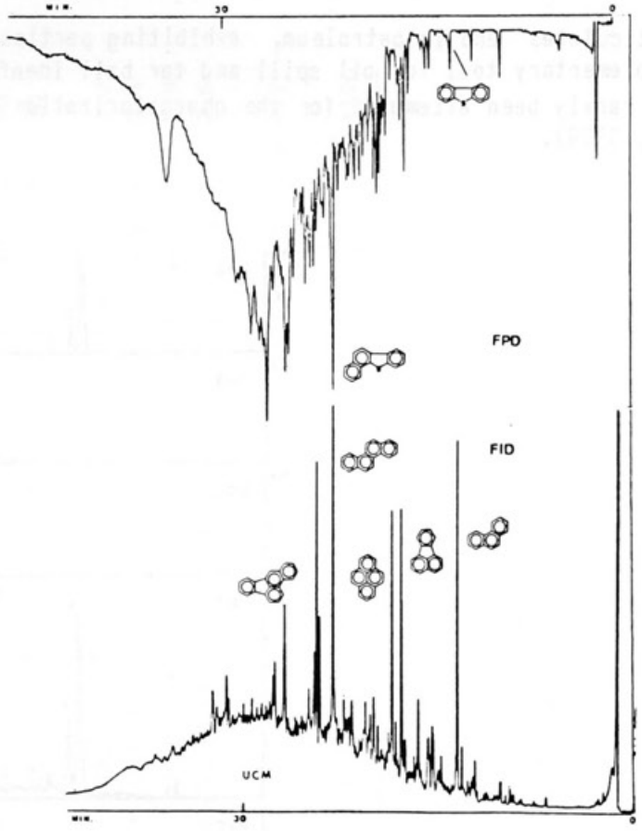


Figure 4. - As figure 3 for station 8.

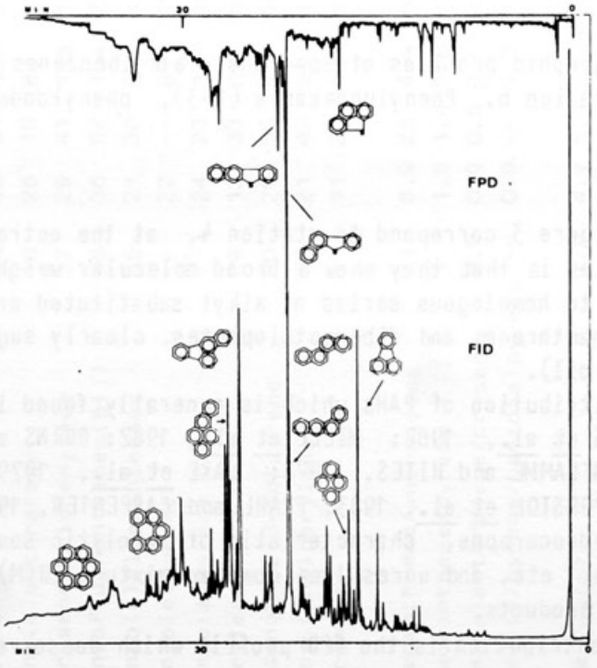


Figure 5. - As figure 3 for station 10.

these compounds is remarkable. They are common in mineral oils, namely lube oils and petroleum (GRIMMER et al., 1981 and 1983) and are absent in atmospheric particulates. We assume that such a profile could reflect a contribution of urban run-off (e.g. street dust, containing aeolian fall-out, asphalt particles, lubricating oils, etc.) washed from roads to coastal sediments.

Finally, traces of geochemically produced hydrocarbons (retene, pimanthrene and other phenanthrene derivatives, indicated by an asterisk) have also been found in these sediments.

The profiles shown in figure 5, correspond to a sediment collected offshore from Valencia. In this case the contribution from fossil fuels is minimal because both profiles (FID and FPD) are devoid of alkylsubstituted species and the UCM. PAHs are typically pyrolytic and OSC show the predominance of phenanthrothiophene and benzo(b)naphtothiophene isomers, that could indicate a coal tar source (LATER et al., 1981).

Even though fingerprinting of PAHs and OSC by gas chromatography has provided evidence of widespread deposition of different pollutant sources into coastal sediments, a more accurate assessment of those can be obtained with quantitative parameters, such as alkyl homologue distributions within each series of compounds (AHD), parent compound distributions (PCD) and specific compounds ratios (LAFLAMME and HITES, 1978; LAKE et al:, 1979: SPORSTOL et al., 1983). AHDs of samples in figures 3-5 are displayed in figure 6. The increasing predominance of pyrolytic inputs from samples A (corresponding to Barcelona harbour: figure 3) to sample C (Valencia· figure 5) is documented by the homologue distributions within the phenanthrene + anthracene and fluoranthene + pyrene series (figure 6). Petroleum contains only small amounts of parent PAHs relative to their alkylated homologues whereas in combustion (pyrolytic) products the parent compounds are at much higher concentrations than their homologues. The bimodal distributions observed in samples A and B may indicate a combined input of petrogenic and pyrolytic hydrocarbons. It is worth mentioning that fluoranthene + pyrene is more indicative of a pyrolytic contribution than phenanthrene (see sample A). On the other hand notice that, as pointed out by SPORSTOL et al:, (1983), alkyldibenzothiophenes do not provide such information.

PCDs as well as several individual PAHs ratios are indicated in table II. The PAH pattern in these sediments is identical to that observed in other recent aquatic sediments where the occurrence of a deposition of fossil fuel combustion products directly from the air or from land by washing has been suggested (LAFLAMME and HITES, 1978: LAKE et al., 1979; WAKEHAM et al., 1980: OVERTON and LASETER, 1980).

Table III. - Selected PAH ratios in different hydrocarbon pollutant sources.

	air particulates(a)	coke oven emissions(b)	coal tar (c)	used motor oil (d)	petroleum (e)
phenanthrene/anthracene	5-30	1-3	20-30	3-5	50
fluoranthene/pyrene	0.5-1.1	1.0-1.5	1.1-1.5	0.5-2.0	0.3-1.0
benz(a)anthracene/crysene	0.35-0.50	0.5-0.9	0.9-1.1	1.0	0.1-0.6
benzo(e)pyrene/benzo(a)pyrene	1.3-2.0	0.3	0.6-0.8	1.1-2.0	3-15

a)Gordon, 1976; Grosjean, 1934 ; Grimmer et al., 1983
b)Lao et al., 1975
c)Lao et al., 1975; Borwitzky and Schomburg, 1979
d)Grimmer et al., 1981; Lake et al., 1979
e)Grimmer et al., 1983; Neff, 1979

In table III we have summarized PAH ratios for some possible source materials. The higher phenanthrene/anthracene ratios (P/A) are shown by petroleum and coal tar (30-50) whereas used motor

oil and coke oven emissions give the lower ones (1-5). Air particulates show intermediate values (15-30). The transect sediments have low P/A (1.2/13.1), although a certain increase is observed for the more offshore samples (see transects 2,5 and 7). As previously stated we may argue that nearshore sediments reflect urban run-off inputs that contain air particulates and used crankease oils adsorbed on to street dust. In more remote areas the deposition of atmospheric particulates could predominate.

Fluoranthene/pyrene ratios are respectively around 1.0 for all sediments except for n° 10 (Valencia). These values are slightly high if related to airborne particulates, so that the proposed combination of pyrolitic and used oil inputs seems to be reinforced. Benzo(e)pyrene/benzo(a)pyrene values are also in accordance with this assumption. Notice that here again sediment n° 10 deviates from normal values (0.6) fitting well with the proposed coal tar input.

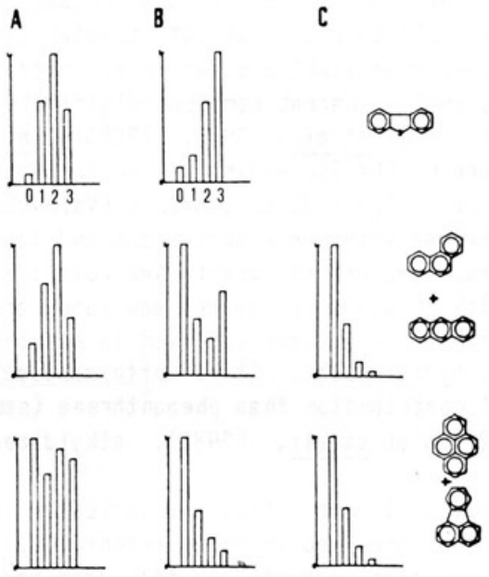


Figure 6. - Alkyl homologue distributions of dibenzotiophenes, phenanthrenes + anthracenes and fluoranthenes + pyrenes in samples shown in figure 3 (A), 4(B) and 5(C).

Particularly characteristic of urban atmospheric emissions are the high concentrations of indeno (1, 2, 3-cd)pyrene, benzo(ghi)perylene and coronene with respect to benzo(a)pyrene (GORDON, 1976: GRIMMER et al., 1983). JELTES (1974) has proposed B(a)P/B(ghi)Pe for differentiating between domestic and automobile sources. Benzo(a)anthracene may arise also to a larger degree from non-automobile sources. It is difficult, however, to confer strong validity on these parameters for the interpretation of data from marine sediments because these compounds undergo relative changes during transport and deposition due to a variety of environmental processes that may modify the original distributions. For example, the solubility of phenanthrene is 20 times that of anthracene, benzo(a)pyrene is more phtoreactive than benzo(e)pyrene, etc. (NEFF, 1979).

The levels of PAH in the sediments studied (Table II) are about the same as those reported by LAFLAMME and HITES (1978) and by PRAHL and CARPENTER (1983) in marine sediments from the Atlantic and Pacific coasts, respectively, although the abundance rises to 1,300-2,300 ng/g of dry sediment with the proximity of urban centres (stations 3, 5, 6 near Barcelona and station 10 off Valencia). The total pyrolytic-like PAHs in recent sediments from open sea areas range from 100-500 ng/g for the North Sea and from 0.1-50 for the Central Atlantic (TISSIER and SALIOT, 1983).

We have found concentrations of 15-70 ng/g of benzo(a)pyrene in sediments under the influence of anthropogenic inputs. DUNN and STICH (1976) reported 7.6-121 ng/g in the vicinity of a sewage treatment plant (Vancouver, Canada) and JENSEN (1984) found 13-54 ng/g in a marine area affected by a refinery effluent (Kalundborg, Denmark).

As far as other Mediterranean areas are concerned, data are very limited. MILLE et al., (1982) and BURNS and VILLENEUVE (1983) found PAH values within the same ranges in samples off Monaco (599-723 ng/g). The values reported by MILLE et al., (1982) for other sites of the French coast are lower, but this may be attributed to the fact that sediments were collected at shallow depths (5-10 m) and therefore possibly containing a major proportion of sand.

Values of benzo(a)pyrene below 5 ng/g can be considered as baseline concentrations for the surface sediments in the Mediterranean (ARNOUX et al., 1982: ALBAIGES, 1982). This contribution may arise from deposition of aeolian transported fossil fuel combustion products.

Conclusion

The detailed analysis of aromatic hydrocarbons in coastal sediments has permitted the recognition of a variety of sources of inputs to the Spanish Mediterranean coast ranging from domestic wastes to urban run-off,airborne particulate deposition and oil spills (including petroleum and coal tars).

However, the PAHs patterns show that urban run-off of street dust may be the most important PAH input to coastal sediments. The concentrations are similar to those reported for other coastal areas situated near anthropogenic sources.

Acknowledgment

We are indebted to Instituto Espanol de Oceanografia (A. Alvarez and J. Ros) for making samples available and to W. Walker and R.W. Risebrough (Bodega Bay Institute, Berkeley). Funding was provided by Consejo Superior de Investigaciones Cientificas. One of us (J.G.) acknowledges the receipt of a postdoctoral fellowship from C.S.I.C.

References

ALBAIGES J. P. ALBRECHT (1979). - Fingerprinting of marine pollutant hydrocarbons by COM-GC-MS. Internat. J. Environ. Anal. Chem. 6, 171-190.

ALBAIGES J. (1980). - Fingerprinting methods for the identification of petroleum pollutants in the Mediterranean Sea. In Analytical Techniques in Environmental Chemistry 1. (Ed. J. Albaigés). Pergamon Press, Oxford. 69-81.

ALBAIGES J., J.ALGABA, J.M. BAYONA and J. GRIMALT (1982). - New perspectives in the evaluation of anthropogenic inputs of hydrocarbons in the Western Mediterranean Coast. Ves Journées Etud. Pollutions. Cannes C.I.E.S.M., 199-206.

ARNOUX A., A. BLANC, A. JORAJURIA, J.L. MONOD, J. TAOSSIAN (1980). - Etat actuel des pollution sur les fonds du secteur de Cortiou (Marseille). Ves Journées Etud. Pollutions. Cagliari. C.I.E.S.M., 459-470.

ARNOUX A., J.L. MONOD, Th. SHEMBRI (1982). - Identification par GC et GCMs des POB, des hydrocarbures linéaires saturés, des hydrocarbures aromatiques polynucléaires et des phtalates dans les sédiments profonds de la Méditerranée Occidentale.

BATES T.S., R. CARPENTER (1979). - Organo-sulfur compounds in sediments of the Puget Sound basin.Geochim. Cosmochim. Acta, 43, 1209-1221.

BORWITSKY H., G. SCHOMBURG (1979). - Separation and identification of polynuclear aromatic compounds in coal tar using glass capillary chromatographic included combined gas-chromatography-mass spectrometry. J. Chromatog., 170, 99-110.

BURNS K.A., J.P. VILLENEUVE (1983). - Biogeochemical processes affecting the distribution and vertical transport of hydrocarbons residues in the coastal Mediterranean. Geochim. Cosmochim. Acta. 47, 995-1006.

CUBERES M.R., C. MARFIL, J.M. BAYONA, J. GRIMALT, J. ALBAIGES (1984). - Hidrocarburos aromaticos policiclicos. IV Aislamiento y separacion en muestras ambientales. Afinidad, 41, 391.

DUNN B.P., H.F. STICH (1976). - Monitoring procedures for chemical carcinogens coastal waters. J. Fish. Res. Bd. Canada. 33, 2040-2046.

EGANHOUSE R.P., D.L. BLUMFIELD, I.R. KAPLAN (1983). - Extractable organic matter in municipal waste waters. 2. Hydrocarbons: molecular characterization. Environ. Sci. Technol. 17, 523-530.

FARRINGTON J.W., B.W. TRIPP (1977). - Hydrocarbons in Western North Atlantic surface sediments. Geochim. Cosmochim. Acta., 41, 1627-1641.

GORDON R.J. (1976). - Distribution of airborne polycyclic aromatic hydrocarbons throughout Los Angeles. Environ. Sci. Technol., 10, 370-373.

GEARING P., J.N. GEARING, J.S. LYTTE (1976). - Hydrocarbons in 60 northeast Gulf of Mexico shelf sediments : a preliminary survey. Geochim. Cosmochim. Acta, 40, 1005-1017.

GRIMALT, J. (1983). - Geoquimica organica de sistemes deltaics. Ph.D. Thesis. Authonomous University. Barcelona.

GRIMALT J., C. MARFIL, J. ALBAIGES (1984). - Analysis of hydrocarbons in aquatic sediments. Internat. J. Environ. Anal. Chem. 18, 183-194.

GRIMMER G., J. JACOB, K.W. NAUJACK, G. DETTBARU (1981). - Profile of the polyciclic aromatic hydrocarbons from used engine oil. Z. Anal. Che., 309, 13-19.

GRIMMER G., K.W. NAUJACK, D. SHNEIDER (1981). - Profile of the polycyclic aromatic hydrocarbons in urban areas. Internat. J. Environ. Anal. Chem. 10, 265-276.

GRIMMER G., J. JACOB, K.W. NAUJACK (1983). - Profile of the polycyclic aromatic compounds from crude oil. Z. Anal. Chem. 314, 29-36.

GROSJEAN D. (1983). - Polycyclic aromatic hydrocarbons in Los Angeles air from samples collected on Teflon, glass and quartz filters. Atm. Env. 17, 2565-2573.

ISHIWATARI R., H. TAKADA, S. YUN, Ed. MATSUMOTO (1983). Alkylbenzene pollution of Tokyo Bay sediments. Nature, (Lond.) 301, 599-560.

JELTES R. (1974). Fingerprinting techniques as aids in the analysis of composite chemical pollutants in the environment. J. Chromatog. Sci., 12, 599-605.

JENSEN K., (1984). - Benz(a)pyrene input and occurrence in a marine area affected by refinery effluent. Water, Air and Soil Poll., 22, 57-65.

KEIZER P.D., J. DALE, D.C. GORDON (1978). Hydrocarbons in surficial sediments from the Scotian Shelf. Geochim Cosmochim. Acta., 42, 165-172.

LAFLAMME R.E., R.A. HITES (1978). - The global distribution of polycyclic aromatic hydrocarbons in recent sediments. Geochim. Cosmochim. Acta., 42, 289-303.

LAKE J.L., C. NORWOOD, C. DINOCK, R. BOWEN (1979). - Origins of polycyclic aromatic hydrocarbons in estuarine sediments. Geochim. Cosmochim. Acta., 43, 1847-1854.

LAO R.C., R.S. THOMAS, J.L. MONKMAN (1975). - Computerized gas chromatographic-mass spectrometric analysis of polycyclic aromatic hydrocarbons in environmental samples. J. Chromatog. 112, 681-700.

LATER D.W., M.L. LEE, K.D. BARTLE, R.C. KONG, D.D. VASSILAROS (1981). - Chemical class separation and characterization of organic compounds in synthetic fuels. Anal. Chem., 53, 1612-1619.

MILLE G., Y.Y. CHEN, H. DON (1982a). - Hydrocarbures présents dans des sédiments superficiels méditerranéens. VIes. Journées Etud. Pollution, Cannes C.I.E.S.M., 191-198.

MILLE G., Y.Y. CHEN, H. DON (1982b). - Polycyclic aromatic hydrocarbons in Mediterranean Coastal sediments. Internat. J. Environ. Anal. Chem., 11, 295-304.

NEFF J.M. (1979). Polycyclic aromatic hydrocarbons in the aquatic environment, Appl. Science, London.

OVERTON E.B., J.L. LASETER (1980). - Distribution of aromatic hydrocarbons in sediments from selected Atlantic, gulf of Mexico and Pacific Outer Continental shelf areas. In petroleum in the Marine Environment . (Ed. Petrakis, F.I. Weiss) Amer. Chem. Soc. n° 185, 327-341.

PHILLIPS D.J.H. (1983). - Application of the mussel watch concept: case histories. Paper presented at the 2nd. International Mussel Watch. Hawai (U.S.A.).

PRAHL F.G., R. CARPENTER (1983). - Polycyclic aromatic hydrocarbons (PAH)-phase associations in Washington coastal sediment. Geochim. Cosmochim. Acta, 47, 1013-1023.

RISEBROUGH R. et al., (1983). - Application of the Mussel Watch Concept in Studies of distribution of hydrocarbons in the Coastal Zone. Mar. Pollut. Bull. 14, 181-187.

SPORSTOL S., N. GJOS, R.G. LICHTENTHALER, K.O. GUSTAVEN, K. URDAL, F. ORELD (1983). Source Identification of Aromatic Hydrocarbons in Sediments Using GC/MS. Env. Sci. and Techn., 17, 282-286.

TISSIER M.J., A. SALITO (1983). - Pyrolytic and naturally occuring PAHs in the marine environment. Advances in Organic Geochemistry 1981 (Ed. M. Bjoroy) J. Wiley, 268-278.

VENKATESSAN M.J., S. BRENNER, E. RUTH, J. BONILLA, I.R. KAPLAN (1980). Hydrocarbons in age-dated sediment was from two basins in the Southern California Bight. Geochim. Cosmochim. Acta, 44, 789-802.

WAKEHAM S.G., C. SHAFFNER, W. GIGER (1980). - Polycyclic aromatic hydrocarbons in recent lake sediments. I Compounds having anthropogenic origins. Geochim. Cosmochim. Acta., 44, 403-413.

PETROLEUM AND CHLORINATED HYDROCARBONS IN BIOTA SAMPLES FROM THE WESTERN MEDITERRANEAN II. FISH SAMPLES

by

J. ALBAIGES, A. FARRAN
P. MARTIN* and M. SOLER
Instituto de Quimica Bio-Organica (CSIC)
Jorge Girona Salgado, 18-26. 08034-Barcelona (Spain)
* Instituto de Investigaciones Pesqueras (CSIC)
P° Nacional, s/n. 08004-Barcelona (Spain)

Abstract

In a previous paper (VIth C.I.E.S.M. Workshop 1982) we reported data on levels of fossil hydrocarbons in benthic organisms (primarily bivalves) collected on the northern Spanish Mediterranean coast. We now report data on hydrocarbons, PCBs and DDTs in three fish species collected at different sites of the Catalan coast, namely off Barcelona, in the north (Palamos) and in the south (Ametlla). The species are Merluccius merluccius, Trachurus trachurus and Mullus barbatus and the analyses were carried out on the muscle, liver and gonads. Hydrocarbons are accumulated primarily in the liver and PCBs and DDTs in gonads.

The levels found are related to the mobility of the species and to the pollutant sources. Mullus and Merluccius collected near Barcelona exhibited higher levels of hydrocarbons whereas those collected south near the Ebro mouth exhibited higher levels of PCBs and DDTs. These values are similar to or higher than those found in related species from other areas of the Mediterranean.

*
* *

Introduction

The NW Mediterranean coast has been considered as one of the most polluted areas in the Mediterranean in terms of BOD (LE LOURD, 1977). However, at present and particularly on the Spanish coast, little information has been generated for the assessment of levels and trends of coastal pollution from organic sources.

In order to improve this kownledge we initiated an investigation on the occurrence of widely distributed organic pollutants such as hydrocarbons and organochlorinated compounds in the different marine compartments. Based on the "mussel watch concept" (GOLDBERG et al., 1978), concentrations of these contaminants were measured in bivalves and the results have recently been reported (RISEBROUGH et al., 1983; ALBAIGES et al., 1982a). Although bivalves were found to be suitable sentinels for the recognition of land-based pollutant sources ("hot spots"), we were also interested to know whether coastal contamination affects pelagic species and causes adverse effects on the recruitment of those of commercial interest.

Table I. - Hydrocarbons, PCB's and DTTs in various organs of fish samples caught in the Western Mediterranean from 7.83 to 9.83. In parenthesis the number of specimens and the mean length. For sample sites see fig. 1. Results in µg/g dry wt.

SPECIES		SITES	SATURATES (FR.I)			AROMATICS			PCB's	DDTs
			UCM	RHC	TOTAL	FR.II	FR.III	TOTAL		
Merlucius merlucius										
Adults										
(1, 395 mm.)	Muscle	A	-	0.5	0.5	5.4	2.0	7.4	0.065	0.011
	Gonads		-	3.1	3.1	15.1	1.8	16.9	0.209	0.064
	Liver		42.3	10.1	52.4	20.8	0.9	21.7	0.948	0.129
(2; 427±39 mm.)	Muscle	B	-	0.9	0.9	3.6	0.8	4.4	0.156	0.024
	Gonads		6.2	1.4	7.6	7.9	2.2	10.1	4.963	0.642
	Liver		91.3	18.2	109.5	73.8	69.6	143.4	3.361	0.635
Intermediates										
(14, 258±56 mm.)	Muscle	A	1.5	0.5	2.0	0.6	1.1	1.7	0.222	0.045
	Liver		86.5	16.3	102.8	64.6	42.6	107.2	3.266	1.253
(6, 263±68 mm.)	Muscle	B	-	0.6	0.6	2.5	1.4	3.9	0.411	0.082
	Liver		121.9	14.3	136.2	40.7	8.6	49.3	9.749	1.792
(9, 248±52 mm.)	Muscle	C	-	1.2	1.2	0.9	1.5	2.4	0.245	0.039
	Liver		34.3	10.1	44.4	33.1	62.2	95.3	7.818	1.699
Juveniles										
(15, 79±20 mm.)	Whole Body	A	5.2	382.3	387.7	2.8	5.1	7.9	0.005	0.001
(7, 115±11 mm.)	Whole Body	B	8.1	0.8	8.9	13.6	3.5	17.1	2.298	0.730
(15, 111±42 mm.)	Whole Body	C	5.8	8.2	14.0	4.4	0.8	5.2	0.654	0.126

Table I (continuation)

SPECIES		SITES	SATURATES (FR.I) UCM	RHC	TOTAL	AROMATICS FR.II	FR.III	TOTAL	PCB's	DDTs
Mullus barbatus										
Adults										
	Muscle		12.6	2.4	15.0	0.6	3.8	4.4	0.307	0.057
(14, 162±15 mm.)	Gonads	A	37.6	9.9	47.5	5.2	10.7	15.9	1.413	0.320
	Liver		63.3	69.6	132.9	101.9	30.5	132.4	1.093	0.280
	Muscle		22.2	3.9	26.1	8.1	1.2	9.3	1.280	0.405
(16, 156±29 mm.)	Gonads	B	-	-	-	-	-	-	-	-
	Liver		7797.6	1488.5	9286.1	516.1	85.5	601.6	2.979	0.939
	Muscle		5.8	1.9	7.7	1.3	9.8	11.1	0.685	0.138
(14, 144±23 mm.)	Gonads	C	65.5	23.9	89.4	31.1	26.3	57.4	8.749	1.771
	Liver		1115.9	101.8	1217.7	94.7	30.2	124.9	2.282	0.499
Juveniles										
(3, 117±6 mm.)	Whole Body	A	7.5	2.1	9.6	1.2	2.1	3.3	0.291	0.035
(6, 57±5 mm.)	Whole Body	B	21.1	9.6	30.7	8.2	25.6	33.8	1.039	0.527
(9, 84±5 mm.)	Whole Body	C	12.3	3.7	16.0	17.7	1.9	19.6	2.084	0.247

Table I (continuation)

SPECIES	SITES		SATURATES (FR.I)			AROMATICS			PCB's	DDTs
			UCM	RHC	TOTAL	FR.II	FR.III	TOTAL		
Trachurus trachurus										
Adults										
	Muscle		11.2	11.1	22.3	0.6	3.6	4.2	0.234	0.027
(12, 192±47 mm.)	Gonads	A	-	-	-	-	-	-	-	-
	Liver		240.9	157.8	398.7	17.6	16.0	33.6	3.406	0.027
	Muscle		1.4	2.9	4.3	10.5	0.4	10.9	0.099	0.026
(12, 183±31 mm.)	Gonads	B	-	-	-	-	-	-	-	-
	Liver		207.8	20.8	228.6	39.9	3.4	43.3	5.434	1.475
	Muscle		5.4	4.3	9.7	1.3	2.4	3.7	0.127	0.067
(13, 184±30 mm.)	Gonads	C	7.7	4.3	12.0	7.8	11.4	19.2	4.547	0.637
	Liver		91.6	137.3	228.9	1.9	5.9	7.8	0.357	0.088
Juveniles										
(19, 81±12 mm.)	Whole Body	A	3.3	400.7	404.0	0.01	3.2	3.2	0.284	0.047
(6, 77 ± 4 mm.)	Whole Body	B	7.2	118.9	126.1	5.4	7.2	13.6	1.027	0.448
(15, 67 ± 4 mm.)	Whole Body	C	6.9	268.7	275.6	1.8	2.3	4.1	-	0.005

For this purpose, the study was continued with the investigation of three fish species currently found in the area: hake (Merluccius merluccius), red mullet (Mullus barbatus) and jurel (Trachurus trachurus). Adult and juvenile specimens were caught during the autumn of 1983 at three different locations along the Catalan coast, as indicated in figure 1 and analysed accordingly for hydrocarbons and organochlorinated compounds.

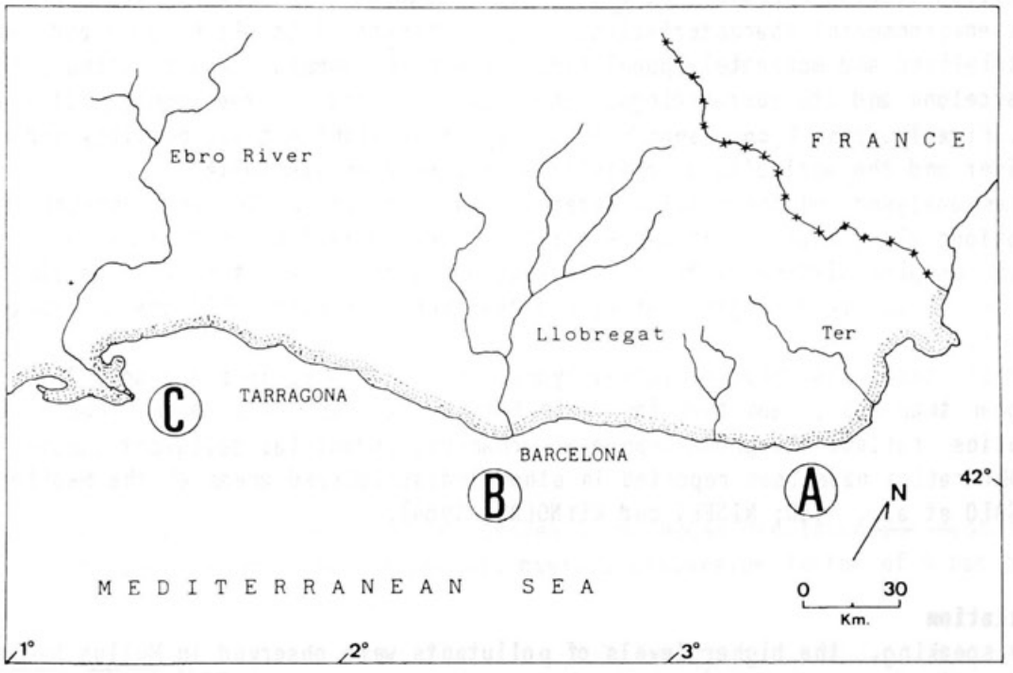


Figure 1. - Sample locations.

Materials and methods

Immediately after collection fishes were wrapped in clean aluminium foil and kept at -20 °C until analysis. The different parts of the fish (tissue, gonads and liver) were carefully separated with a scalpel and homogenized. Samples from fishes of the same species were pooled in order to reduce the number of analyses and obtain mean values of the pollutant concentrations (BERNHARD, 1976).

The analytical methodology for hydrocarbons has been reported elsewhere (ALBAIGES et al., 1982a). This basically encompasses saponification of the homogenate (6N aq. NaOH), extraction (ethyl ether) and chromatography (5 % water deactivated silica-alumina). The fraction eluted with n-hexane contains the saturated hydrocarbons that are analysed by glass capillary GC (20 m. SE-52). The second fraction, eluted with hexane-methylene chloride (9:1), contains the PCBs and the DDTs. the latter in the DDE form because of the previous saponification of the homogenate. This fraction is analysed by GC with an ECD (Carlo Erba FV 2,200) using a packed column (2 m. x 3 mm. i.d.; 1.5 % OV-17 and 1.95 % OF-1 on Supelcoport 100/120) at 190° C and argon/5 % methane as carrier. Quantitation was accomplished by comparison with reference solutions of Aroclor 1,260 and p,p'-DDE, in iso-octane. Aromatic hydrocarbons that are included in the second and the third (hexane-methylene chloride, 8:1) chromatographic fractions were analysed by UV-fluorescence using a Kuwait crude oil aromatic fraction os standard quantitation. The detailed operating conditions are given elsewhere (SOLANAS et al., 1982).

All results are expressed in ug/g on a dry weight basis and are not corrected for recoveries, which were always better then 85 per cent.

Blanks carried through the entire analytical procedure showed no signals attributable to contamination during the handling procedure.

Results and discussion

As in previous studies (ALBAIGES et al., 1982a) samples were obtained from three areas reflecting different environmental characteristics. Zone A (figure 1) is off Palamos and can be considered as non-industrialized and moderately populated, except in summer. Zone B reflects the influence of the city of Barcelona and its surroundings, including the port and the highly polluted rivers Besos and Llobregat. Finally, zone C corresponds to an important fishing area, possibly under the influence of the Ebro river and the agricultural activities developed in its delta.

The samples analysed and the results obtained (on a dry wt. basis) are reported in table I. With only few exceptions all samples exhibited significant concentrations of hydrocarbons, PCBs and DDTs. The unresolved complex mixture (UCM) of saturated hydrocarbons and the two aromatic fractions are considered to be of pollutant origin whereas the resolved saturates (RHC) are attributed primarily to biogenic sources.

The overall data show that pollutant hydrocarbon concentrations are more than one order of magnitude higher than PCBs, and that the ratio between PCBs and DDTs ranges from 3 to 8 (mean value 5). These ratios reflect the predominance of urban and industrial pollutant inputs in the area. Similar PCBs/DDT ratios have been reported in other industrialized areas of the Mediterranean and the Atlantic (FOSSATO et al., 1980; NISBET and REYNOLDS, 1984).

Bioaccumulation

Generally speaking, the higher levels of pollutants were observed in Mullus barbatus, followed by Merluccius merluccius and Trachurus trachurus. This may be explained by the habit of mullet of feeding on the bottom, thus being exposed to the pollutants in the sediment.

We have found in sediments from the Catalan continental shelf concentrations of hydrocarbons and PCBs ranging, respectively, from 10-100 ug/g 6-300 ug/g dry wt., the higher values corresponding respectively to areas near Barcelona - Tarragona and the Ebro mouth (ALBAIGES et al., 1982b; GRIMALT, 1983; BAYONA, 1984). Notice, however, that while the concentrations of hydrocarbons in fish tissues are of the same order of magnitude as in sediments, those of PCBs are much higher. On the other hand, although levels of hydrocarbons and organochlorinated compounds in adult specimens are consistently higher than in juveniles, they exhibit relative differences. For example, the ratio total aromatics/PCBs is about 10 for juveniles whereas it may reach 100 for adults. All these features obviously imply a different mechanism of accumulation or a different metabolic behaviour of these pollutants in marine biota.

Whether hydrocarbons are incorporated primarily by ingestion or through the gills is to be proved, although we have found that the hydrocarbon concentrations of plankton that correlates well with those of the fish stomach contents, are usually high (ALBAIGES et al., 1982a). In any case LEE et al. (1972) demonstrated that they are temporary stored in the liver from where they are metabolized and excreted. In fact, several authors have reported the accumulation of hydrocarbons in the liver (MIDDLEDITCH, 1979; SERRAZANETTI, 1980). The results shown in table I are consistent with these findings. Hydrocarbons are distributed among the different tissues as follows: liver > gonads > muscle. We assume that liver and muscle contents could reflect, respectively, acute and chronic pollutant inputs.

This is not the case for the organochlorinated compounds which are hardly metabolized. Due to their lipophilicity they are accumulated in tissues primarily according to their fat contents (BRUGGEMAN, 1982). Then, differences between gonads and liver are not so large and although the liver still exhibits the higher accumulation factor the distribution is muscular tissue < gonads < > liver.

The accumulation of DDTs and PCBs in gonads may be a feature to be considered from the possible effects on the viability of the eggs. The fact that adult and juvenile specimens exhibit for example, different total aromatics/PCBs ratios may be explained according to the different metabolic pathways of both contaminants. On the other hand, at present there is no clear evidence that petroleum hydrocarbons can be magnified in a marine food-chain as occurs with organochlorinated compounds. In this respect, we have found that fish tissues contain a great an amount of hydrocarbons as bivalves caught in the same area (ALBAIGES et al., 1982a, RISEBROUGH et al., 1983).

	PCBs	DDTs	Location	References
Mullus barbatus	4400	148	Mallorca (bay)	Chacartegui,1980
	738-3458	105-425	Genova	Contardi et al,1980
	(2115)	(233)		
	1-2101	0.3-197	C.Adriatic	Dujmov et al,1978
	(303)	(60)		
	528 220	160 92	N.Adriatic	Fossato et al,1980
	4640	1020	Rovinj	Smodlaka et al,1980
	2812	284	N.Aegean	Kilikidis et al,1980
Merlucius merlucius	325	67	S.Adriatic	Vilicic et al,1978
	2040	172	N.Aegean	Kilikidis et al,1980
Trachurus trachurus	136-1148	64-520	Rovinj	Smodlaka et al,1980
		560	Black Sea	Serbanescu et al,1980

Table II. - Concentrations of PCBs and DDTs in tissues of selected Mediterranean fish species (in ng/g dry wt). When results were reported on a fresh wt. basis a conversion factor of 4 has been applied.

Geographical distribution

Mullus barbatus and Merluccius merluccius are stationary species, so that they would reflect geographical patterns of pollution. Effectively comparing the same tissues and particularly the liver for different stations, the high degree of hydrocarbon pollution in zone B can be inferred. This is also observed for the migratory Trachurus trachurus.

The lower values correspond to zone A and can be considered as the Baseline levels for the area and are similar for both and juvenile specimens (5-12 ug/g and 2-7 ug/g of muscular tissue for saturated and aromatic hydrocarbons respectively).

Unfortunately, the information regarding hydrocarbon contents in Mediterranean fishes is almost inexistent (U.N.E.P., 1981). However, levels of hydrocarbons in tissues of marine organisms from areas apparently not exposed to petroleum contamination usually range from 0.4-40 ug/g dry wt. (MALINS, 1977). Therefore, from our data it cannot be concluded that the higher concentration of hydrocarbons existing in the Mediterranean with respect to other oceanic areas (LE LOURD, 1977) may be testified by the hydrocarbon concentrations in muscular tissues of pelagic fishes.

As far as PCBs and DDT contents are concerned, the higher concentrations have again been found in Mullus barbatus and Merluccius merluccius from zone B, though in this case the values for zone C are not so different as they were for hydrocarbons. This confirms provious observations based on the concentrations of PCBs anf DDts in mussels (RISEBROUGH et al., 1983) and in sediments (GRIMALT, 1983), according to which the Ebro river is an important source of these pollutants that requires continued monitoring.

With some exceptions, attributable to local pollution sources, it can be said that the actual values fall within others recently reported in the literature for pelagic fishes (e.g. North Atlantic: SIMS et al., 1977; HAGEL and TUINSTRA, 1978; BREVICK et al., 1978). In table II we have summarized for comparison the results of other studies carried out in the Mediterranean with the same species as the present ones.

Conclusions

The first set of data on hydrocarbons, PCBs and DDTs in Mullus barbatus, Merluccius merluccius and Trachurus trachurus caught at three stations of the Catalan coast and reported in this paper suggest that the amounts of pollutants observed are related to local sources for stationary species (M. Barbatus, M. merluccius) and probably correspond to baseline levels for the migratory ones (T. trachurus). Thus, higher levels of hydrocarbons are found in the area off Barcelona, whereas PCBs and DDTs are also high in the area near the Ebro mouth. However, baseline levels fall within other oceanic areas, including other parts of the Mediterranean.

Hydrocarbons are largely accumulated in the liver whereas organochlorinated compounds are both in the liver and the gonads. From our data it is not clear whether hydrocarbons are accumulated along the food web. However, adults contain much higher concentrations than juveniles, a situation which is different for PCBs and DDTs which are already present in significant concentrations in juveniles. These values are slightly lower than others reported for pelagic species from the same area in 1970. Despite this apparent decrease in concentrations a continued monitoring of hydrocarbons, PCBs and DDTs in stationary species from the areas selected along the Catalan coast would be desirable.

Acknowledgement

Thanks are due to Fundacion Areces and Consejo Superior de Investigaciones Cientificas for sponsoring this research.

References

ALBAIGES J., A. GALLIFA, J. GRIMALT, M. SOLER (1982a). - Hydrocarbons in biota samples from the Western Mediterranean. VIes. Journées Etud. Pollutions, Cannes C.I.E.S.M., 215-218.

ALBAIGES J., J. ALGABA, J.M. BAYONA, J. GRIMALT (1982b). - New perspectives in the evaluation of anthropogenic inputs of hydrocarbons in the Western Mediterranean coast.VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M., 109-206.

BAYONA J.M. (1984). - Hidrocarburs com a marcadors geoquimics a la plataforma continental mediterrania. Ph.D. Thesis. Authonomous University of Barcelona.

BERNHARD M. (1976). - Manual of methods in aquatic environment research. Part 3. Sampling and analysis of biological material. F.A.O. Fish. Techn. Reps. 158-124.

BREVICK E.M., J.E. BJERCK, N.J. KVESETH (1978). - Organochlorines in Cod fish from harbours along the Norwegian coast. Bull.. Env. Contam. Toxicol., 20. 715-720.

BRUGGEMAN W.A. (1982). - Hydrophobic interactions in the aquatic environment. The book of Environmental Chemistry. Vol. 2B, 83-102.

CHACARTEGUI G. (1980). - Niveaux de pollution dans les eaux littorales des îles Baléares. Ves. Journées Etud. Pollutions. Cagliari. C.I.E.S.M., 521-528.

CONTARDI V., G. ZANICCHI, D. MAZZOWE, B. MAGROCOSMA, (1980). - Fluctuations saisonnières de DPC, DDT et métabolites dans les organismes de la mer Ligure. Ves. Journées Etud. Pollutions, Cagliari, C.I.E.S.M., 335-340.

DUJMOV J., T. VUCETIC, M. PICER, N. PICER (1978). Some results of the monitoring of chlorinated hydrocarbons in organisms from the Central Adriatic. IVes. Journées Etud. Pollutions, Antalya, C.I.E.S.M., 137-141.

FOSSATO V.U., L. CRABOLEDDA (1980). - Chlorinated hydrocarbons in organisms from the Italian Coast of the Northern Adriatic Sea. Ves. Journées Etud. Pollutions, Cagliari C.I.E.S.M., 169-174.

FOWLER S.W. et al. (1978). - Heavy metals and chlorinated hydrocarbons in pelagic organisms from the open Mediterranean Sea. IVes. Journées Etud. Pollutions, Antalya, C.I.E.S.M., 155-158.

GOLDBERG E.D., V.T. BOWEN, J.W. FARRINGTON, G. HARVEY, J.H. MARTIN, P.L. PARKER, R.W. RISEBROUGH, W. ROBERTSON, E. SCHNEIDER, E. GRAMBLE (1978). - The mussel watch. Environ. Conservat. 5,101-125.

GRIMALT J. (1983). - Geoquimica organica de sistemes deltaics. Ph.d. Thesis. Authonomous University of Barcelona.

HAGEL P., L.G.M. TUINSTRA (1978). Trends in PCB contamination in Dutch Coastal and Island Fishery Products 1972-1976. Bull. Env. Contam. Toxicol. 19, 671-676.

KILIKIDIS S.D., J.E. PSOMAS, A.P. KAMARIORNOS, A.G. PANETSOS (1980). - Geo-Monitoring of DDT, PCBs and other chlorinated hydrocarbons in marine organisms from the North Aegean Sea. Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M, 301-306.

LE LOURD Ph. (1977). - Oil pollution in the Mediterranean Sea. Ambio, 6, 317-320.

MALINS C.D. (1977). Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms. Academic Press.

NEFF J.M., D. DIXIT, J.W. ANDERSON (1976). Accamulation and release of petroleum-derived aromatic hydrocarbons by four species of marine animals. Mar. Biol., 38, 279-289.

NISBET I.C.T., L.M. REYNOLDS (1984). - Organochlorine residues in common terns and associated estuarine organisms. Massachussets, U.S.A., 1971-81. Mar. Env. Res., 11, 33-36.

RISEBROUGH R.W., B.W. DELAPPE, W. WALKER, B.R.T. SIMONEIT, J. GRIMALT, J/ ALBAIGES, J.A. GARCIA, A. BALLESTER, M.G. MARINO (1983). - Application of the Mussel Watch Concept in studies of the distribution of hydrocarbons in the coastal zone of the Ebro Delta. Mar. Pollut. Bull., 14, 181-187.

SERBANESCU D., I. BILAL, R. MIHNEA (1980). - Derivés du DDT et du HCH chez quelques espèces de poissons du Danube et de la zone marine pre-deltaique. Ves. Journées Etud. Pollutions, Cagliari, C.I.E.S.M. 569-572.

SERRAZANETTI G.P., E. CARPENE, P. CORTESI, G. CRISETIG (1980). - Distribution des hydrocarbures aliphatiques dans les tissus d'Engraulis encrasicholus L. de la mer Adriatique. Ves. Journées Etud. Pollutions, Cagliari, C.I.E.S.M., 361-368.

SIMS G.G., J.R. CAMPBELL, F. ZEMLYAK, J.M. GRAHAM (1977). Organochlorine residues in fish and fishery products from the Northwest Atlantic. Bull. Env. Contam. Toxicol. 18, 697-705.

SMODLAKA N., M. DEVESCORI, I. IVANCIC (1980). - Comparison of two methods for analyses of chlorinated hydrocarbons in marine organisms. Ves. Journées Etud. Pollutions, Cagliari, C.I.E.S.M., 43-48.

SOLANAS A.M., R. PARES, C. MARFIL, J. ALBAIGES (1982). - A comparative study of chemical and microbiological monitoring of pollutant hydrocarbons in urban aquatic environments. Internat. J. Environ. Anal. Chem., 12, 141-151.

U.N.E.P. (1981). - Selected bibliography on the pollution of the Mediterranean Sea. 106 pp.

VILICIC D., N. PICER, M. PICER, B. NAZNSKY (1978). - Monitoring of chlorinated hydrocarbons in biota and sediments of South Adriatic coastal waters. VIes. Journées Etud. Pollutions, Antalya, C.I.E.S.M., 143-146.

PELAGIC TAR IN THE WESTERN MEDITERRANEAN 1981-82

by

J.D. DE ARMAS
Instituto Espanol de Oceanografia
Laboratorio de Canarias P.O. Box 1373
S. Cruz de Tenerife (Islas Canarias)

Abstract

In the cruises ALBORAN 81 (October 1981) anf ALSAREV 82 (May 1982), quantitative monitoring of particulate oil pollution in the Alboran Sea and adjacent areas was carried out. Tar balls within a wide range of sizes were present in all the samples. Concentrations of tar ranged from 0.01 mg/m^2 to 25.61 mg/m^2. In the Alboran Sea, the average concentration was 0.80 mg/m^2 and in the adjacent area, 3.60 mg/m^2. Pelagic tar samples from 15 stations on cruise ALBORAN 81 have been analyzed by gas chromatography to obtain an indication of the nature of tar balls.

*
* *

Introduction

The oceans are contamined by waste disposal proceeding from human activities. Among the most important contaminations are the residues from petroleum and its derivatives, which have been discharged from land sources or from ships, specially those used for the transport of petroleum.

The Alboran Sea and adjacent area is a zone of intense maritime traffic, including oil-tankers. These vessels clean their tanks in such a way that a great quantity of hydrocarbons of petroleum origin enter the ocean.

The quantitative collection of pelagic tar (petroleum residues) found on the surface of the Mediterranean Sea began in 1969, (HORN et al., 1970).

In January, 1975, the Spanish Institute of Oceanography began a programme of investigation into the contamination of the Mediterranean by hydrocarbons derived from petroleum. As from this date, several expeditions have been carried out with a view to obtaining a wider knowledge of the pollution of the zone.

In this paper we present the results obtained from the cruises ALBORAN 81 (October, 1981) and ALSAREV 82 (May, 1982), carried out in the Western Mediterranean on board the R/V Cornide de Saavedra.

Methods

A total of 49 samples were taken during these cruises from October, 1981 and May, 1982.

The surface samples were collected by means of a modified neuston sampler (SAMEOTO et al., 1969). This sampler consists of an aluminium open-ended box with a nylon plankton net. The sampler

was normally towed at 4 knots over a distance of one nautical mile, covering approximately a sea surface of 740 m2 to depth of 10-30 cm.

After each tow, the tar particles were removed by means of tweezers and spatula and were washed twice with distilled water, they were then dried at room temperature and weighed.

The extracts in n-hexane of the particles chosen were analysed in a gas Chromatograph Hewlett Packard model 5840 A equipped with columns of 2.5 m x 3.2 mm i.d. (3.8 % SE-30 on Chromosorb W, 80-100). The temperature was programmed from 100° C to 300° C (at 10° C/ min) using nitrogen as carrier gas.

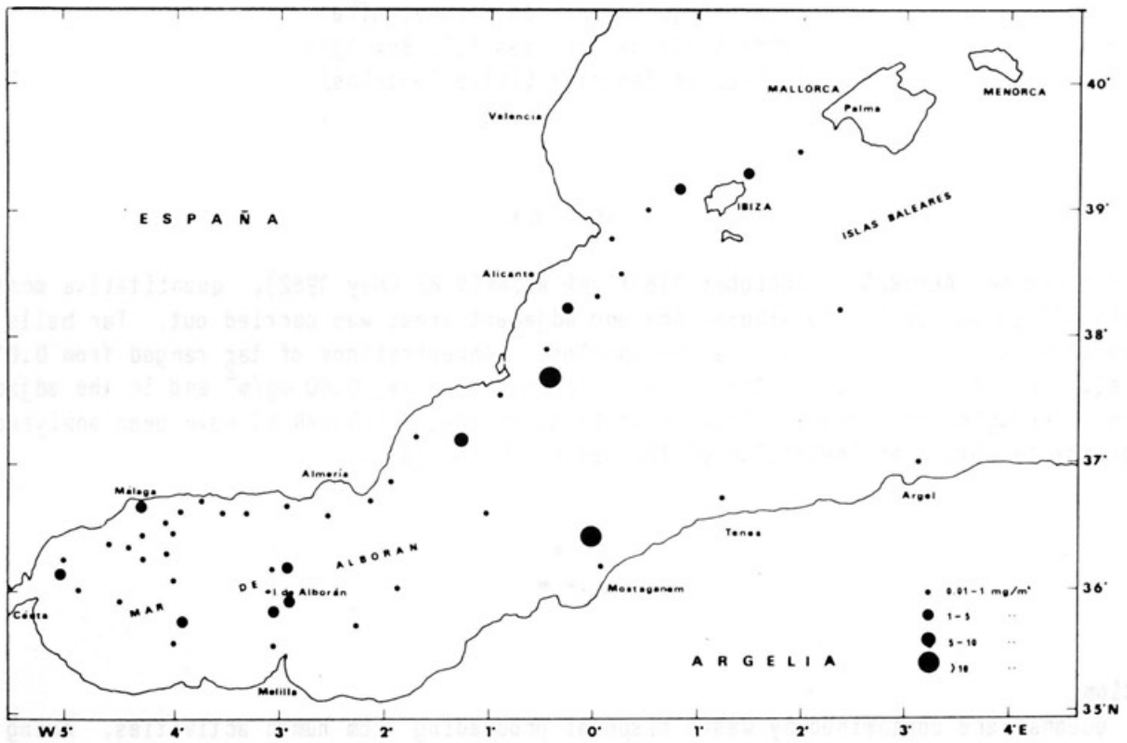


Figure 1.- Locations from which samples were collected 1981-82 inclusive. Sizes of black circles indicate relative amount of tar.

Results and discussion

Besides a varied quantity of particles of tar the content of the samples consisted of, plankton, seaweed fragments, fish and crustacean larva. terrestrial insects, small pieces of wood, plastic and other floating debris.

Figure 1 gives the distribution of the quantities of tar obtained in the Western Mediterranean. As can be seen in each one of the 49 tows carried out, particles of tar were collected showing great variability with values ranging from a concentration of 0.01 mg/m2 to 25.61 mg/m2 with an arithmetic mean of 1.65 mg tar/m2.

On grouping values by geographical zones we observed that for the Alboran Sea, the mean value found was 0.80 mg tar/m2; this value is lower than the values pointed out by HORN et al. (1970) and MORRIS et al. (1975) of 11.0 and 6.5 mg/m2 respectively and on the contrary, slightly higher than the value pointed out by ROS and FARACO (1978) who in the ALBORAN 76 expedition found a mean value of 0.56 mg tar/m2.

As for the adjacent area which we have considered as the Balearic Sea we found a mean value of 3.60 mg tar/m2 which is similar to the one reported by ROS and FARACO (1978) of 3.89 mg tar/m2 during the CONMEDOC II expedition of 1977, and higher than those found by HORN et al., (1970) and MORRIS et al., (1975) in the Balearic Sea of 2.40 mg tar/m2 and 0.50 mg tar/m2 respectively.

The maximum values of 25.61 mg/m2 and 19.80 mg/m2 were obtained in areas of intense tanker traffic off the Spanish and Algerian coast.

Likewise in figure 1 can be observed an area of greater contamination in the vicinity of the Isle of Alboran which must also be due to maritime traffic.

In figure 2, diagrams of the frequency distribution of the logarithms of the values obtained are represented, and table I gives the results of the concentration of tar in the areas mentioned and of the logarithmical transformations of the results.

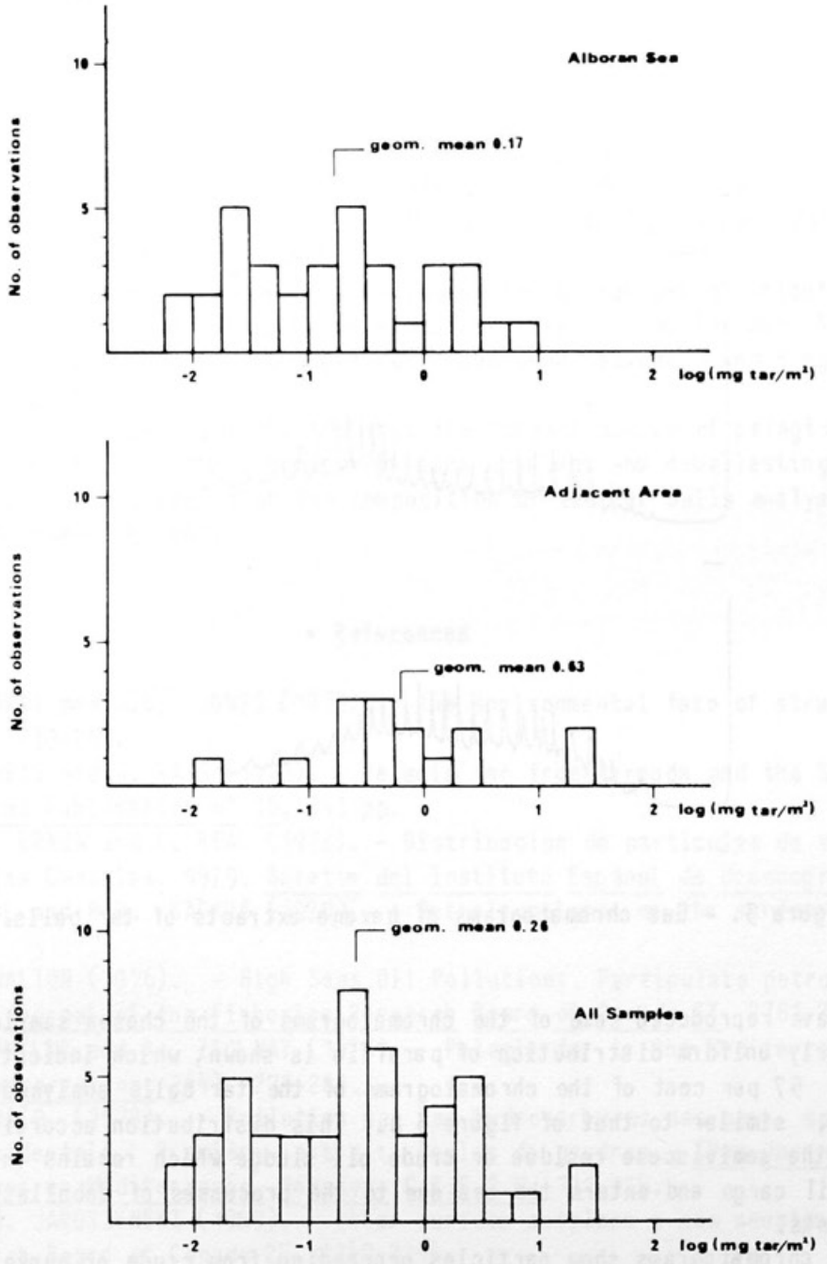


Figure 2. - Statistical distribution of observations.

From the data obtained, a great variability is observed and consequently a high standard deviation which is indicative of the rate of the patchiness of the distribution of the particles of tar and because of which , the geometric mean will be more representative of the central tendency of the distribution that the arithmetic mean.

With a view to obtaining more information about the possible sources of tar particles, tar balls proceeding from 15 stations on cruise Alboran 81, were analysed by Gas Chromatography. The chromatograms show very pronounced paraffinic components as indicate by BUTLER et al. (1973). Likewise a variation is observed between the resolved an unresolved components of the different samples probably due to the different origin, age and time of exposition to the elements.

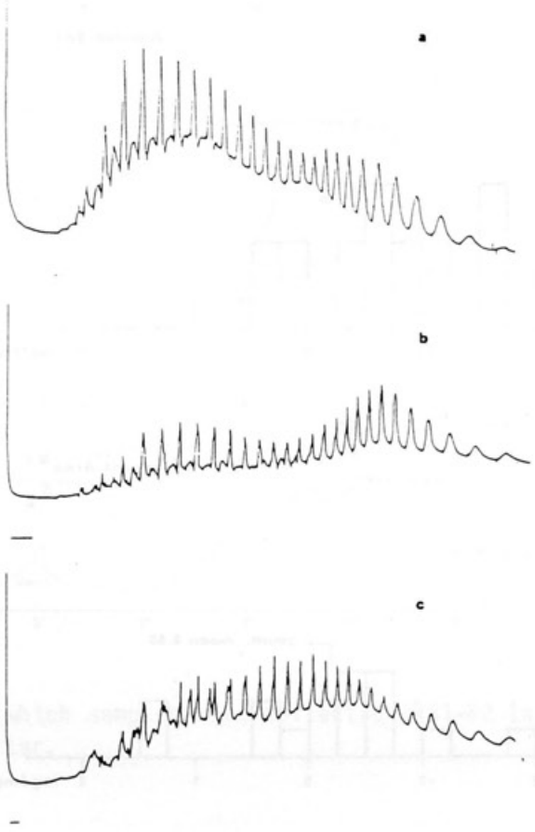


Figure 3. - Gas chromatograms of hexane extracts of tar balls.

In figure 3 we have reproduced some of the chromatograms of the chosen samples. In figure 3 a. a chromatogram of a fairly uniform distribution of paraffin is shown, which indicates that the tar ball is relatively fresh. 57 per cent of the chromatograms of the tar balls analysed presented a bimodal distribution of peaks, similar to that of figure 3 b. This distribution according to BLUMER et al. (1973) is typical of the semiviscose residue or crude oil sludge which remains in the tanks after the discharge of crude oil cargo and enters the sea due to the processes of deballasting and cleaning of the aforementioned tanks.

The rest of the chromatograms show particles proceeding from crude or bunker oil more or less degraded. (Fig. 3 c)

Table I
Concentration of tar in the surface waters of the Western Mediterranean including the log-transformed data.

	n° of samples	Avg. concn. (mg/m2)	± SD	Avg. log. concn.	Geom. mean (mg/m2)
Alboran Sea	34	0.80	1.39	-0.77	0.17
Adjacent area	15	3.60	7.85	-0.20	0.63
W. Mediterr.	49	1.65	4.59	-0.59	0.25

Conclusions

We can conclude that according to the data there are not significant statistical differences between the results obtained during the cruise ALBORAN 76 on the Alboran Sea and the values pointed out in this paper for the waters of the same zone.

On grouping the concentration values and following the categories distribution used by WONG et al. (1976), 30.6 per cent of the samples contained less than 0.1 mg tar/m2; 42.8 per cent had a concentration between 0.1 and 1 mg/m2; 20.5 per cent contained between 1 and 5 mg/m2 and 6.1 per cent contained more than 5 mg tar/m2.

The chromatographical analysis indicates that the largest source of pelagic tar in the Western Mediterranean continues to be the discharge of tank washings and deballasting by the crude oil tankers. Likewise there is evidence that the composition of the tar balls analysed bear no relation to the location of the sampling zone.

References

BLUMER M., M. EHRHARDT and J.H. JONES (1973). - The environmental fate of stranded crude oil. Deep Sea Research 20, 239-259.

BUTLER J.N., B.F. MORRIS and J. SASS (1973). - Pelagic tar from Bermuda and the Sargasso SEA. Bermuda Biological Special Publication n° 10, 343 pp.

DE ARMAS J.D., J.G. BRAUN and F. REAL (1982). - Distribucion de particulas de alquitran pelagico en aguas de las Islas Canarias. 1979. Boletin del Instituto Espanol de Oceanografia. (In press).

HORN M.H., J.M. TEAL and R.H. BACKUS (1970). - Petroleum lumps on the surface of the sea. Science 168, 245-246.

LEVY E.M. and A. WALTON (1976). - High Seas Oil Pollution: Particulate petroleum residues in the North Atlantic. Journal of the Fisheries Research Board of Canada 33, 2781-2791.

MORRIS B.F., J.N. BUTLER and A. ZSOLNAY (1975). - Pelagic tar in the Mediterranean Sea, 1974-1975. Environmental Conservation, 2(4), 275-281.

ROS J. and F. FARACO (1978). - Pollution par les hydrocarbures des eaux superficielles de la Méditerranée Occidentale. Première partie : Boules de goudron. IVes Journées d'études sur les pollutions marines en Méditerranée, Antalya, C.I.E.S.M. 111-115.

SAMEOTO D.D. and L.O. JAROSZYNSKI (1969). - Otter surface sampler: a new neuston net. Journal of the Fisheries Research Board of Canada 25, 2240-2244.

WONG C.S., D.R. GREEN and W.J. CRETNEY (1976). - Distribution and source of pelagic tar on the Pacific Ocean. Marine Pollution Bulletin, 7(6), 102-106.

SUIVI PLURIANNUEL DES COMPOSES PHENOLIQUES DANS LA ZONE LITTORALE DE L'ADRIATIQUE CENTRALE

par

Josip DUJMOV
Institut d'Océanographie et de Pêche - Split (Yougoslavie)

Résumé

Au cours des six dernières années, nous avons effectué des investigations permanentes sur la teneur en phénols des eaux se trouvant à proximité des rejets des eaux résiduaires des centres urbains et industriels les plus importants de la zone de l'Adriatique centrale (Sibenik, Split, Dubrovnik). Les échantillons de l'eau marine, destinés à l'analyse ont été pris au moins trois fois par an, les investigations comprenant la détermination des phénols dans les eaux profondes, intermédiaires et superficielles.

*
* *

Introduction

Les phénols ont trouvé une forte utilisation et une large application en différents domaines de l'activité humaine. Ils sont également présents dans les eaux résiduaires de toute une série d'installations industrielles, ainsi que dans les eaux résiduaires ménagères (RUBIN and McMICHAEL, 1975 : BAIRD et al, 1976 ; NORUP, 1972 : GAMES and HITES, 1977 : PORTMANN, 1974). La détermination des phénols complète l'image de la pollution marine par des polluants organiques.

Méthodes et techniques analytiques en usage

Au cours de la période 1978-1983, on a effectué 69 analyses sur chacune des trois localités : Split (entrée du port), Dubrovnik (devant le port de Gruz) et celle de Šibenik (au fond du Chenal de Šibenik) : les échantillonnages ont été effectués à trois niveaux.

La détermination des phénols a été faite par l'application de la méthode amino-4-antipyrine (STANDARD METHODS, 1980) : la courbe d'étalonnage d'après le phénol (C_6H_5OH), et les mensurations ont été effectuées à 460 nm, sur spectrophotomètre Pye Unicam SP 600.

Il est à noter que les résultats de l'analyse comprennent les composés de phénols au total ceux donc, qui sont naturellement présents dans l'eau de mer, ainsi que ceux résultant d'une activité humaine.

La précision de la méthode est de l'ordre de 10% pour des concentrations inférieures à 10 ug/l C_6H_5OH.

Résultats et discussion

Les phénols ont été, sur la côte adriatique orientale, très intensivement recherchés de 1970 à 1974. Les concentrations de ces polluants organiques ont été déterminées dans la couche superficielle, sur la colonne d'eau de mer par profondeurs, dans certains organismes végétaux et animaux de

DUBROVNIK

Année	1978				1979				1980						1981			1982			1983.				
Mois / Profon.	VII	VII	IX	XI	IV	VI	VII	IX	I	IV	VI	VII	IX	XI	V	VII	X	VII	VIII	IX	VII	VIII	XI	M	$M_{pl.ann.}$
0	6,4	18,6	3,6	0,8	1,9	11,0	20,0	4,0	0	7,4	10,0	17,0	0	3,0	0	19,0	24,0	14,5	8,8	8,4	0	19,0	8,0	$8,9^{\pm}7,7$	
20	10,2	10,1	5,0	0,9	3,2	8,8	8,6	6,0	0	6,0	6,6	8,0	8,2	2,9	6,0	19,5	14,0	11,1	4,4	4,0	1,5	15,0	1,5	$6,9^{\pm}4,9$	$7,8^{\pm}6,2$
35	14,4	7,0	5,1	0,8	2,0	10,4	14,0	9,1	4,2	11,0	12,0	12,8	18,0	0,9	0	19,5	14,0	2,2	7,3	1,2	1,5	4,6	0	$7,5^{\pm}6,1$	
$M_{ann.}$	$6,9^{\pm}5,6$				$8,2^{\pm}5,3$				$7,1^{\pm}5,6$						$12,9^{\pm}8,8$			$6,8^{\pm}4,4$			$5,7^{\pm}6,9$				

SPLIT

0	6,1	11,2	4,0	1,0	5,0	5,2	13,6	7,4	1,4	17,2	5,0	1,3	8,2	3,2	3,5	7,0	5,8	8,4	4,2	4,4	5,2	12,0	4,8	$6,3^{\pm}4,0$	
20	12,2	4,2	3,9	1,0	1,6	8,0	7,6	4,2	1,0	6,8	5,2	27,3	8,3	2,2	2,4	10,3	6,7	2,5	6,3	4,3	7,0	2,4	8,6	$6,3^{\pm}5,5$	$7,5^{\pm}6,2$
35	10,0	2,2	6,0	6,2	4,0	20,0	10,8	6,8	2,6	17,2	38,3	17,0	6,4	3,5	4,4	15,2	8,8	16,1	12,8	10,0	6,0	6,0	3,0	$10,1^{\pm}8,1$	
$M_{ann.}$	$5,7^{\pm}3,8$				$7,8^{\pm}5,0$				$9,6^{\pm}10,1$						$7,1^{\pm}3,9$			$7,7^{\pm}4,5$			$6,1^{\pm}2,9$				

SIBENIK

0	8,0	17,0	15,2	3,4	3,4	20,8	7,0	5,5	2,2	6,5	20,2	12,6	7,0	4,0	11,0	26,0	14,0	6,4	17,2	1,5	2,0	8,0	4,0	$9,7^{\pm}6,9$	
20	16,0	3,6	3,8	6,6	2,0	22,0	6,0	9,2	0	8,0	12,0	8,0	2,0	1,0	12,0	28,0	18,0	7,2	14,0	3,0	2,2	6,0	5,5	$8,5^{\pm}7,1$	$10,5^{\pm}10,5$
35	9,5	10,0	7,0	2,6	3,8	34,0	6,0	11,0	3,0	6,5	65,0	5,4	2,2	3,3	11,5	20,0	30,0	16,2	26,8	3,0	11,0	11,2	8,0	$13,3^{\pm}14,3$	
$M_{ann.}$	$8,5^{\pm}5,1$				$10,9^{\pm}9,7$				$9,4^{\pm}14,7$						$18,9^{\pm}7,4$			$10,6^{\pm}8,4$			$6,4^{\pm}3,4$				

Tableau I. - Résultats des investigations sur la quantité de phénols dans la région de l'Adriatique Centrale pour la période 1978-1983 (en µg/l) avec les valeurs des moyennes des différences.

la zone littorale et dans le sédiment (JARDAS et MUNJKO, 1972, 1972a, 1973, 1973a, 1974 : STILINOVIĆ et al, 1977 ; PAVLETIĆ, 1982). Malheureusement, les échantillons d'eau de mer ont été prélevés périodiquement, rarement plusieurs fois au même endroit, ainsi que les gammes des concentrations obtenues qui ont été élevées (de 0 à 600 µg/l).

Sur la base des expériences de recherches précédentes, dans ce travail nous exposons les résultats du monitorage pluriannuel des phénols sur les trois stations les plus menacées de la partie centrale de la côte orientale de l'Adriatique. Les résultats des investigations pour chaque localité sont présentés sous forme de tableau (Tab. 1) et de figure (Fig. 1).

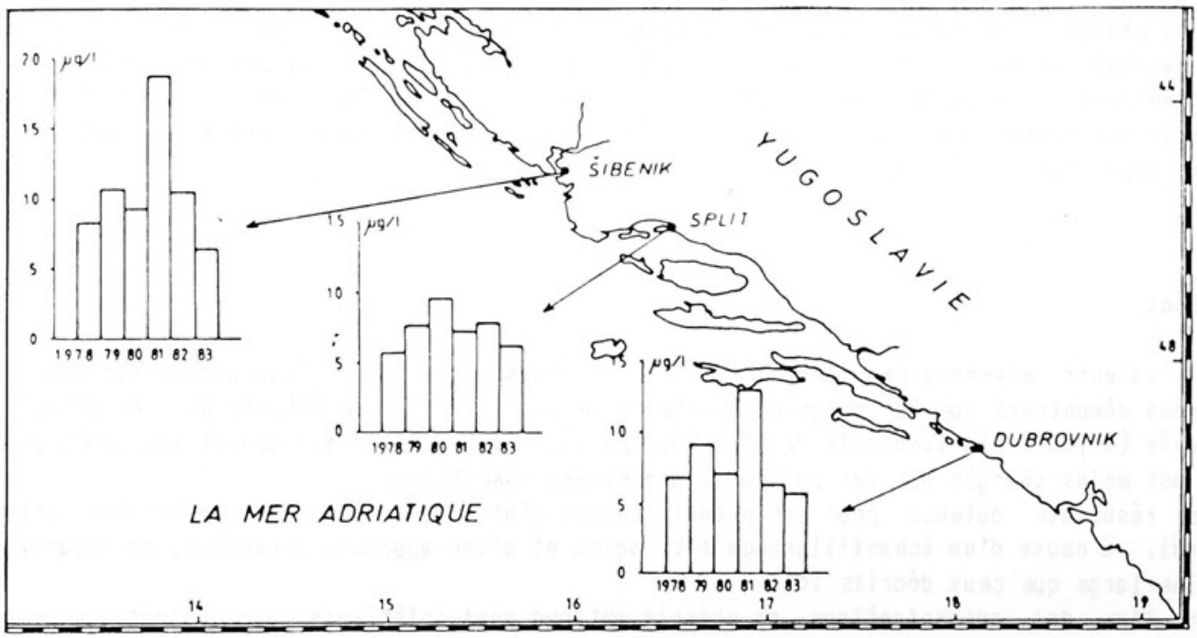


Figure 1. - Evolution des concentrations moyennes annuelles en phénols sur les localités recherchées.

Localité de Šibenik

Prises dans leur ensemble, les concentrations moyennes annuelles en phénols totaux en mer, dans la période analysée, n'ont pas considérablement varié, en s'élevant de 6,4 à 10,9 µg/l. Ce n'est qu'en 1981 qu'a été déterminée une moyenne plus élevée de 18,9±7,4 µg/l. L'afflux des eaux urbaines déversées est donc évident, de sorte que même les quantités des phénols écartées, par biodégradation, se trouvent constamment compensées. Compte tenu du nombre d'habitants (235.398) et des installations industrielles, il serait logique de s'attendre aux concentrations maximales en phénols dans les eaux littorales de Split. Cependant le caractère enfermé de la localité de Šibenik (nombre d'habitants : 79.705), la faible influence du large, ainsi que de la matière organique du sédiment marin, (JARDAS and MUNJKO, 1974) contribuent au fait que les concentrations maximales en phénols totaux sur la colonne d'eau de mer, en comparaison avec les autres localités recherchées, sont justement enregistrées dans la région de Šibenik (10,5± 10,1 µg/l).

Localité de Split

Il est fort connu que les grands ports et les agglomérations urbaines présentent des sources plus fortes de la pollution marine. Split présente les deux puisque c'est en même temps la plus grande ville et le plus grand port de la région recherchée ; cependant, les données pluriannuelles démontrent que sur cette localité les concentrations des matières de phénols sont moins élevées en comparaison avec la région de Šibenik. Alors que les autres villes de l'Adriatique centrale polluent la mer littorale par les eaux résiduaires puisqu'elles possèdent un système mal réglé du

déversement de ces eaux, cette question, à Split, a été partiellement réglée par la construction d'un canal sous-marin, relativement long qui, pour le moment, sert à rejeter les eaux résiduaires de la partie sud-est de la ville dans le chenal de Brač.

Les concentrations en phénols s'élevaient dans la région de Split de 1,0 à 38,5 µg/l, et la moyenne pluriannuelle pour toute la colonne d'eau était de 7,5±6,2 µg/l.

Localité de Dubrovnik

Effectuée sur six années, la moyenne annuelle des concentrations en phénols totaux sur la localité de Dubrovnik s'élevait à 7,8±6,2 µg/l, si l'on prend en considération la colonne d'eau tout entière, de la surface au fond, elle est au niveau de la moyenne déterminée pour la localité de Split. Les investigations pluriannuelles démontrent que la charge polluante de la couche superficielle par les phénols dans cette région est la plus élevée (8,9±7,6 µg) : dans la couche intermédiaire, on note les concentrations les plus basses (6,9±4,9 µg) alors que dans la couche profonde les valeurs des concentreations augmentent de nouveau (7,5±6,4 µg/l). Cette image de la distribution verticale des phénols est probablement la conséquence de la répartition des eaux résiduaires plus légères dans la partie supérieure de la colonne.

Conclusions

Les valeurs moyennes des concentrations en phénols totaux dans l'eau marine sur les localités recherchées démontrent que la charge polluante de la mer littorale de Šibenik par les phénols est la plus élevée (moyenne pluriannuelle de 10,5±10,1 µg/l), alors que la mer devant les ports de Split et de Gruž est moins chargée par ces polluants organiques spécifiques.

Les résultats obtenus pour les phénols totaux s'accordent avec les recherches précédemment faites qui, à cause d'un échantillonnage hétérogène et d'une approche extensive, se situent dans une gamme plus large que ceux décrits ici.

Les taux des concentrations en phénols obtenus sont inférieurs aux valeurs provoquant la toxicité sublétale chez les organismes marins les plus sensibles.

Références

R.B. BAIRD, G.L. CARMONA and L.R. JENKINKS, (1976). - The direct-injection G.L.C. analysis of xylenols in industrial wastewaters. Bull. Environ. Contam. Toxicol., 17/6/: 764-767.

L.M. GAMES and A.R. HITES, (1977). - Composition, treatment efficiency and environmental significance of dye manufacturing plant effluents. Anal. Chem. , 49/9/ : 1433-1440.

I. JARDAS, i I MUNJKO, (1972). - Kompleksno istraživanje Jadranskog mora.Suvremena biologija, 13 : 18-19

I. JARDAS and I. MUNJKO, (1972a). - Preliminary observation of oil and phenol distribution in the Central Adriatic. Biljeske-Notes, 29 : 1-7.

I. JARDAS and I. MUNJKO, (1973). - Determination of phenols in the sea water and some sea organisms in the Kaštela Bay. Ekologija, 8/1/ : 199-205.

I. JARDRAS, i I. MUNJKO, (1973a). - Mineralna ulja i fenoli u morskoj vodi i njihov utjecaj na neke organizme uz istočnu obalu Jadrana, Morsko ribarstvo, 25/4/ : 159-164.

I. JARDAS, i I. MUNJKO, (1974). - Sinteza dosadasnjih rezultata istrazivanja uija i fenola u moru uz istocnu obalu Jadrana.Pomorski zbornik, , 12 : 423-451.

B. NORUP, (1972). - Toxicity of chemicals in paper factory effluents. Water research, 6: 1585-1588.

Z. PAVLETIC, (1982). - Organska polucija mora oko nekih većih naselja na jugoslavenskoj obali Jadrana. Acta Adriat., 23/1,2/ : 319-328.

E.J. PORTMANN, (1974). - Persistent organic residues. In :"Disharge of sewage from sea outfalls" Paper N°. 13 : 123-130.

F.S. RUBIN and C.F. McMICHAEL, (1975). - Impact of regulations on coastal conversion plants. Env. Sci. Technol., 9/2/ : 112-117.

STANDARD METHODS, (1980). - Chloroform extraction method. Ed. A.E. GREENBERG, American Public Health Addociation : 510-512.

L. STILINOVIĆ, I. MUNJKO, I.JARDAS and R.MIKLIČAN, (1977). - Phenols in sea water, sediments and some marine organisms of the interdidal zone of the eastern Adriatic coast. Thalassia Jugoslavica, 13/1,2/: 63-67.

SURVIVAL OF SALMONELLA STRAINS AND ESCHERICHIA COLI IN THE MARINE ENVIRONMENT

by

Dragica FUKS and Massimo DEVESCOVI
"Rudjer Boskovic" Institute
Center for Marine Research Rovinj
52210 Rovinj (Yugoslavia)

Abstract

The effect of organic nutrients and temperature on the survival of some Salmonella strains and Escherichia coli was determined in field experiments at two locations in coastal waters off Rovinj (northern Adriatic Sea). The results suggested that sea temperature may exert an important control on the magnitude of the decay rates of the bacteria tested, while a large amount of organic nutrients may overcome the bactericidal effect of sea temperature and light on the same bacteria. There was no significant difference between the survival time of all tested bacteria.

*
* *

Introduction

The common practice of disposing of untreated sewage and other wastes in the sea is of growing concern nowadays, as it may result in contamination of sellfish areas and bathing beaches with pathogenic micro-organisms. These micro-organisms may survive sufficiently long in sea-water to be transmitted to man, for instance through shellfish which have been exposed to pollution. Oysters and mussels concentrate bacteria suspended in the water, so that, unless purified, they offer a risk of infection. The relatively low incidence of disease among swimmers in polluted areas indicates that a process of self-purification, as a result of a combination of physical . chemical and biological factors, takes place in sea-water. Extensive studies have beer carried out on the survival of Escherichia coli, coliforms and faecal straptococci in sea-water (1, 2, 4, 8-10, 13-15), but little work has been done to determine the fate of salmonellas entering the bathing areas through sewage or other means.

The purpose of this work was to determine the effect of organic nutrients and temperature on the survival of some Salmonella strains and E. coli in a marine environment, and in laboratory-performed experiments with natural sea-water.

Materials and methods

Chamber design

The dialysis culture chamber was constructed of three plexiglass rings (6 cm inner diameter) held together by steel bolts (10). The inner ring forms a 20-ml growth chamber which is enclosed by 0.45-um-porosity membranes (Millipore) held in a circular frame of 6.5-mm-thick plexiglass. The total

surface area of the membranes in the chamber was 56.8 cm^2, and the surface area to volume ratio was 2.84. Two hypodermic needles were fitted into the top of the central space to allow filling and withdrawal of samples. The plexiglass parts of the chambers were sterilized by autoclaving, and the membranes were irradiated with ultra-violet light.

Bacterial inocula and enumeration

In the experiments the following organisms were used: Escherichia coli B, Salmonella typhimurium, Salmonella enteritidis, Salmonella newport. These test organisms were selected because they are the most frequently isolated from faecal materials of the local population. The test organisms were cultured in liquid Dextrose broth (Beef extract - 3 g, Peptone - 5 g, Dextrose - 10 g, NaCl - 5 g, KH_2PO_4 - 1 g, K_2HPO_4 - 3 g, in 1 litre distilled water) to log-phase and transferred to separate dialysis chambers. The population of enteric bacteria after dilution ranged from 10^7-10^9 n/ 1ml. A replicate of 1 ml sample was withdrawn from each container with a sterile syringe.

The enumeration of bacteria was done by the spread plate method using Dextrose nutrient agar for Salmonellas and E. coli, and Marine agar (Peptone - 5 g, Yeast Extract - 1 g, $FePO_4$ - 0.01 g, Agar - 15 g, in 1 litre of 75 % aged sea-water) for heterotrophic marine bacteria. E. coli and Salmonellas were incubated at 37 °C for 24 hours, and heterotrophic bacteria at 25 °C for three days. Colonies appearing on the plate were counted.

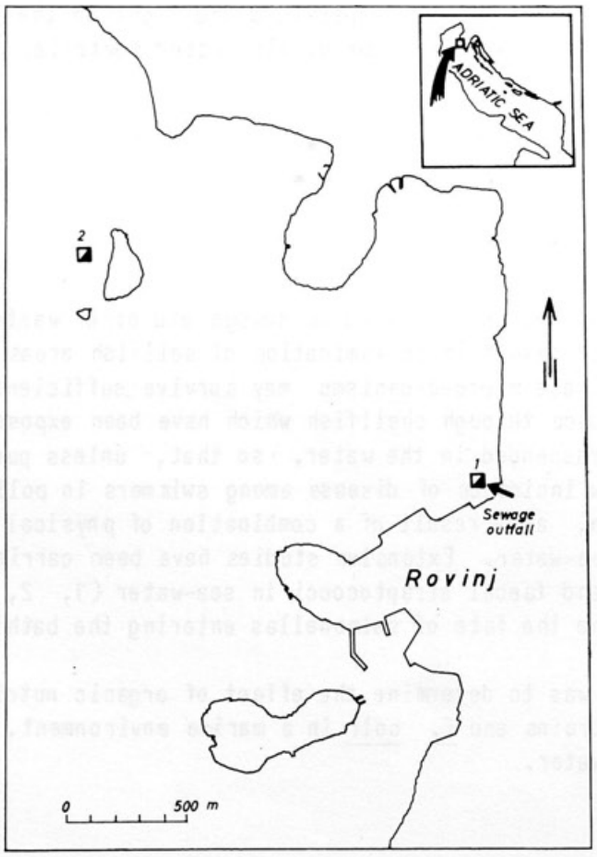


FIGURE 1. Area of "in situ" experiments.

Field experiments

Two experiments were performed at stations 1 and 2. Station 1 was situated in the vicinity of a sewage outfall, while station 2 was about 2 km from station 1 (Figure 1).

Chambers loaded with suspensions of washed cells were hung on a metal construction immersed at half a metre depth and anchored at both stations. The experiments started between 9.00-10.30 a.m. All experiments were conducted in sunny weather, and "summer" and "winter" temperatures of the sea. Samples of bacteria from the chamber were selected at 1, 2, 4, 6, 24 and 28 hours from the beginning of the experiment.

The following environmental parameters were measured in the sea at stations 1 and 2 (three times during the experiment): sea temperature, pH, oxygen, salinity, BOD_5, nutrients (P-tot, PO_4, NH_3, NO_2, NO_3), particulated organic matter, light intensity and heterotrophic marine bacteria. All environmental parameters were analysed by standard oceanographic methods.

Laboratory experiments

Bacterial survival experiments were also performed in laboratory conditions. The chambers were immersed in the water from stations 1 and 2 that filled the glass vessels which were suspended in a refrigerated water bath to control the temperature. Two experiments with sea-water from station 1 and 2, at two different temperatures (12 °C and 23 °C), were performed. All environmental parameters controlled in field experiments were also measured in laboratory experiments.

Die-off rate

The disappearance or die-off rate, known as T_{90} (i.e. the time required for a 90 per cent reduction in bacterial number), was estimated by linear regression of the logarithm microbial count. The use of the exponential reduction model to correlate the measured bacterial concentration in the time was quite satisfactory. The coefficient correlation always had an absolute value near one (r = 0.88-0.99 in field experiments, r = 0.78-0.99 in laboratory experiments).

Results and discussion

Results of relevant environmental parameters measured in the experiments are presented in table I. The registered amount of dissolved oxygen, BOD_5, nutrients and particulated organic matter confirmed a significant influence of the nearby fish-plant sewage outfall on the sea at station 1. A remarkable difference, two to three orders of magnitude, between the numbers of registered heterotrophic marine bacteria revealed favourable environmental conditions at station 1 compared to station 2. Light intensity was registered during the first six hours of the experiments. The registered daylight intensity varied according to the season of the year (summer, winter) and the nature of radiation (natural, artificial).

Rates of survival of the tested bacteria exposed to *in situ* conditions are shown in table II. A marked decrease in the survival of the tested bacteria is registered at station 1 during a higher sea temperature exposure, while at a lower sea temperature (12 °C) all bacteria survived longer. At station 2; where low organic nutrients were registered, die-off rates of all bacteria were significantly greater, at low temperature (13 °C), as well as at a high temperature exposure (22 °C), compared to station 1.

Rates of survival of the tested bacteria exposed to sea-water in laboratory experiments are given in table III. During the laboratory exposure to sea-water collected from stations 1 and 2, all tested bacteria survived somewhat longer at both temperatures, 12 °C and 23 °C, compared to *in situ* exposure.

T_{90} values for the survival indicated that *Salmonella* survivals were somewhat higher in all experiments compared to *E. coli*. None of the differences, however, were significant.

The decrease noticed in the survival of enteric bacteria could be a result of seasonal changes in physical parameters, such as temperature, and solar radiation, as well as changes in chemical and biological parameters, such as organic nutrients, populations of predators, or the production of inhibitory substances.

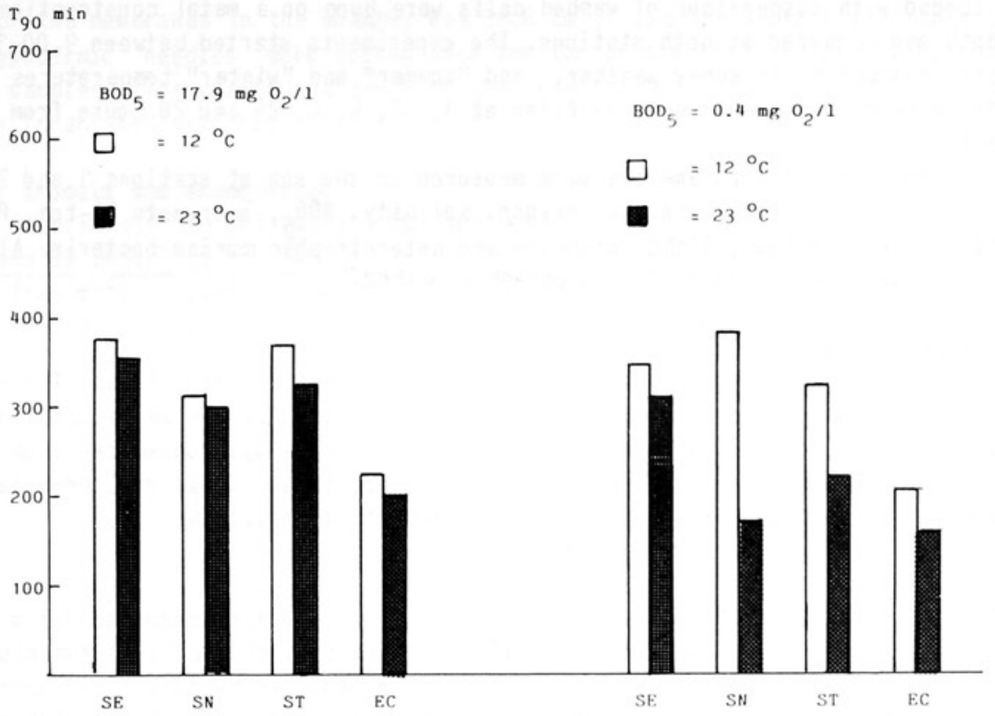


FIGURE 2. T_{90} of S. enteritidis (SE), S. newport (SN), S. typhimurium (ST), and E. coli (EC) in laboratory experiments at two different temperatures and BOD_5, and identical light intensity (0.4 W m^{-2}).

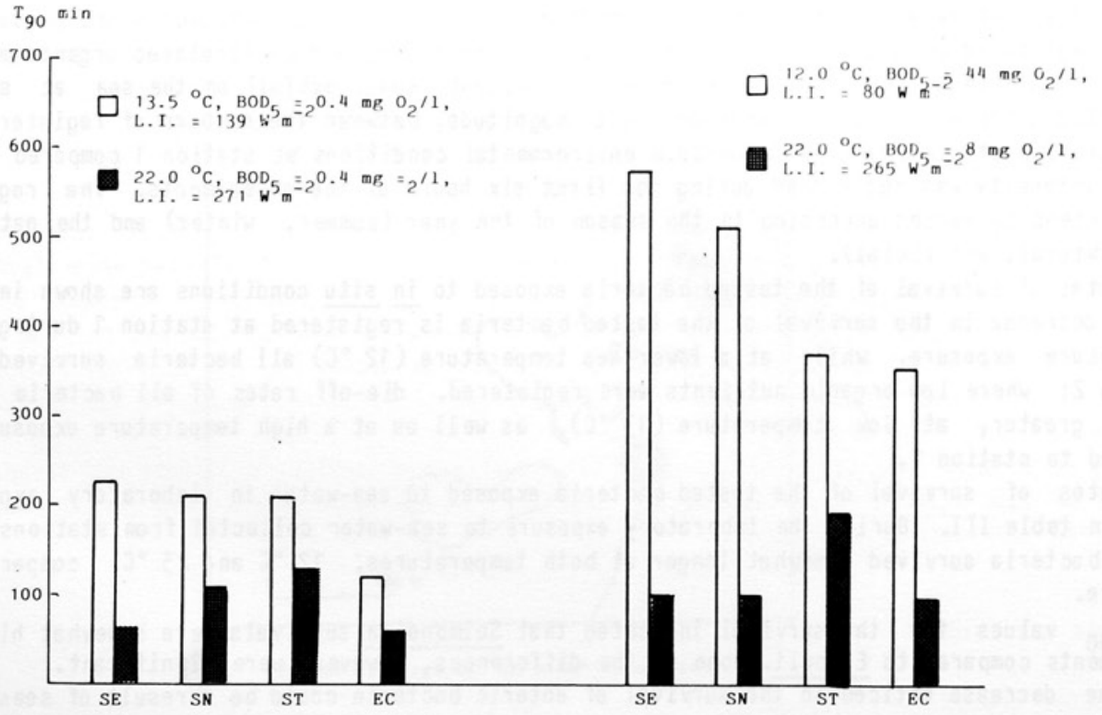


FIGURE 3. T_{90} of S. enteritidis (SE), S. newport (SN), S. typhimurium (ST) and E. coli (EC) in field experiments in à function of temperature and light intensity (L.I.).

In laboratory studies (2, 6, 9, 11, 15). the survival time of pure cultures of coliform bacteria in sea-water or fresh water has been shown to decrease with increasing temperature. The present study confirms and extends these studies to pure cultures of Salmonella strains. Our results suggest that temperature may exert an important control on the magnitude of decay rates of Salmonellas, and E. coli, particularly at low levels of organic nutrients (Figure 2). The effect of temperature on the decay rate may be related to its effect on the metabolism. At low organic nutrient levels and low temperatures, the toxic material may be metabolized at slower rates and prolong the survival. GRAHAM and SIEBURTH (5) found that increasing the incubation temperature from 15 °C to 25 °C without adding organic nutrients led to the decline of Salmonella typhimurium and Escherichia coli in artificial sea-water.

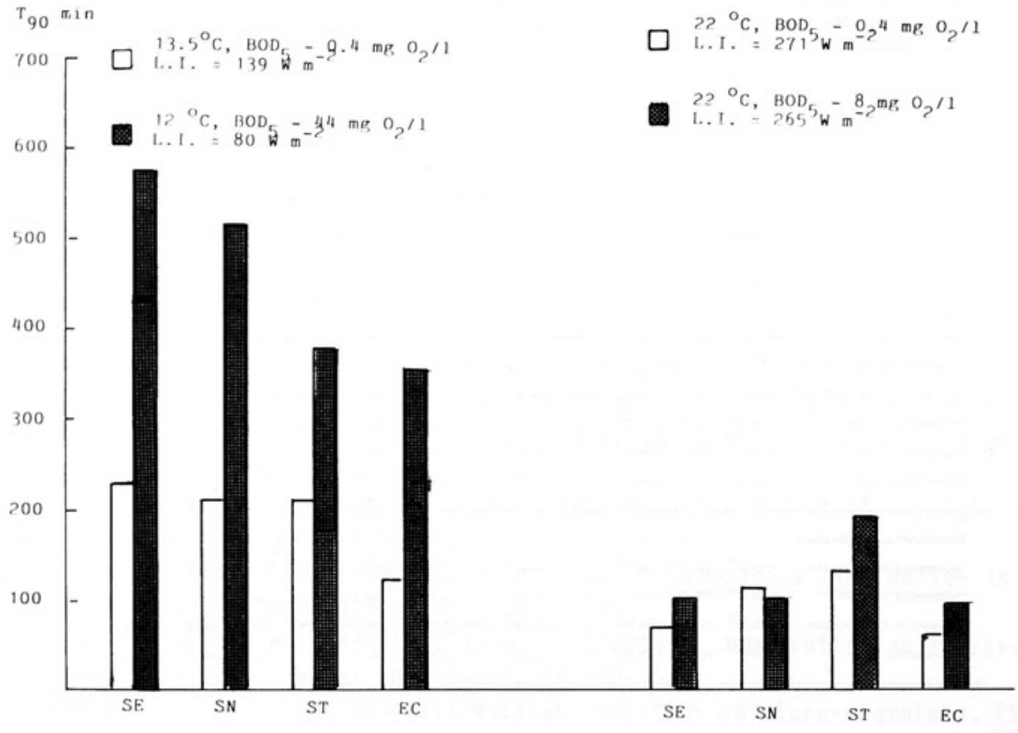


FIGURE 4. T_{90} of S. enteritidis (SE), S. newport (SN), S. typhimurium (ST) and E. coli (EC) in field experiments in a function of BOD_5 at identical light intensity (L.I.) and temperature.

Numerous laboratory studies have demonstrated that near-UV light can be lethal or inhibitory to growth in a great variety of bacteria (7). Several researchers, however, provided evidence that visible light can decrease the survival of enteric bacteria in sea-water (3, 4). The results of our field experiments confirmed that the lower light intensity and sea temperature prolonged the survival of all tested enteric bacteria (Figure 3). These effect are enhanced with increasing amounts of organic nutrients (BOD_5).

The survival rate of all enteric bacteria tested significantly increased when BOD_5 increased from 0.4 to 44 mg O_2/l, while a slight increase in the survival rate was noticed when BOD_5 increased from 0.4 to 8 mg O_2/l (Figure 4). This is in accordance with the idea put foward by SAVAGE and HANES (12), that above a certain initial level of BOD_5, sea-water temporarily loses its toxicity and the survival of enteric bacteria is prolonged.

TABLE I Environmental parameters' variations measured at field experiments.

Station	No 1		No 2	
Temperature (°C)	21.5-22.0	11.9-12.2	21.6-22.4	13.2-13.6
pH	8.00-8.25	7.70-8.10	8.24-8.38	8.00-8.12
Salinity (°/oo)	33.77-36.16	35.52-36.92	35.51-36.71	37.82-38.03
Oxygen (ml/l)	2.99-4.30	3.48-4.48	4.01-5.62	4.82-5.58
Oxygen (%)	58.9-86.3	57.8-74.6	80.5-113.4	84.1-95.9
BOD_5 (mg O_2/l)	7.23-8.21	44.24-44.72	0.15-0.79	0.20-0.43
Particulated organic material (mg/l)	3.18-16.17	16.18-60.84	0.64-0.87	0.46-2.12
Heterotrophic bacteria (n/l ml)	0.2-2.5x10^5	2.6-3.9x10^5	250-345	1000-1330
Light intensity (W m^{-2})	265	80	271	139
Nutrients (µmol l^{-1})				
P-tot	3.13-50.15	28.0-35.5	0.14-0.28	0.36-0.39
PO_4-P	1.64-7.45	22.5-35.0	0.01-0.06	0.13
NH_4-N	19.03-55.38	65.0-102.1	0.14-0.59	0.98-2.64
NO_2-N	0.16-0.42	0.06-0.16	0.01-0.03	0.20-0.25
NO_3-N	0.02-3.50	4.29-10.53	0.24-0.57	0.56-1.12

TABLE II Survival of tested bacteria during "in situ" exposure to sea water at stations No 1 and No 2.

Station	No 1						No 2					
Time (hours) / Concentration	0 n/l ml	%	6 n/l ml	%	24 n/l ml	%	0 n/l ml	%	6 n/l ml	%	24 n/l ml	%
Experimental temperature	11.9-12.2 °C						13.2-13.6 °C					
Salmonella enteritidis	4.6x10^8	100.0	3.2x10^8	69.6	1.1x10^8	23.9	4.0x10^9	100.0	7.8x10^7	1,9	5,4x10^6	0.1
Salmonella newport	9.1x10^8	100.0	3.4x10^8	37.4	1.6x10^8	17.6	4.2x10^9	100.0	7.6x10^8	7.6	3.7x10^5	0.01
Salmonella typhimurium	3.8x10^8	100.0	5.8x10^7	15.3	9.9x10^6	2.6	2.6x10^9	100.0	6.8x10^7	2.6	3.1x10^6	0.1
Escherichia coli	1.9x10^8	100.0	3.9x10^7	20.5	1.1x10^7	5.8	3.0x10^8	100.0	3.0x10^6	1.0	1.5x10^5	0.05
Experimental temperature	21.4-22.0 °C						21.6-22.4 °C					
Salmonella enteritidis	1.6x10^9	100.0	4.9x10^6	0.3	5.4x10^5	0.03	1.5x10^9	100.0	2.5x10^5	0.02	1.5x10^4	0.001
Salmonella newport	2.4x10^9	100.0	3.4x10^6	0.1	1.5x10^5	0.01	2.0x10^9	100.0	1.1x10^7	0.6	4.1x10^5	0.02
Salmonella typhimurium	1.2x10^8	100.0	8.0x10^5	0.7	1.0x10^5	0.08	2.8x10^9	100.0	4.5x10^7	1.6	4.6x10^5	0.02
Escherichia coli	2.0x10^8	100.0	3.7x10^5	0.2	1.2x10^5	0.06	2.8x10^9	100.0	3.0x10^5	0.01	1.0x10^2	<0.001

TABLE III Survival of tested bacteria during laboratory exposure to sea water collected at stations No 1 and No 2.

Station	No 1						No 2					
Time (hours) / Concentration	0 n/l ml	%	6 n/l ml	%	24 n/l ml	%	0 n/l ml	%	6 n/l ml	%	24 n/l ml	%
Experimental temperature	12 °C						12 °C					
Salmonella enteritidis	1.5x10^9	100.0	1.7x10^8	11.3	6.6x10^7	4.5	4.2x10^9	100.0	2.4x10^8	5.7	1.1x10^8	2.6
Salmonella newport	1.9x10^9	100.0	1.3x10^8	6.8	5.1x10^7	2.7	1.1x10^9	100.0	5.3x10^8	48.2	1.1x10^8	10.0
Salmonella typhimurium	6.1x10^8	100.0	7.4x10^7	12.1	2.0x10^6	0.3	2.2x10^9	100.0	1.5x10^8	6.8	1.9x10^7	0.9
Escherichia coli	1.4x10^9	100.0	3.7x10^7	2.6	1.8x10^5	0.01	1.7x10^8	100.0	2.5x10^7	13.8	2.7x10^4	0.02
Experimental temperature	23 °C						23 °C					
Salmonella enteritidis	5.1x10^7	100.0	6.5x10^6	12.7	2.3x10^5	0.5	1.6x10^9	100.0	1.8x10^8	11.3	3.9x10^6	0.2
Salmonella newport	1.0x10^8	100.0	2.3x10^7	23.0	1.3x10^6	1.3	2.6x10^9	100.0	3.8x10^7	1.5	7.4x10^5	0.03
Salmonella typhimurium	3.9x10^7	100.0	4.1x10^6	10.5	4.1x10^5	1.1	1.9x10^9	100.0	5.8x10^7	3.1	5.7x10^5	0.06
Escherichia coli	7.5x10^7	100.0	4.7x10^6	6.3	3.3x10^4	0.04	3.5x10^8	100.0	3.5x10^6	1.0	6.0x10^4	0.02

Conclusions

The survival of S. enteritidis, S. newport and S. typhimurium was somewhat higher in all experiments compared to E. coli, but none of the differences were significant.

The results suggested that sea temperature may exert important control on the magnitude of decay rates of Salmonella strains and E. coli.

In situ experiments revealed that a large amount of organic nutrients may overcome the bactericidal effect of sea temperature and light on tested Salmonella strains and E. coli.

Acknowledgements

Assistance for the work reported has been received from the Selfmanagement Community of Interest for Scientific Research of S.R. Croatia, and from the U.N.E.P. Mediterranean Monitoring and Research Programme.

References

1. ANDERSON I.C., M.W. RHODES and H.I. KATOR (1983). - Seasonal variation in survival of Escherichia coli exposed in situ in membrane diffusion chambers containing filtered and nonfiltered estuarine water. Appl. Environ. Microbiol. 45: 1,877-1,883.
2. CARLUCCI A.F. and D. PRAMER (1960). - An evaluation of factors affecting survival of Escherichia coli in sea-water. II. Salinity pH and nutrients. Appl. Microbiol. 8: 247-250.
3. GAMESON A.D.H. and D.J. GOULD (1975). - Effects of solar radiation on the mortality of some terrestrial bacteria in sea-water, p. 209-214. In: A.L.H. Gameson (Ed.),Discharge of Sewage from Sea Out-falls. Pergamon Press, New York.
4. GAMESON A.D.H. and J.R. SAXON (1967). - Field studies on the effect of daylight on mortality of coliform bacteria. Water Res. 1: 279-295.
5. GRAHAM J.J. and J.M.N. SIEBURTH (1973). - Survival of Salmonella typhimurium in artificial and coastal sea-water. Rev. Intern. Ocean. Med. 29: 5-29.
6. HANES N.G., G.A. ROHLIC and W.B. SARLES (1965). - Effects of temperature on survival of indicator bacteria in water.J.N. Engl. Water Work Assoc. 80: 13-16.
7. JAGGER J. (1976). - Effects of near-ultraviolet radiation on micro-organisms. Photochem. Phtobiol. 23: 451-454.
8. KAPUCINSKI R. and R. MITCHEL (1981). - Solar radiation induces sublethal injury in Escherichia coli in sea-water. Appl. Envirorm. Microbiol. 41: 607-674.
9. LESSARD E.J. and J.McN. SIEBURTH (1983). - Survival of natural sewage populations of enteric bacteria in diffusion and batch chambers in the marine environment. Appl. Environm. Microbiol.45: 950-959.
10. McFETERS G.A. and D.G. STUART (1972). - Survival of coliform bacteria in natural waters: Field and laboratory studies with membrane filter chambers. Appl. Microbiol. 24: 805-811.
11. ORLOB G.T. (1956). - Viability of sewage bacteria in sea-water. Sewage Ind. Wastes 28: 1,147-1,167.
12. SAVAGE H.P. and N.B. HANES (1971). - Toxicity of sea-water to coliform bacteria. Journal WPCF. 43: 854-861.
13. SCARPINO P.V. and D. PRAMER (1962). - Evaluation of factors affecting the survival of Escherichia coli in sea-water. VI. Cysteine. Appl. Microbiol. 10: 436-440.
14. SLANETH L.W. and C.H. BARTLEY (1965). - Survival of faecal streptococci in sea-water. Health Lab. Sci. 2: 142-148.
15. VERSTRATE W. and J.P. VOETS (1976). - Comparative study of E. coli survival in two aquatic ecosystems. Water Res. 10: 129-136.

OCCURRENCE OF PSEUDOMONAS AERUGINOSA AND SALMONELLA IN VALENCIA COASTAL WATERS

by

José Luis ALONSO MOLINA*
Inmaculada PERIS AGUIRRE **
and Inmaculada AMOROS MUNOZ***
Catedra Ingenieria Sanitaria*, Catedra de Puertos** y I.H.M.N.***
E.T.S.I.C.C. y P. Universidad Politecnica de Valencia
B.P. 2012 Valencia (Espana)

Abstract

Pseudomonas aeruginosa is an opportunistic pathogen that causes skin, eye and ear infections, and Salmonella may produce gastro-enteritis.

The occurrence of Pseudomonas aeruginosa and Salmonella has been studied in Cullera and Gandia coastal waters (Valencia).

The Pearson-coefficients between the bacteriological faecal indicators and Pseudomonas aeruginosa were calculated and also the ratio PA: FC x 100.

*
* *

Introduction

In the coastal regions of the province of Valencia, there are some tourist villages whose populations increase by large numbers in the summer. Among these villages the most important are Cullera and Gandia (figure 1); the sewage from these two villages have no depuration treatment and consequently some of the beaches may occasionally be polluted. The main source of pollution is the Jucar river in Cullera and the river Serpis in Gandia.

To control recreational water quality, the E.E.C. (1) uses the faecal contamination indicators: total coliforms, faecal coliforms and faecal streptococci, besides Salmonella (pathogenic bacteria).

The bacteria indicating faecal contamination prevent us from the possibility of finding pathogenic bacteria, although the best indicator will be the pathogenics themselves (2).

The MED VII programme has identified skin, ear, and nose infections, and to a lesser degree diarrhoea as the most common troubles among bathers in Spanish coastal waters (12). In this present work, we study the presence of Salmonella in Cullera and Gandia, the pathogenic bacteria that produces gastro-enteritis (3) and Pseudomonas aeruginosa,opportunist pathogenic (4) associated with ear (5) and skin (6) infections. This bacteria has been proposed as a water indicator (7) (8) (9) (10) and is ubiquitous in water and soil (11).

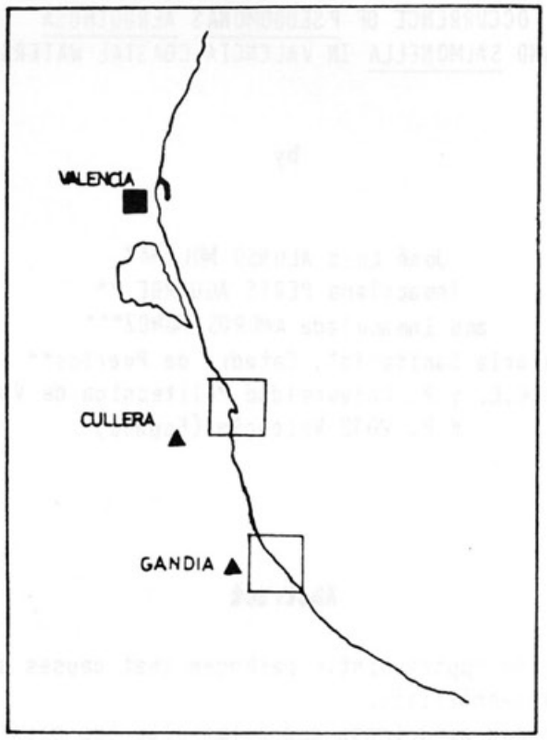


Figure 1. - Location of Cullera and Gandia.

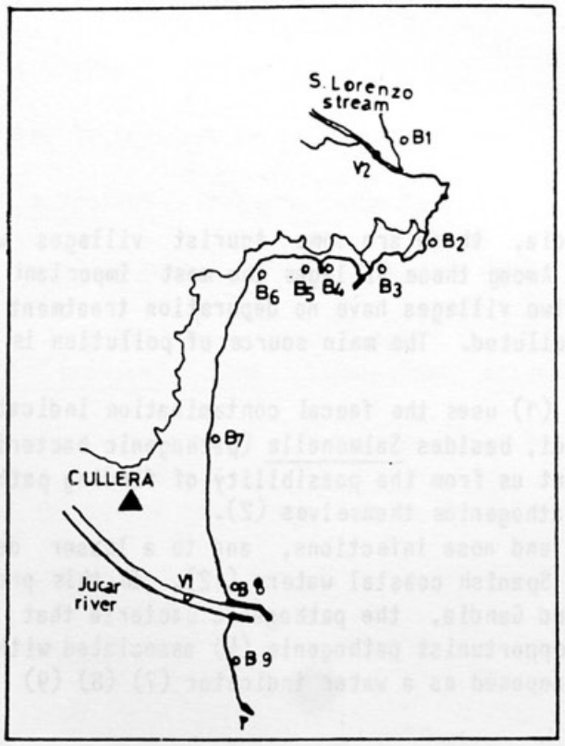


Figure 2(a). - Location of sampling sites in Cullera.

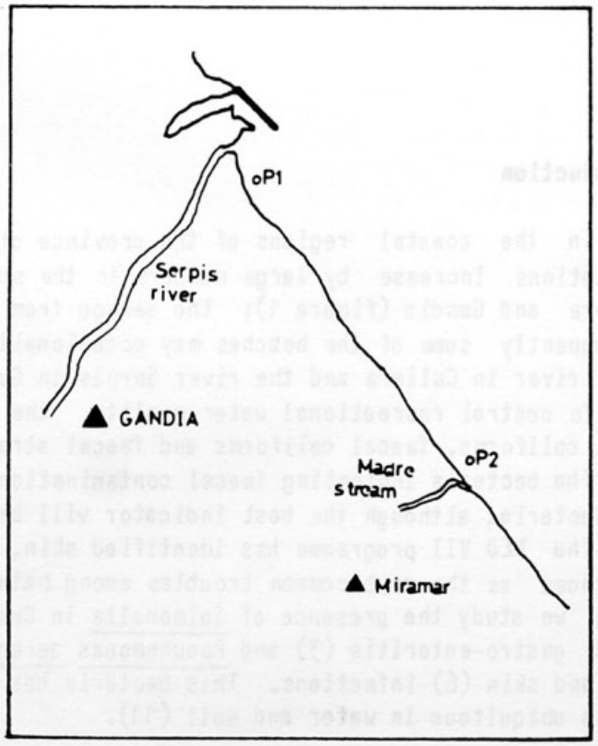


Figure 2(b). - Location of sampling sites in Gandia.

Materials and methods

Water samples were collected once a month: in the river Jucar and on the beaches of Cullera for a period of 19 months (4 April 1982 to 27 March 1984); in the polluted stream S. Lorenzo (Cullera) during the period 13 April 1983 to 27 March 1984 and on Gandia beaches from 12 January to 20 July 1982.

The distribution of sampling sites in the area of Cullera is given in figure 2(a) and in Gandia in figure 2(b).

Samples were transported at 4° C from the sampling site to the laboratory. The period of time between collecting the samples and initiating water tests never exceeded 3 h.

Enumeration of indicator organisms. - Total coliforms: series of fermentation tubes (10.1 and 0.1 ml) of lauryl tryptose broth (Difco) were used to determine the most probable number (MPN) of coliforms (13). The tubes were incubated at 37 ° C for 48 h. Faecal coliforms: all presumptive tubes which were positive as defined in Standard Methods (13), were transferred into EC broth (Difco) and incubated for 24 h. at 44.5 ± 0.2 (13). Faecal streptococci: the membrane filtration technique was used, with KF-Streptococcus (Merek). The membranes were incubated at 37° C for 48 h (13).

Pseudomonas aeruginosa: the asparagine medium was used throughout this study for the presumptive enumeration by the MPN technique (13). Appropriate dilutions of water samples were poured into five tubes and were incubated for 48 h at 41.5° C. The tubes showing pigment production, superficial pellicle formation and/or turbidity after 24 to 48 h of incubation were interpreted as presumptively positive. The primary confirmatory test was carried out by transferring inocula from positive asparagine broth tubes into Cetrimide agar (Merek) (14) and incubated for 48 h at 41.5° C.

Salmonella: R10/43° C with novobiocin (Sigma) medium (15) was used for enrichment. Water samples were introduced into this medium and incubated at 43° C for 24 h. After enrichment, the cultures were inoculated into two plating media with different selective powers: Bismuth Sulphite agar (Merck) and Hektoen agar (Merck) and incubated for 24 h at 37° C.

Biochemical identification of Ps. aeruginosa and Salmonella

Ps. aeruginosa: isolated colonies demonstrating Pseudomonas-like characteristics were transferred to nutrient agar tubes. These cultures were identified as Ps. aeruginosa on the basis of a positive test and growth on agar TSI (Difco). Other biochemical tests were performed on some isolates with the API 20E (Analytab Products), N/F System (Corning laboratories) and Galeries Pasteur pour l'identification de Pseudomonas (Inst. Pasteur).

Salmonella: from each plate, presumptive colonies showing the typical morphology of salmonellas were purified by subculture on the same medium. The salmonellas recovered were identified by the following tests: urease, growth on agar TSI, phenyl alanine desaminase and tryptophan desaminase. For the final characterization of Salmonella, the identification systems API 20E and API Z (Analytab Products) were used.

Statistical analysis

Correlation coefficients of Bravais-Pearson were calculated with the "Start Enter Program" (Boalox Informatica). This programme tests the coefficient correlation for significance at the 95 % and 99 % level. The bacteriological counts had been subjected to a $\log_{10}$ transformation and the computer used had been the ZX Spectrum

Results

Pseudomonas aeruginosa: the isolation of this bacterium was negative on the beaches of Cullera during the sampling period. However in the river Jucar and the S. Lorenzo stream (an irrigation channel) was detected an evolution in the content of Ps. aeruginosa. This evolution can be observed for the river Jucar (VI) in figures 3 and 4, and for the S. Lorenzo stream in figure 5. The highest

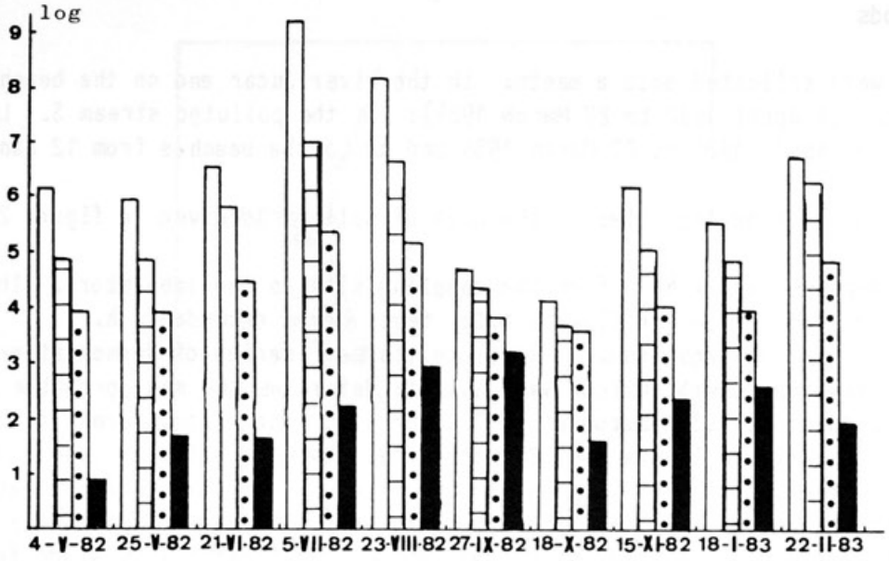


Figure 3. - Total coliform, Fecal coliform, Fecal streptococci and Ps. aeruginosa densities at the Jucar river (Cullera), sampling period 4 May 82 to 22 February 83.

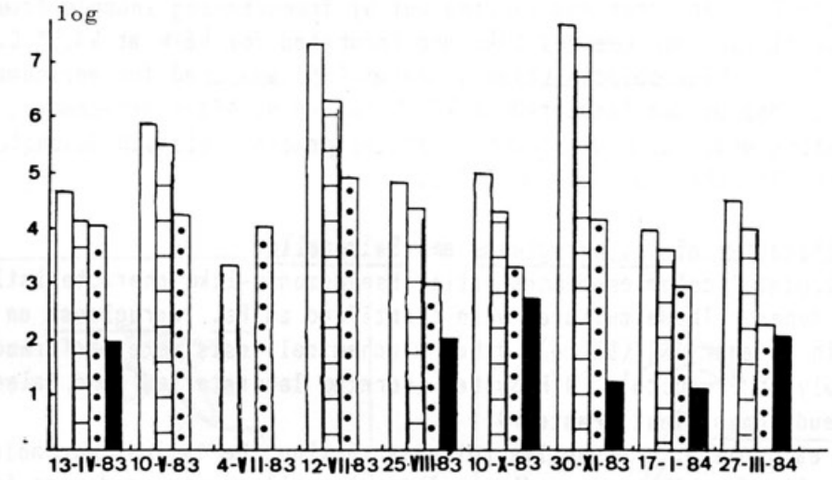


Figure 4. - Total coliform, Fecal coliform, Fecal streptococci and Ps. aeruginosa densities at the Jucar river (Cullera), sampling period 13 April 83 to 27 March 84.

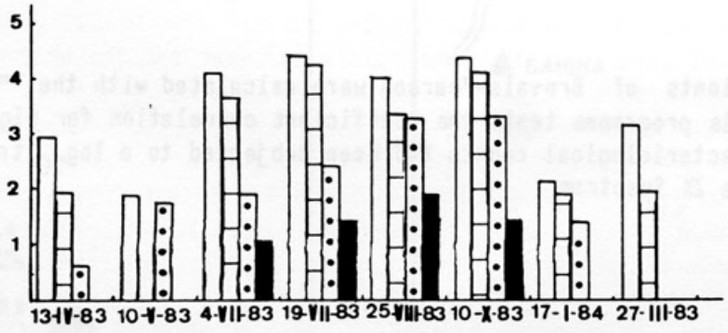


Figure 5. - Total coliform, Fecal coliform, Fecal streptococci and Ps. aeruginosa densities at the irrigation channel S. Lorenzo (Cullera), sampling period 13 April 83 to 27 March 84.

□ Total coliform /100 ml
⊟ Fecal coliform/100 ml
⊡ Fecal streptococci/100 ml
■ Ps. aeruginosa/100 ml

count was obtained in VI on 27 September '82 with 1609/100 ml and in V2 (S. Lorenzo) on 25 August '83 with 94/100 ml.

Ps. aeruginosa was also found in the Gandia area, on the beaches of Venecia (P1) and Miramar (P2): at P1 in the sampling of 25 April '82, 85/100 ml with a density of faecal coliforms of 11,000/100 ml; at P2 in the sampling of 7 July '82, 1/100 ml and a concentration of faecal coliforms of 29 x 10^4/100 ml; and on 20 July '82 a density of Ps. aeruginosa of 1/100 ml and faecal coliforms of 93 x 10^4/100 ml (table 3).

The ratio PA: FC/100 (Ps. aeruginosa/Faecal Coliform) can be used as orientation in order to know if the place selected for sampling is next to a drain of faecal contaminated waters or not (16). In the river Jucar the interval for this ratio was 0.0007 - 6.9 and in the S. Lorenzo stream 0.2 - 4 (table 2). On the beaches of Gandia, in the three samplings that it has been possible to calculate, the ratio gives values less than 1 (table 3).

The study of the correlations between germs indicating faecal contamimation and Ps. aeruginosa have only been calculated in the river Jucar, because it is the only sampling place with a sufficient number of the four parameters. As can be seen in table 1.

Table 1
Correlations between bacteriological parameters in the river Jucar (Cullera)

Parameters	Pearson coefficient	Significance level (%)
Ps. aeruginosa -Total Coliform	0.06	N.S.*
" -Faecal Coliform	0.01	N.S.
" -Faecal Strep.	0.17	N.S.
Total Coliform -Faecal Coliform	0.96	1 %
" " -Faecal Strep.	0.76	1 %
Faecal Coliform -Faecal Strep.	0.73	1 %

* N.S. = not significant

There is no correlation between Ps. aeruginosa and the germs indicating faecal contamination; and there is significant correlation between the indicators.

Table 2
Interval of the ratio PA:FCx100 in the river Jucar (V1) and in S. Lorenzo irrigation channel (V2), Cullera.

Sampling Station	Sampling date	Count/100 ml Pseudomonas aeruginosa	Count/100 ml Faecal coliforms	PA:FC X100
V1	30-XI-83	17	23×10^6	0.00007
	27-IX-82	1609	23×10^3	6,9
V2	4-VII-83	13	49×10^2	0,2
	25-VIII-83	94	23×10^2	4

Salmonella: You cannot find it, as has occurred with Ps. aeruginosa in waters of the beaches of Cullera bay. Its presence has been detected in the river Jucar and in the S. Lorenzo stream. In V1 in samplings of the 5-VII-82, 23-VIII-82, 13-IV-83, 19-VII-83, 25-VIII-83, 10-X-83 and 30-XI-83; in V2 it has been isolated on the following dates: 13-IV-83, 25-VIII-83 and 10-X-83. In Gandia it has been found on the two beaches, in P1 in sampling of the 24-VII-82 and in P2 in samplings of the 26-V-82, 7-VII-82 and 20-VII-82 (table 3).

Table 3
Occurrence of Ps. aeruginosa and Salmonella in Gandia, and ratio PA:FCx100

Sampling Station*	Sampling date	Ps. aer.	Count/100 ml TC	FC	EF	Mean recovery Salmonella	PA: FCX 100
P1	20-IV-82	85	$43x10^3$	$11x10^3$	30	negative	0,77
P2		-	$11x10^2$	$11x10^2$	170	negative	-
P1	26-V-82	0	980	340	0	negative	-
P2		0	$37x10^3$	$11x10^2$	1880	positive	-
P1	24-VI-82	0	$11x10^4$	$93x10^2$	137	positive	-
P2		-	$11x10^2$	460	240	negative	-
P1	7-VII-82	0	4	0	4	negative	-
P2		1	$46x10^6$	$29x10^5$	$53x10^2$	positive	0,00003
P1	20-VII-82	0	460	93	11	negative	-
P2		1	$11x10^5$	$93x10^4$	$11x10^2$	positive	0,00003

* sampling stations shown in fig. 2(b)
TC = total coliform, FC = faecal coliform, EF = faecal streptococci
Ps. aer = Pseudomonas aeruginosa.

In all cases the faecal coliform level has been higher than 1.000/100 ml.

Discussion

The Ps. aeruginosa concentrations in V1 have varied from 0-1609/100 ml and in V2 from 0-94; the density in V1 has been higher than that found in river waters by CIAN (18), who registered 1-260 germs per 100 ml. This might be because we used the MPN technique and CIAN uses membrane filtration, and the MPN technique in highly polluted waters produces counts greater than those found by membrane filtration (19). Comparing these two techniques in counts of Ps. aeruginosa, CARSON (31) with the MPN reaches higher counts of this bacterium, but LEVIN and CABELLI (16) register higher counts of Ps. aeruginosa with membrane filtration. BRODSKY (14) with the culture media mPA-B and mPA-C (membrane filtration) obtains results within the confidence limits of the MPN technique.

In any sampling in V1, V2, P1, P2, the Ps. aeruginosa concentration has been greater than the coliform density: that coincides with the results obtained by CIAN (18), DUTKA (21), DRAKE (22) and is the opposite of the results obtained by HIGHSMITH (17).

BONDE (23) and DE VICENTE (24) always isolate Ps. aeruginosa when the faecal coliform level is higher than 1000/100 ml: and we also obtained counts of Ps. aeruginosa when this level is higher than 1000/100 ml, except in V1 for samplings of 10 May, 4 July and 19 July, 1983.

In the waters off Gandia beaches it has been possible to detect Ps. aeruginosa because there are sewages outlets on these beaches the nutrients from which help the survival of this bacterium in littoral waters (16), although LAURENCE (25) has observed that Ps. aeruginosa does not need the presence of nutrients for their multiplication in water.

The relation PA:FCx100 has been lower than 20 (16) which proves the existence of waters with faecal contamination (drains) next to the places where the samples were taken.

The absence of a correlation between the indicators of faecal contamination and Ps. aeruginosa, invalidates the use of this bacterium as an indicator: CABELLI (16), when he examines the relationship between this bacterium and faecal coliforms in estuarine waters, concludes that Ps. aeruginosa is a poor indicator of faecal contamination. DE VICENTE (24) points out that the evolution of Ps. aeruginosa in water will be better represented by faecal streptococci than by coliforms.

In V1 infective doses, higher than 100/100 ml (26) were found and the bathers were exposed to ear and eye infections in eight samplings.

The Salmonella isolation technique used has demonstrated that it has the ability to detect this germ in coastal waters: with this procedure, ALCAIDE (15) observed a better recovery of Salmonellas from water than from selenite broth. In regard to estuarine environments, CARNEY (28) indicates that Salmonella may not survive or may become debilitated or altered by high salt concentration and other environmental influences, and less harsh media are required for its recovery from estuarine and coastal waters. GELDREICH (29) has observed that in estuarine environments with 200 faecal coliforms per 100 ml, Salmonella ocurrence may range from 6.5-31 per cent: in the river Jucar, Salmonella was isolated when faecal coliforms densities were higher than 1000/100 ml. On Gandia beaches Salmonella was isolated due to the influence of sewage discharges: in coastal waters receiving waste waters 10 to 20 Salmonella montevideo per litre were obtained (30).

Conclusion

It is necessary to control pathogenic bacteria with as enteric origin such as Salmonella and those which are potentially pathogenic like Ps. aeruginosa, in recreational waters. These bacteria, together with the germ indicators, are of assistance to the development of valid criteria for the use of recreational waters.

References

(1.) Directive du Conseil C.E.E. du 15 juillet (1980). - Journal Officiel des Communautés Européennes. n° 1229, 30-8-80.

(2.) World Health Organization (1977). - Health Criteria and Epidemiological Studies related to Coastal Water Pollution. Regional Office for Europe, Copenhagen.

(3.) LECLERC H., F. GAVINI et C. OGER (1981). - Les indicateurs bactériens dans le contrôle bactériologique de l'eau : exigences et limites. Journal Français d'Hydrologie, 12(2), n° 35, 213-228.

(4.) AYLIFFE G.A., D.R. BARRY, J.L. LOWBURY, M.J. ROPER-HALL and W.M. WALKER (1966). - Postoperative infection with Pseudomonas aeruginosa in an eye hospital. Lancet, 1, 1113-1117.

(5.) WRIGHT D.N. and J.M. ALEXANDER (1974). - Effects of water on the bacterial flora of swimmer'ears. Archives Otolarynology, 99,(1) Jan. 15-18.
(6.) Mc CAUSLAND W.J. and P.J. COX (1975). - Pseudomonas infection traced to motel whirlpool. Journal environmental Health, 37, 455-459.
(7.) REITLER R. and R. SELIGMANN (1957). - Pseudomonas aeruginosa in drinking water. Journal of Applied Bacteriology, 20,(2), 145-150.
(8.) BONDE G. (1963). - Bacterial Indicators of Water Pollution. Tecknisk Forlag. Copenhagen.
(9.) DUTKA B.J. (1973). - Coliforms are an inadequate index of water quality. Journal of Environmental Health, 36, 39-46.
(10.) GELDREICH E.E. (1981). - Current status of microbiological water quality criteria. ASM News, 47, (1), 23-27.
(11.) Bergey's Manual of Determinative Bacteriology. (1974). - 8ª ed. William & Wilkins, Baltimore.
(12.) MUJERIEGO R. and S. MURIAS (1980). - Sanitary and aesthetic quality of the coastal waters of Spain. Progress Water Technology, 12,(1), 119-127. IAWPR Pergamon-Press.
(13.) APHA-AWWA-WPCF (1980). - Standard Methods for the Examination of Water and Wastewater. 15 th. ed. Washington.
(14.) BRODSKY M.H. and B.W. CIEBIN (1978). - Improved medium for recovery and enumeration of Pseudomonas aeruginosa from water using membrane filters. Applied and Environmental Microbiology, 36, July, 36-42.
(15.) ALCAIDE E., J.P. MARTINEZ, P. MARTINEZ-GERMES and E. GARAY (1982). - - Improved Salmonella recovery in moderate to highly polluted waters. Journal of Applied Bacteriology, 53, 143-146.
(16.) CABELLI V.J., H. KENNEDY and M.A. LEVIN (1976). - Pseudomonas aeruginosa-faecal coliform relationships in estuarine and fresh recreational waters. Journal Water Pollution Control Federation, 48,(2), 367-376.
(17.) HIGHSMITH A.K. and R.L. ABSHIRE (1975). - Evaluation of a Most-Probable Number technique for the enumeration of Pseudomonas aeruginosa. Applied Microbiology, 30,(4), 596-601.
(18.) CIAN L. et J. DEVOUCOUX (1981). - Recherche de Pseudomonas aeruginosa dans l'eau. Méthodologie et application à l'analyse bactériologique des eaux de rivière. Journal Français d'Hydrologie, 12, (2), n° 35, 195-212.
(19.) GRABOW W.O.K. (1981). - Membrane filtration: a South African View. In Membrane Filtration Applications, Techniques and Problems.
(20.) LEVIN M.A. and V.J. CABELLI (1972). - Membrane filter technique for enumeration of Pseudomonas aeruginosa. Applied Microbiology, 24, 862-870.
(21.) DUTKA B.J. and K.K. KWAN (1977). - Confirmation of the single M.F. procedure for enumeration of Ps. aeruginosa densities in water. Applied and Environmental Microbiology, 33,(2), 240-245.
(22.) DRAKE C.H. (1966). - Evaluation of culture media for the isolation and enumeration of Ps. aeruginosa. Health Laboratory Science, 3,(1), 10-19.
(23.) BONDE G.J. (1977). - Bacterial indication of water pollution. In: Advances in Aquatic Microbiology (Vol. 1). Ed. by M.R. Droop and H.W. Jannasch, 273-363. Academic Press, London.
(24.) DE VICENTE A., J.J. BORREGO and F. ARRABAL (1982). - An initial study of the inactivation of Pseudomonas aeruginosa in sea-water and its comparison with the usual bacterial indicators of faecal pollution. XXVIIIth Congress and Plenary Assembly, Cannes, Dec. 2-11. Marine Microbiology & Biochemistry Scientific Committee.
(25.) LAURENCE R.A., F.M. GOSSELIN at A.J. DRAPEAU (1981). - Survie comparée de Pseudomonas aeruginosa et de Escherichia coli en eau froide. Annales Microbiologie (Inst. Pasteur), 132B, 445-463.
(26.) HOADLEY (1968). - On the significance of Pseudomonas aeruginosa in surface waters. New Engl. Water Works Assoc., 82, 99-111.
(27.) GELDREICH E.E. (1975). - Handbook evaluating water bacteriological laboratories, 2nd ed., U.S. Environmental Protection Agency, Washington D.C.

(28.) CARNEY J.F., C.E. CARTY and R.R. COLWELL (1975). - Seasonal occurrence and distribution of microbial indicators and pathogens in the Roder River of Chesapeake Bay. Applied microbiology, 30,(5), 771-780.

(29.) GELDREICH E.E. (1974). - Bacterial populations and indicator concepts in feces, sewage, stormwater and solid wastes. In: Indicators of Viruses in Water and food, ed. by Gerald Berg, 51-97. Ann Arbor Science Pub, Mich.

(30.) BUTTIAUX R. and T. LENRS (1953). - Survie des salmonelles dans l'eau de mer. Bulletin Académie Nat. Médecine, 137: 457.

(31.) CARSON L.A., N.J. PETERSON, M.S. FAVERO, I.L. DOTO, D.E. COLLINS, M.A. LEVIN (1975). - Factors influencing detedtion and enumeration of Ps. aeruginosa by MPN and MF Techniques. Applied Microbiology, 30,(6): 935-941.

ETUDE DE LA CONTAMINATION VIRALE DE L'EAU DE MER AU LARGE D'UN EMISSAIRE APRES REJET D'EAUX USEES TRAITEES

par

Chantal PIETRI et Pierre STROMBONI
Unité 40 de l'INSERM
1 avenue Jean Lorrain
06300 Nice (France)

Résumé

Les auteurs ont étudié, au large d'un émissaire après rejet d' eaux usées traitées, la répartition de la charge virale dans l'eau de mer à différentes profondeurs. La fréquence d'isolement des particules virales est faible : sur 19 échantillons d'eau de mer, 4 sont positifs. Les concentrations virales trouvées sont faibles : elles varient de 2,9 à 5,9 NPPUC (Nombre le Plus Probable d'Unités Cytopathogènes) pour 500 ml d'eau de mer. A l'inverse des bactéries fécales, aucune distribution homogène des particules virales n'est observée dans l'eau de mer. Aucune relation statistique entre les titres viraux et les titres bactériens n'a pu être établie.

Abstract

The viral contamination of sea water at different depths in the area near the discharge of treated domestic sewage was investigated. The isolation frequency of viral particles was low : on 19 samples of sea water, 4 are positive. Viral concentrations were not substantial : they varied from 2,9 to 5,9 MPNCU (Most Probable Number Cytopathic Unit) in 500 ml of sea water. In the contrary to fecal bacteria, no homogeneous distribution of viral particles in sea water was observed. No statistical relation between those microorganisms was established.

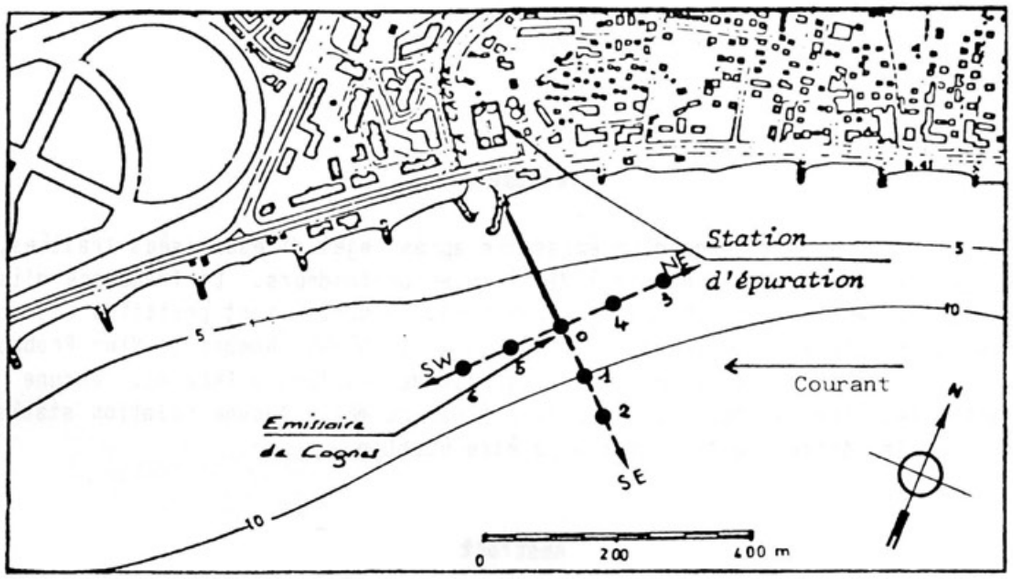
*
* *

Introduction

L'estimation de la qualité sanitaire des eaux de mer sur le plan microbiologique est une préoccupation importante en Santé Publique (4). Actuellement la recherche des bactéries fécales constitue le meilleur test rapide pour connaître la salubrité de ces eaux. Cependant, l'épidémiologie des maladies à transmission hydrique souligne l'existence des infections virales, indépendamment de celles d'origine bactérienne. En effet, les traitements physico-chimiques ou biologiques appliqués aux eaux usées ne suffisent pas à éliminer les virus véhiculés par voie hydrique (18, 27). Ces derniers appartenant essentiellement aux groupes des Entérovirus, des Adénovirus et des Réovirus, se

retrouvent alors dispersés dans le milieu récepteur (21, 13, 28). Même si les risques liés à la présence des virus en eau de mer sont difficiles à évaluer, il est prouvé cependant que certaines maladies gastro-intestinales en découlent (20, 8, 9). La recherche de ces virus est donc une nécessité. D'ailleurs, la circulaire du 1er octobre 1971 (1) stipule qu'il faut effectuer cette détermination complémentaire dans le cas où l'eau est souillée bactériologiquement.

Dans ce but, nous avons recherché, dans l'eau de mer à différentes profondeurs, la répartition de la charge virale au large d'un émissaire. De plus, pour évaluer la représentativité en eau de mer des germes test de contamination fécale par rapport aux virus d'origine entérique, nous avons étudié également, dans la même zone d'étude, la répartition de la charge bactérienne fécale.



● : Points de prélèvements

Figure 1. - Emplacement de l'émissaire et de la station d'épuration biologique de Cagnes-sur-mer. Position des radiales et des point de prélèvements.

Matériel et méthodes

Sites hydrologiques

Nos expériences ont été réalisées, comme le montre la figure 1, dans la zone côtière correspondant au rejet des eaux usées traitées de la station d'épuration biologique de Cagnes-sur-Mer (Alpes-Maritimes).

La station comprend 4 bassins de décantation primaire, 4 bassins d'oxydation et 4 bassins de décantation secondaire. Les caractéristiques physico-chimiques de l'effluent et de l'eau de mer dans la zone de rejet sont inscrites dans le tableau 1.

Prélèvements

Trois radiales de prélèvements ont été choisies, comme le montre la figure 1 (une au sud-est, au droit de l'émissaire ; une au nord-est ; une au sud-ouest). Les points de prélèvements ont été positionnés sur ces radiales à 50 mètres et à 100 mètres par rapport au panache de l'émissaire.

En chaque point, il a été effectué, à l'aide d'une bouteille à renversement, des prélèvements

d'eau de mer de 10 litres en surface, à mi-profondeur près du fond. De plus, pour servir de témoin, un prélèvement de 10 litres d'eau usée traitée a été effectué, au moment de l'expérimentation, dans la rigole d'évacuation de la station après la sortie du bassin de décantation secondaire.

Type d'eau \ Paramètres physicochimiques		D.B.O. (mg/l)	D.C.O. (mg/l)	M.E.S.T. (mg/l)	pH	Salinité %
Effluent		19 ± 4*	49 ± 19*	12 ± 8*	7,1 ± 0,9*	–
Eau de Mer	Surface	–	–	–	8,46**	37,50**
	Milieu	–	–	–	8,45**	38,10**
	Fond	–	–	–	8,54**	38,23**

*: Intervalles de confiance à 95 % déterminés à partir de 7 échantillons (16).

**: Moyennes obtenues à partir de 6 échantillons.

TABLEAU 1

Caractéristiques physico-chimiques de l'eau de mer dans la zone de rejet et de l'effluent.

Examens virologiques

Après transport au laboratoire, les échantillons d'eau (mer et effluent) sont concentrés selon la méthode d'adsorption-élution sur filtres en microfibre de verre (29, 19, 6). L'adsorption des particules virales sur la microfibre de verre est réalisée par filtration de l'eau acidifiée à pH 3,5 après adjonction de chlorure d'aluminium (5,0. 10-4 M).

L'étape d'élution des virus ainsi adsorbés se fait par broyage des filtres en microfibre de verre dans 30 ml de tampon boraté pH 9, additionné de 3 % d'extrait de boeuf. Après centrifugation du mélange à 3000 trs/mn pendant 15 mn, le surnageant est recueilli et constitue l'éluat à titrer.

La méthode utilisée pour le titrage des particules virales est celle du Nombre le Plus Probable, utilisant 5 inoculum par dilution. Les titres obtenus sont exprimés en Nombre le Plus Probable d'Unités Cytopathogènes pour 500 ml d'eau de mer ou d'eau usée.

Examens bactériologiques

Sur chaque échantillon, nous avons effectué les recherches des Coliformes fécaux (C.F.) et des Streptocoques fécaux (S.F.). Ces numérations sont réalisées selon la technique décrite par BUTTIAUX (7, 11) ; après filtrations successives, d'une part de 1 et 10 ml d'eau de mer, d'autre part de 1 ml des différentes dilutions étudiées, sur membranes filtrantes de 0,45 mμ de porosité, ces dernières sont disposées dans des boites de Pétri contenant du milieu de Chapman pour les C.F. et du milieu de Slanetz et Bantley pour les S.F. ; les numérations des C.F. et des S.F. se font respectivement après incubation de ces boites à 44 ± 1°C pendant 24 heures et à 37 ± 1°C pendant 48 heures. Les différents titres bactériens obtenus sont exprimés en nombre de C.F. et de S.F. pour 10 ml d'échantillon d'eau de mer ou d'eau usée.

Examens statistiques des résultats

Pour chaque radiale de prélèvement, des différences statistiques ont été recherchées par comparaison des intervalles de confiance entre les différents titres viraux et bactériens trouvés.

Points de prélèvements		Type d'eau	Niveau de prélèvement et profondeur (m) en chaque point	Titres viraux (NPPUC/500 ml)	Titres bactériens (Nombre/10 ml)	
					C.F.	S.F.
Témoin		Eau usée	–	5,8 (1,5 - 22,0)	6300 (2530 - 13000)	400 (100 - 1020)
Point 0		Eau de mer	0	3,0 (1,5 - 16,5)	2640 (1760 - 3790)	200 (20 - 720)
RADIALE S.E.	Point 1 situé à 50 m	"	Surface : 0 Milieu : −4,80 Fond : −9,50	0 0 0	227 (147 - 335) 37 (26 - 50) 264 (176 - 379)	20 (10 - 28) 3 (2 - 5) 2 (1 - 4)
	Point 2 situé à 100 m	"	Surface : 0 Milieu : −5,80 Fond : −12,50	0 0 0	273 (184 - 389) 28 (19 - 40) 2 (1 - 4)	64 (25 - 131) 4 (2 - 6) 1 (0 - 2)
RADIALE N.E.	Point 4 situé à 50 m	"	Surface : 0 Milieu : −4,60 Fond : −10,00	0 0 0	273 (184 - 389) 33 (23 - 45) 13 (7 - 23)	31 (21 - 43) 4 (2 - 6) 3 (2 - 6)
	Point 3 situé à 100 m	"	Surface : 0 Milieu : −4,80 Fond : −10,00	0 5,9 (2,9 - 27,0) 0	209 (132 - 314) 28 (19 - 39) 15 (9 - 25)	33 (23 - 45) 6 (2 - 13) 4 (2 - 6)
RADIALE S.W.	Point 5 situé à 50 m	"	Surface : 0 Milieu : −4,10 Fond : −8,50	2,9 (1,5 - 16,5) 0 0	500 (162 - 1170) 15 (12 - 20) 20 (12 - 30)	54 (20 - 119) 11 (8 - 14) 3 (2 - 5)
	Point 6 situé à 100 m	"	Surface : 0 Milieu : −3,80 Fond : −7,00	0 0 5,5 (1,5 - 22,4)	191 (118 - 292) 15 (9 - 25) 8 (6 - 11)	18 (11 - 28) 2 (1 - 4) 1 (0 - 2)

( ) : Intervalles de confiance à 95 %

C.F. : Coliformes écaux ; S.F. : Streptocoques fécaux.

TABLEAU 2

Titres viraux et bactériens trouvés dans les divers prélèvements en fonction de la distance en surface et en profondeur.

Résultats

Les valeurs des titres viraux et bactériens trouvés dans les divers prélèvements, en fonction de la distance en surface et en profondeur, sont portées dans le tableau 2.

A partir de ces résultats, pour chaque radiale de prélèvements, nous avons recherché la signification statistique des titres viraux et bactériens :

- d'une part, en fonction de la distance du point de prélèvement par rapport au panache,
- d'autre part, en fonction de la profondeur dans l'eau de mer.

Ces recherches sont condensées dans le tableau 3.

Type de micro-organismes	Radiale	Δ S entre les points			ΔS point situé à 50 m			ΔS point situé à 100 m		
		0 et 50 m	50 m et 100 m	0 et 100 m	Surf. et Milieu	Milieu et Fond	Surf. et Fond	Surf. et Milieu	Milieu et Fond	Surf. et Fond
VIRUS	S.E.	–	–	–	–	–	–	–	–	–
	N.E.	–	–	–	–	–	–	–	–	–
	S.W.	NS	–	–	–	–	–	–	–	–
C.F.	S.E.	S	NS	S	S	S	NS	S	S	S
	N.E.	S	NS	S	S	NS	S	S	NS	S
	S.W.	S	NS	S	S	NS	S	S	NS	S
S.F.	S.E.	NS	NS	NS	S	NS	S	S	S	S
	N.E.	NS	NS	NS	S	NS	S	S	NS	S
	S.W.	NS	NS	NS	S	S	S	S	NS	S

ΔS : Différence statistique ; S : significatif ; NS : non significatif ; Surf. : surface
–: impossible à déterminer.

TABLEAU 3

Signification statistique des titres viraux et bactériens trouvés en fonction de la distance et de la profondeur.

L'examen des valeurs des concentrations virales (tableau 2) souligne la présence de virus dans l'effluent (5,8 NPPUC/500 ml) et montre que l'analyse des 19 prélèvements d'eau de mer s'est avérée positive pour 4 d'entre eux. Les concentrations virales trouvées sont faibles : elles varient entre 2,9 et 5,9 NPPUC pour 500 ml d'eau de mer.

Compte tenu du petit nombre de résultats positifs, les différences statistiques entre les titres viraux trouvés sont impossibles à déterminer pour la majorité des cas. Pour un cas seulement, il existe une différence statistique non significative (Radiale S.W., entre le point 0 et le point 5). Notons également qu'aucune différence statistique significative n'existe avec la concentration virale de l'effluent (5,8 NPPUC/500 ml).

Donc, malgré le quadrillage de la zone de rejet, par les différents points et niveaux de prélèvements sur les 3 radiales étudiées, il n'a pas été observé de distribution homogène des virus dans le milieu.

De ce fait, ni la distance du point de prélèvement par rapport au panache, ni le niveau de prélèvement dans l'eau de mer pour chaque point envisagé, ne semblent jouer un rôle sur le mode de dispersion des virus.

L'examen des valeurs des concentrations bactériennes trouvées dans les divers échantillons d'eau de mer, nous conduit à faire les remarques suivantes :

- Il existe une forte dilution de la charge bactérienne dans l'émissaire : dans le panache, il y a environ deux fois moins de C.F. (2.640 pour 10 ml) et de S.F. (22 pour 10 ml) que dans l'effluent à la sortie de la station d'épuration (C.F. : 6.300 pour 10 ml; S.F. : 400 pour 10 ml).

- Il existe pour les C.F., quelque soit la radiale de prélèvements, une réduction de la charge bactérienne en fonction de la distance (50 et 100 m) par rapport au panache ; des différences

statistiques significatives existent entre les différentes concentrations bactériennes trouvées en ces points : par exemple, pour la radiale S.W., les concentrations en C.F. sont de 2.640 pour 10 ml dans le panache, 500 pour 10 ml au point 5 et 191 pour 10 ml au point 6.

Les différences de concentrations en C.F. entre les prélèvements faits à 50 m et à 100 m sur ces 3 radiales sont moins marquées ; elles ne traduisent pas une diminution significative du nombre de germes : par exemple, pour la radiale N.E., 273 C.F. pour 10 ml au point 4 et 209 pour 10 ml au point 3. Une dilution plus faible des eaux du panache, entre les points 50 et 100 m, par rapport à la dilution des eaux à la sortie de l'émissaire (point 0) peut expliquer en partie cette constatation. Pour les S.F., les concentrations trouvées dans les divers échantillons d'eau de mer sont faibles : sur la radiale S.W., il y a 200 S.F. pour 10 ml dans le panache, 54 S.F. pour 10 ml au point 5 et 18 S.F. pour 10 ml au point 6. Mais les différences de concentrations ne sont pas suffisamment marquées pour être significatives.

- Il existe pour la majorité des points de prélèvements positionnés à 50 et 100 m sur les 3 radiales, une diminution des concentrations en C.F. et en S.F. dans l'eau de mer de la surface vers le fond : des différences statistiques significatives existent entre les concentrations bactériennes trouvées dans l'eau de mer de surface avec, d'une part celles trouvées à mi-profondeur et d'autre part celles trouvées près du fond. Par exemple, sur la radiale S.E. au point 2, les concentrations en C.F. sont respectivement de 273, 28 et 2 pour 10 ml d'eau de mer, aux différents niveaux précités.

Les différences entre les concentrations en C.F. et en S.F. trouvées dans l'eau de mer à mi-profondeur et près du fond sont moins marquées. Il y a pratiquement autant de cas de signification statistique que de cas de non-signification. Des facteurs sont à prendre en considération pour expliquer cela : tout d'abord, l'existence d'un courant de profondeur, d'ouest en est, créé par la sortie des eaux de la Cagne (cf : Fig. 1), qui peut perturber l'évolution des eaux à ce niveau ; ensuite, l'existence de sédiments vaseux sur le fond marin dans la zone de rejet qui sont en fait des réservoirs importants de bactéries (3); sous l'influence de courants et des turbulences de l'eau de mer dans la zone de rejet, on peut supposer un relargage dans l'eau sus-jacente des micro-organismes adsorbés.

- Il nous est difficile, à partir de ces résultats bruts, de comparer globalement les concentrations en C.F. et en S.F. sur chaque radiale de prélèvements. Il semble cependant, qu'en surface de l'eau de mer les concentrations bactériennes sont plus fortes sur la radiale S.W. (par exemple : 500 C.F. pour 10 ml au point 5) que sur les deux autres radiales (par exemple : radiale N.E., 273 C.F. pour 10 ml au point 4; radiale S.E., 277 C.F. pour 10 ml au point 1), tout au moins à 50 mètres du panache. Cette constatation n'est pas surprenante compte tenu de l'influence, sur les eaux du panache, du courant général de dérive portant à l'ouest dans la zone de rejet.

A partir de notre étude, aucune recherche de corrélation statistique entre les titres viraux et les titres bactériens n'a pu être faite : la majorité des échantillons analysés ne contenait pas le virus ; cette absence de virus contraste avec la fréquence d'isolement des bactéries fécales. Notons que les concentrations virales (3,0 NPPUC/500 ml) trouvées dans les eaux polluées du panache (2.640 C.F. et 200 S.F. pour 10 ml) sont du même ordre de grandeur que celles trouvées à 100 mètres du panache (5,9 NPPUC/500 ml), dans une eau de mer de bonne qualité bactériologique (par exemple : point 3, 28 C.F. et 6 S.F. pour 10 ml).

Discussion

L'absence de distribution homogène des virus dans le milieu, mise en évidence dans notre étude, est en contradiction avec les travaux de HUGUES et coll. (17) réalisés sur le même site d'expérimentation. Leurs résultats montrent, dans l'eau de mer en surface, une diminution des concentrations virales au fur et à mesure que l'on s'éloigne du panache sur une radiale de prélèvements au droit de l'émissaire, jusqu'à une distance de 140 mètres. Cette divergence de résultats n'est qu'apparemment contradictoire : d'une part, les expérimentations ont été réalisées à deux époques différentes ;

d'autre part, le manque d'homogénéité du milieu marin explique le caractère aléatoire de la recherche virale en eau de mer autour du panache.

Notre étude montre que dans la zone de rejet étudiée, aucune relation n'existe entre la présence de bactéries témoins de contamination fécale et la présence ou l'absence de particules virales ; des virus sont mis en évidence dans l'eau de mer quand les bactéries fécales s'y trouvent à un taux faible. Ce qui est en contradiction avec les travaux de KATZENELSON et coll.(24), qui estiment qu'à partir de 13 échantillons d'eau de mer, si le nombre d'Escherichia coli pour 100 ml est inférieur à 540, aucun virus ne peut être mis en évidence. D'autres auteurs (21, 16) par contre, trouvent des virus même quand les quantités de C.F. sont faibles (80 C.F. pour 100 ml) ce qui va dans le même sens que nos propres résultats. Des études récentes (14) établissent des corrélations statistiques précises entre le taux de C.F. et de S.F. et le taux de virus dans les eaux côtières de Tel Aviv : les coefficients de corrélation trouvés entre les virus et les C.F. d'une part; les virus et les S.F. d'autre part sont respectivement de $r = 0,77$ et $r = 0,64$ avec une probabilité de $p < 0,01$ et $p < 0,1$. Par contre, d'autres travaux réalisés sur le même site d'étude que le nôtre, n'ont pas permis non plus de mettre en évidence de corrélation statistique précise (17).

Ces divergences de résultats s'expliquent en partie par des différences de méthodologie : volume de l'échantillonnage, méthode de concentration des virus et technique de mise en évidence.

De ce fait, aucune relation précise valable en toute circonstance n'a pu, à ce jour, être mise en évidence entre les virus et les bactéries fécales (22, 10, 8, 15, 23). L'estimation de la représentativité des germes témoins de contamination fécale par rapport aux virus d'origine entérique est complexe compte tenu des différences de résistance propre à chacun de ces micro-organismes au contact du milieu marin.

Comme l'indiquent les résultats des travaux antérieurs (25, 2) et les résultats de travaux réalisés par d'autres auteurs (12, 26) la survie des virus en eau de mer est longue. Par contre, la survie des bactéries fécales est courte : elles sont détruites plus ou moins vite par les conditions biochimiques inhérentes au milieu marin (3) ; Escherichia coli, par exemple, est une des bactéries les plus sensibles au contact du milieu marin (5).

De plus, il faut tenir compte du facteur diffusionnel : la dispersion des bactéries fécales dépend de la diffusion des eaux résiduaires dans le milieu marin et de phénomènes de sédimentation existant au voisinage des points de rejet des émissaires (3) ; par contre, le mode de dispersion des virus ne semble pas s'expliquer par ces facteurs.

Notre étude faite dans une zone de rejet d'eaux usées, ne nous permet pas de préjuger de la qualité virologique d'une eau de baignade avoisinante : elle montre, cependant, l'existence d'une source de contamination virale pour l'environnement.

Il est difficile d'évaluer le risque que cette contamination induit en Santé publique car les doses minimales infectantes pour l'homme sont peu connues (20). Mais, il ne faut pas mésestimer le risque potentiel lié à la présence des virus dans l'eau de mer, même à de faibles concentrations.

Les normes actuelles préconisent l'absence de particule virale (1) dans 10 litres d'eau de mer. Notons que les valeurs que nous avons trouvées dépassent ces normes alors que la qualité bactériologique de l'eau de mer était bonne.

Un effort doit être fait, soit pour standardiser les méthodes d'études de la pollution virale marine, soit pour rechercher un bio-indicateur de salubrité représentatif à la fois de la contamination virale et de la contamination bactérienne fécale d'une eau de mer, afin d'éviter tout risque en Santé publique.

Références

1. Anonyme. - Normes de qualité des eaux de baignade. Directive des Communautés Européennes du 8 décembre 1975. J.O. des Communautés Européennes du 5 février 1976.
2. C. ATTREE-PIETRI, J.P. BREITTMAYER, C. MINGHELLI, (1980). - Etude comparée de la résistance de 3 souches virales en eau de mer et en eau de rivière. Chemosphère, 9, 719-724.

3. M. AUBERT, J. AUBERT, (1973). - Pollutions marines et aménagement des rivages. Rev. Int. Océanogr. Méd., Suppl., 3-309.
4. G.A. BAGDASARJAN et coll., (1979). - Les virus humains dans l'eau, les eaux usées et le sol. Rapport technique de l'O.M.S.
5. A. BIANCHI, V. MIRCEA, (1976). - La disparition d'Escherichia coli en eau de mer. Expérimentations en enceintes dialysantes. Rapp. Comm. Int. Mer Médit., 23, 95-96.
6. J.L. BLOCK, J.C. JORET, M. MORLOT, J.M. FOLIGUET, (1978). - Recherche des enterovirus dans les eaux superficielles par adsorption-élution sur microfibre de verre. Tech. et Sc. Municipales, 3, 181-184.
7. R. BUTTIAUX, (1958). - Surveillance et contrôle des eaux d'alimentation. III : la standardisation des méthodes d'analyse de l'eau. Rev. Hyg. Méd. Soc., 6, 159-170.
8. V.J. CABELLI, (1977). - Bacterial indicators. Health Hazards Associated with water, A.S.T.M. STP 635 A.W., Hoadley and Dukta Eds., American Society for testing and materials, 222-238.
9. V.J. CABELLI, (1981). - Epidemiology of enteric viral infections. In: viruses and waste-waters treatment. Edited by M. Godard and M. Butler, Pergamon Press, Oxford 291-304.
10. V.J. CABELLI, M.A. LEVIN, A.P. DUFOUR, L.J. Mc CABE, (1974). - The development of criteria for recreational waters, Infl. Gameson Eds., Discharge of Sewage from sea outfalls, Pergamon Press, 63-73.
11. H. CASSAGNE, (1966). - Milieux de culture, Eds. de la Tourelle, Saint-Mandé.
12. F. DENIS, (1974). - Les virus pathogènes pour l'homme dans les eaux de mer et les mollusques : survie, bilan, recherche. Med. Mal. Infect., 4, 325-335.
13. T.D. EDMOND, G.E. SCHAIBERGER, C.P. GERBA, (1978). - Detection of enteroviruses near deep marine sewage outfalls. Marine Pollut. Bull., 9, 246-249.
14. B. FATTAL, R.J. VASL, E. KATZENELSON, H.I. SHUVAL, (1983). - Survival of bacterial indicator organisms and enteric viruses in the mediterranean coastal waters of Tel Aviv. Water Res., 17, 397-402.
15. C.P. GERBA, S.M. GOYAL, R.L. LABELLE, I. CECH, G.F. BOGDAN, (1979). - Failure of indicator of bacteria to reflect the occurence of enteroviruses in marine water. Am. J. Public Health. 69, 1116-1119.
16. B. HUGUES, M.A. BOUGIS, M. PLISSIER, M. ANDRE, D. LAURENT, A. PAGLIARDINI, (1979 a). - Evaluation de la contamination virale marine après rejet d'un effluent par un émissaire en mer. Zbl. Bakt. Hyg. I. Abt. Orig., B 169, 253-264.
17. B. HUGUES, J.R. LEFEBVRE, M. PLISSIER, (1981). - Distribution of viral and bacterial densities in sea-water near a costal discharge of treated domestic sewage. Zbl. Bakt. Hyg. I. Abt. Orig., B 173, 509-516.
18. B. HUGUES, M. PLISSIER, M. ANDRE, A. PAGLIARDINI, D. LAURENT, (1979 b). - Evaluation de la charge virale dans les eaux d'une station d'épuration biologique au moyen de deux méthodes de concentration des virus par adsorption-élution sur poudre de verre et sur microfibre de verre. Water Res., 13, 1117-1123.
19. B. HUGUES, M. PLISSIER, A. PAGLIARDINI, D. LAURENT, M. ANDRE, (1979 c). - Application de la méthode d'adsorption-élution sur filtre en fibre de verre pour déterminer la charge virale à l'entrée et à la sortie d'une station d'épuration biologique. J. Franç. Hydrol., 10, 21-28.
20. I.A.W.P.R.C. Study group on water virology, (1983). - The health significance of viruses in water. Water Res., 17, 121-132.
21. E. KATZENELSON, (1977). - Concentration and indentification of viruses from sea water. Rev. Int. Océanogr. Méd., 48, 9-16.
22. F. LUCENA, C. FINANCE, J. JOFRE, J. SANCHO, L. SCHWARTZBROD, (1982). - Viral pollution of superficial waters (river waters and sea water) from the urban area of Barcelona (Spain). Water Res., 16, 173-177.
23. Y. MARZOUK, S.M. GOYAL, C.P. GERBA, (1980). - Relationship of virus and indicator bacteria in water and waste water of Israel. Water Res., 14, 1585-1590.

24. L. PETRILLI, S. (DE) FLORA, (1977). - Correlation between animals viruses and bacteria in coastal sea water and sediments. Rev. Int. Océanogr. Méd., 48, 33-36.
25. C. PIETRI, J.P. BREITTMAYER, (1976). - Etude de la survie d'un enterovirus en eau de mer. Rev. Int. Océanogr. Méd., 41-42, 77-86.
26. M. PLISSIER, B. HUGUES, (1978). - Survie comparée "in situ" de poliovirus I, adénovirus V, Salmonella paratyphi B, Escherichia coli, Bandda albicans en eau de mer et en eau de rivière. Rapport 76.100, Ministère de la qualité de la vie et de l'environnement.
27. D. ROLLAND, (1981). - Flux de virus entériques dans les eaux d'une station d'épuration biologique. Thèse de doctorat de 3e cycle, Université de Metz.
28. G.G. RUITER, R.S. FUJIOKA, (1978). - Human enteric viruses in sewage and their discharge into the ocean. Water Air Soil Pollut., 10, 95-103.
29. B. SARRETTE, C.D. DANGLOT, R. VILLAGINES, (1977); - A new and simple method for recuperation of enteroviruses from water. Water Res., 11, 355-358.

THE OCCURRENCE OF STAPHYLOCOCCUS AUREUS AND PSEUDOMONAS AERUGINOSA IN COASTAL WATER OF ISRAEL

by

Yona YOSHPE-PURER and Sicilia GOLDERMAN
The A. Felix Public Health Laboratory
Ministry of Health
P.O.B. 8255, Tel Aviv (Israel)

Abstract

During the summer of 1983 (June to October) 356 samples of coastal water from 33 beaches, most of them monitored for faecal coliforms within the framework of MED POL-PHASE II, were also monitored for total coliforms and Pseudomonas aeruginosa, and 340 of them for Staphylococcus aureus as well. Although 317 samples (89 %) contained faecal coliforms, their number was quite low in most samples, and the geometric mean of faecal coliforms in all beaches was less than 100 per 100 ml of water.

Pseudomonas aeruginosa were found in 180 samples (50 %), 98 % of which were positive for total coliforms and 92 % for faecal coliforms.

Staphylococcus aureus were present in 220 samples (64.6 %), 94 % of which contained total coliforms and 87 % contained faecal coliforms. The number of S. aureus exceed considerably that of the faecal coliforms in 13 samples and in 26 samples (7 % of all samples monitored) faecal coliforms were absent.

Preliminary laboratory experiments on survival of P. aeruginosa and S. aureus in marine water are presented.

*
* *

Introduction

The bacterial indicators of pollution in recreational waters, namely total coliforms, faecal coliforms and faecal streptococci assess mainly faecal contamination, hence the possible occurrence of enteric pathogens. However, infections in these waters are not limited to enteric diseases but extend to the skin, ear, nose and throat, and most organisms that cause infection in these organs do not originate in the intestinal tract of warm-blooded animals. STEVENSON (1) found that ailments of the upper respiratory tract represented about 50 per cent of illnesses recorded in bathers while gastro-intestinal disturbances were only about 20 per cent. MUJERIEGO et al. (2) observed that the most frequent complaints recorded in recreationists in coastal waters of Spain were skin diseases (morbidity rate of 2 per cent), followed by ear and eye infections (1.5 per cent) while intestinal infections were less than 1 per cent. FOSTER and HANES (3) maintain that the large majority of

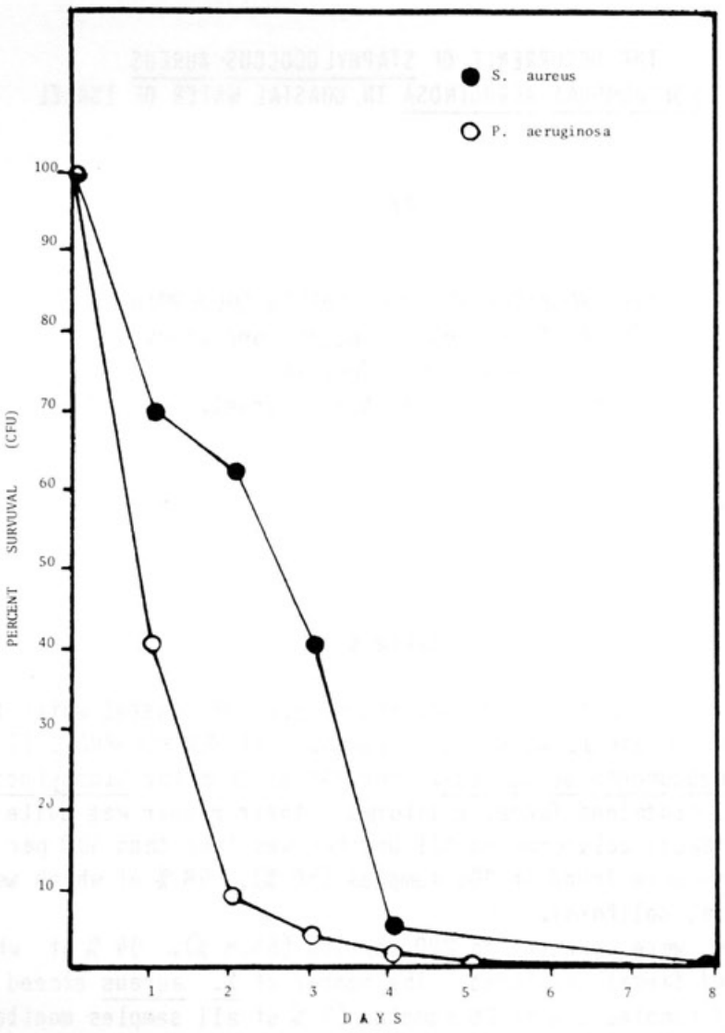


Figure 1. - Survival of S. aureus and P. aeruginosa in sea water.

illnesses that are related to swimming are transmitted by contact, not ingestion, but the problem of disease spread by contact has been relatively ignored.

Two species of bacteria have been recommended as additional indicators of pollution in recreational waters, Staphylococcus aureus and Pseudomonas aeruginosa.

All strains of coagulase positive Staphylococcus aureus are potential pathogens causing a wide range of infections. They are found in nasal membranes, hair follicles, skin and perineum of warm-blooded animals (4) and their origin in bathing waters is undoubtedly human activity. They were found to be shed by bathers under all conditions of swimming (5) and were recommened as an index of pollution by bathers is swimming pools (5, 6), since they are more chlorine-resistant than coliforms. Being salt-tolerant they may survive in the marine environment, constituting a potential health hazard to bathers in crowded beaches.

Numerous cases of folliculitis and otitis due to Pseudomonas aeruginosa, that were caused by bathing in contaminated water, have been recorded (7-11). Several authors suggest that P. aeruginosa may be a valid additional parameter for recreational waters (6, 12), especially in warm estuarine environments (13).

There is a dearth of information regarding the occurrence of S. aureus and P. aeruginosa in marine waters. The aim of this project was to investigate the occurrence of these micro-organisms in our coastal waters and, if possible, attempt to estimate their value as indicators of marine pollution. The results of the first part of this investigation are presented here.

Materials and methods

All monitoring was done by membrane filtration. For total coliforms m-Endo agar was employed and for faecal coliforms m-Fc agar, according to the reference methods of U.N.E.P./WHO.

For coagulase positive S. aureus we used the medium of MINTZER-MORGENSTEN and KATZENELSON (14) slightly modified. Suspected colonies were picked and confirmed for haemolysis, coagulase and catalase production.

For P. aeruginosa we employed mPA-C medium, which is the BRODSKY and CIEBIN (15) modification of the original m-PA medium introduced by LEVIN and CABELLI (16) and improved by DUTKA and KWAN (17).

Of the 356 samples examined, 318 were from 30 beaches monitored routinely and included in the MED POL-PHASE II. These samples were collected by the municipal sanitary inspectors, in sterile containers supplied by our laboratory, during the morning (6.30 to 11 AM) and brought to the laboratory within one to two hours. The water temperature at the time of sampling ranged from 21° to 28 °C. Thirty-eight additional samples were from three beaches on which we collaborated with the environmental Health Department of the Hebrew University in Jerusalem in their epidemiological study. These samples were collected on Friday afternoons and Saturdays, when the beaches were most crowded, and refrigerated till we examined them on Sunday morning.

Survival experiments were conducted on four strains of S. aureus and five strains of P. aeruginosa. The bacteria were grown on nutrient agar slants, suspended in membrane-filtered sea-water and known amounts of suspension were added to 100-500 ml portions of filtered sea-water in brown bottles, to give a final concentration of 10^3 organisms per ml. They were kept at room temperature which was around 22 °C during the day and 10 °C at night. Samples were withdrawn at 0 time and at various intervals, plated on nutrient agar and incubated at 36 ± 1c for 24 h. Colonies were counted and the number of colony forming units per ml was calculated. When the number of organisms decreased and larger amounts had to be inoculated (1-100 ml) the samples were filtered through MF and the filters were placed on nutrient agar plates. For P. aeruginosa parallel experiments were set up with filtered tap water.

Results

The results of the individual beaches were grouped according to the geographical regions from north to south. The data are presented in tables 1 - 4.

The number of organisms in the majority of samples was quite low, so it did not seem valid to calculate means and try to establish a numerical relationship betweeen the various organisms. This will be done at the end of the second season for the data from the most populated beaches. At this stage we preferred to indicate the number of positive samples for each organisms and the range of the number of organisms per 100 ml of water. The number of samples that contained S. aureus or P. aeruginosa with and without total and faecal coliforms was also indicated.

Of the total 356 samples monitored, 317 (89 per cent) contained total coliforms, 286 (80 per cent) contained faecal coliforms and in 180 samples (50 per cent) P. aeruginosa was found. In six additional samples P. aeruginosa was absent in 100 ml of water but grew in Rappaport's enrichment medium, which we employed for isolation of salmonella, where 500 ml of water were filtered. These samples were considered negative as there was less than one organism per 100 ml of water. Of the 180 samples that contained P. aeruginosa 98 per cent were also positive for total coliforms and 92 per cent for faecal coliforms.

S. aureus were monitored in 340 samples and 220 of them (64.6 per cent) were positive. Total and faecal coliforms were present in 94 and 87 per cent of the 220 samples, respectively (the percentage varied in each group of beaches as seen from tables 1 - 4). In 13 samples the number of S. aureus exceeded considerably that of the coliforms and in 26 samples (7 per cent of the total number monitored) S. aureus were present and faecal ccliforms were absent. The majority of these samples

Table 1: P. aeruginosa (Pa) and S. aureus (Sa) in water from beaches of the Nathania region and their relation to the presence of total coliforms (Tc) and faecal coliforms (Fc). All figures given under "range" are number of organisms per 100 ml of water.

Station No.	No. of Samples	Tc		Fc		P. aeruginosa		No. of samples						S. aureus		No. of samples						Samp. not monitored for S. a.
		Pos Samp	Range	Pos Samp	Range	Pos Samp	Range	Tc+ Pa+	Tc+ Pa-	Tc- Pa+	Fc+ Pa+	Fc+ Pa-	Fc- Pa+	Pos Samp	Range	Tc+ Sa+	Tc+ Sa-	Tc- Sa+	Fc+ Sa+	Fc+ Sa-	Fc- Sa+	
M25	15	11	1-30	10	2-28	3	2-120	2	9	1	2	8	1	2	2-12	2	9		2	8		
M26	15	14	2-76	11	2-66	6	1-20	6	8		6	5		7	2-18	6	8	1	5	6	2	
M27	14	10	2-150	9	2-40	7	3-140	6	4		6	3		7	2-56	4	4	3	4	3	3	2
M28	15	10	4-40	9	1-10	6	2-44	5	5	1	5	4	1	7	2-13	5	5	2	5	4	2	
M29	17	16	4-230	14	1-260	7	1-36	6	10	1	5	9	2	10	1-160	9	5	1	9	3	1	2
M30	17	15	2-240	12	4-190	11	1-32	10	5	1	8	4	1	12	2-120	11	2	1	9	1	3	2
M31	17	16	2-210	14	2-50	9	2-30	9	7	1	8	6	3	10	2-31	9	5	1	7	5	3	2
M32	14	11	4-220	10	2-120	5	2-44	5	6		5	5		6	2-94	5	3	1	5	2	1	3
M33	14	12	2-160	10	2-12	5	2-60	7	5		6	4		8	2-20	8	1	1	7		1	3
M34	17	14	2-50	12	2-60	8	4-44	7	7		5	7	2	8	1-56	7	5	1	6	4	2	2
Total	155	129		111		67		63	66	5	56	55	10	77		66	47	12	59	36	18	16
%		83		71.6		43							6.5	55.4 *				8.6 *			12.9 *	

*Calculated from a total of 139 samples, since in 16 samples the amount of water did not suffice for all tests and Staphylococci were not monitored.

Table 2: P. aeruginosa (Pa) and S. aureus (Sa) in water from beaches of Tel Aviv region and their relation to the presence of total coliforms (Tc) and feacal coliforms (Fc). All figures given under "range" are number of organisms per 100 ml of water.

Station No.	No. of Samples	Tc		Fc		P. aeruginosa		No. of Samples						S. aureus		No. of Samples					
		Pos Samp	Range	Pos Samp	Range	Pos Samp	Range	Tc+ Pa+	Tc+ Pa-	Tc- Pa+	Fc+ Pa+	Fc+ Pa-	Fc- Pa+	Pos Samp	Range	Tc+ Sa+	Tc+ Sa-	Tc- Sa+	Fc- Sa+	Fc+ Sa-	Fc- Sa+
M36	3	3	4-20	3	2-20	1	6	1	2		1	2		0			3			3	
M37	4	4	4-44	4	4-44	2	5-79	2	2		2	2		2	1-4	2	2		2	2	
M38	4	4	6-14	4	2-12	4	1-6	4			4			1	2	1	3		1	3	
M39	4	4	10-54	4	3-52	3	2-10	3	1		3	1		2	2-5	2	2		2	2	
M40	5	5	2-330	5	2-15	1	8	1	4		1	4		0			5			5	
M41	7	6	2-400	5	2-300	2	3-60	2	4		2	3		5	2-80	5	1		5		
M42	11	10	8-100	10	6-160	10	1-200	9	1	1	9	1	1	10	1-120	10			10		
M43	7	6	2-90	6	4-90	5	1-10	5	1		5	1		5	8-190	5	1		5	1	
M45	11	11	2-200	9	6-72	6	1-28	6	5		6	3		7	1-11	7	4		5	4	2
M46	6	5	24-80	5	10-66	4	2-8	3	2	1	3	2	1	5	10-36	5			5		
M47	7	7	6-200	7	1-160	3	1-4	3	4		3	4		5	2-360	5	2		5	2	
M48	7	7	4-320	7	2-240	3	2-6	3	4		3	4		6	13-200	6	1		6	1	
Total	76	72		69		44		42	30	2	42	27	2	48		48	24		46	23	2
%		94.7		90.8		57.9							2.6	63.2							2.6

Table 3: P. aeruginosa (Pa) and S. aureus (Sa) in water from beaches of Bat - Yam and Rishon - Lezion region and their relation to the presence of total Coliforms (Tc) and feacal (Fc). All figures given under "range" are number of organisms per 100 ml water.

Station No.	No. of Samples	Tc		Fc		P. aeruginosa		No. of Samples						S. aureus		No. of Samples					
		Pos Samp	Range	Pos Samp	Range	Pos Samp	Range	Tc+ Pa+	Tc+ Pa-	Tc- Pa+	Fc+ Pa+	Fc+ Pa-	Fc- Pa+	Pos Samp	Range	Tc+ Sa+	Tc+ Sa-	Tc- Sa+	Fc+ Sa+	Fc+ Sa-	Fc- Sa+
M49	8	8	10-720	6	10-720	6	5-80	6	2		6	2		5	8-120	5	3		5	3	
M50	8	7	2-140	5	2-60	3	2-6	3	4		3	2		6	1-20	6	1		5		1
M51	8	8	4-140	5	4-42	3	1-9	3	5		2	3	1	5	1-21	5	3		3	2	
M52	15	13	4-720	10	8-300	8	2-42	9	4		8	2		11	1-78	11	2		9	1	2
M53	14	12	12-500	12	4-110	6	2-25	6	6		6	6		12	1-54	11	1	1	11	1	1
M54	13	13	8-160	13	2-140	7	1-44	7	6		7	6		12	2-100	12	1		12	1	
M55	13	12	6-200	11	4-90	5	4-36	5	7		5	6		9	1-38	9	3		9	2	
M56	8	5	6-200	4	2-10	0			5			4		2	4-100	1	4	1		4	2
Total	87	78		68		38		39	39		37	31	1	62		60	18	2	54	14	6
%		89.6		78.2		43.7							1.1	71.3							6.9

were from the Nathania region beaches (18 samples, 11.6 per cent of the samples from that area). two were from Tel Aviv and six from Bat-Yam and Rishon-Lezion.

The survival rate of S. aureus in sea-water at room temperature was about 70 per cent after one day, 63 per cent after two days and ca. 41 per cent after three days. After four days the survival rate dropped to 1 - 7 per cent and after eight days it was 0.1 per cent.

In P. aeruginosa the average survival rate in marine water was 41 per cent after one day, 8 per cent (2 - 18 per cent) after two days, ca. 2 per cent (0.14 - 4.2 per cent) between four to six days and after 9 - 11 days 0.5 per cent (0.05 - 1.0 per cent). In tap water there was growth of 3-4 orders of magnitude after a lag period of about one day, reaching 10^7 CFU per ml after two to three days.

Discussion

Recreational waters, particulary those of marine bathing beaches, which are seldom swallowed in considerable amounts, are apt to be a source of infection by contact rather than ingestion. Yet, their sanitary surveillance is based on bacteria originating in the intestinal tract of warm-blooded animals, as in drinking water. It was felt for some time that additional bacterial parameters, which are potential pathogens and originate in other parts of the human body or in the environment, may contribute to the assurance that these waters are free of pathogens. The two ubiquitous potential pathogens that can serve this purpose are S. aureus and P. aeruginosa.

The main source of S. aureus in recreational waters is the bathers, who harbour the bacteria in their nasal membranes, on their skin and other parts of the body that come in contact with the water during bathing and swimming. We isolated them from numerous swimming pools, as did other workers (6, 18). In the marine water we found coagulase positive S. aureus in 64.6 per cent of samples, varying from 56 per cent in one group of beaches to 92 per cent in onother. This percentage is quite high in view of the fact that some beaches are located in scarcely populated areas and others were sampled in the early morning hours when the number of bathers was small. The number of organisms in the positive samples was quite low (the highest was 360 per 100 ml) and could hardly be considered hazardous. However, it is reasonable to assume that in crowded areas, during the period of heavy bathing load, their concentration was much higher and the bacteria we isolated were only the few survivors of the original population. The survival rate of S. aureus in brown bottles at room temperature was ca. 41 per cent in three days and only 0.1 per cent in eight days. In the open sea, with the effect of solar radiation and the enormous dispersion factor the decline would be much more pronounced. Therefore samples taken on the following day, or even a few hours after the period of heavy bathing load, may contain only a small fraction of the bacteria that were present at that time. This assumption is also borne out by the fact that on beaches sampled on Friday afternoons or Saturdays, when the bathing load was at its greatest utmost, the isolation rate was 92 per cent (table 4). in spite of the fact that the water was stored for 24-36 hours before inoculation, while on the beaches of the Nathania region (table 1) that were sampled in the early morning hours (6.30 - 9.00) of weekdays. it was only 56 per cent. We hope to gain more information on the subject from the second part of this study which will concentrate on the most populated beaches.

P. aeruginosa is widely distributed in nature. It is found in soil streams, fresh water and the alimentary canal of man and domestic animals. The organism can be recovered from stools of about 10 per cent of normal humans and consequently is frequently found in sewage, where the count may exceed 10^7 per 100 ml (19). It is probably not a marine organism but can be recovered from sea-water near outfalls and polluted river outlets (19). P. aeruginosa proliferates in distilled water and in buffered distilled water their number rose from 10^2 to 10^7 in 48 h at 37 °C (20). We obtained similar results in filtered tap water at room temperature. In sea-water P. aeruginosa did not grow and the die-off rate was 94 - 99.9 per cent in three to four days. According to VASCONCELOS and SWARTZ (21) P. aeruginosa die off more rapidly in sea-water then E. coli and S. aureus. This explains their absence in 50 per cent of the samples we monitored and their low numbers in the positive samples. Of

Table 4: P. aeruginosa (Pa) and S. aureus (Sa) in water from beaches studied in collaboration with Envinon. Health Dep. of Hebrew University. All figures given under "range" are number of organisms per 100 ml water.

Beach	No. of Samples	Tc		Fc		P. aeruginosa		No. of Samples						S. aureus		No. of Samples					
		Pos Samp	Range	Pos Samp	Range	Pos Samp	Range	Tc+ Pa+	Tc+ Pa-	Tc- Pa-	Fc+ Pa+	Fc+ Pa-	Fc- Pa+	Pos Samp	Range	Tc+ Sa+	Tc+ Sa-	Tc- Sa+	Fc+ Sa+	Fc+ Sa-	Fc- Sa+
Gordon	18	18	12-900	18	2-110	17	1-200	17	1		17	1		18	4-230	18			18		
Sheraton	13	13	6- $>10^3$	13	2- 10^3	7	1-46	7	6		7	6		10	1-210	10	3		10	3	
Rishon Lezion	7	7	40-3300	7	12-130	7	2-21	7			7			7	8-78	7			7		
Total	38	38		38		31		31			31	7		35		35	3		35	3	
%		100		100		81.6								92							

the 180 samples that contained P. aeruginosa 98 per cent and 92 per cent were also positive for total and faecal coliforms, respectively. It seems, at this stage, that in sea-water, monitoring for P. aeruginosa would not add much to the information gained by coliforms on the sanitary quality of marine bathing beaches. The subject will be re-evaluated at the end of the project, when numerical relationships between the various micro-organisms are established.

References

1. STEVENSON A.H. (1953). - Studies on bathing water quality and health. Am. J. Public Health, 43: 529-538.
2. MUJERIEGO R., J.M. BRAVO and M.T. FELIN (1982). - Recreation on coastal waters: Public Health implications. VIth Workshop on Marine Pollution of the Mediterranean, Cannes, Dec. 2-4.
3. FOSTER D.H., N.B. HANES and S.M. LORD Jr (1971). - A critical examination of bathing water quality standards. J. Water Poll. Cont. Fed. 43: 2,229-2,241.
4. Bergey's Manual of Determinative Bacteriology (8th edition) (1975). - R.E. Buchaman and N.E. Gibbons Eds. William and Wilkins Co. Baltimore.
5. ROBINTON E.D. and E.W. MOOD (1966). - A quantitative and qualitative appraisal of microbial pollution of water by swimmers. J. Hyg. Camb. 64: 489-491.
6. FAVERO M.S., C.H. DRAKE and J.B. RANDALL (1964). - Use of Staphylococci as indicators of swimming pool pollution. Public Health Rep. 79: 61-70.
7. MMWR (1983). - An outbreak of Pseudomonas folliculitis associated with a waterslide. 32(32): 425-527.
8. ALOMAR A., V. AUSINA, J. VERNIS and J.M. DEMORAGAS (1982). - Pseudomonas folliculitis. Cutis 30: 405-409.
9. KHABBAZ R.F., T.W. Mc KINLEY, R.A. GOODMAN et al. (1983). - Pseudomonas aeruginosa serotype O:9, new cause of whirlpool dermatitis.Am. J. Med. 74: 73-77.
10. VAN KEULEN P.H.J. and S.A. KOHLER (1983). - Labyrinthistis due to Pseudomonas aeruginosa after whirpool bathing. Med. Tijdscher. Geneeskd. 127: 381-383.
11. HAVELAAR A.H., M. BOSMAN and J. BORST (1983). - Otitis externa by Pseudomonas aeruginosa associated with whirlpools. J. Hyg. Camb. 90: 489-498.
12. HOADLEY A.W. (1968). - On the significance of Pseudomonas aeruginosa in surface water. J. New Eng, Water Works Assn. 83: 99 (Cited by FOSTER et al.).
13. GELDREICH E.E. (1974-5). - Microbial criteria concepts for coastal bathing waters. Ocean Management 3: 225-248.
14. MINTZER-MORGENSTERN L. and E. KATZENELSON (1982). - A simple medium for isolation of coagulase-positive Staphylococci in a single step. J. Food Protec. 45: 218-222.
15. BRODSKY M.H. and B.W CIEBIN (1978). - Improved medium for recovery and enumeration of Pseudomonas aeruginosa from water using membrane filters. App. Environ. Microbiol. 36: 36-42.
16. LEVIN M.A. and V.J. CABELLI (1972). - Membrane filter technique for enumeration of Pseudomonas aeruginosa, Appl. Microbiol, 24: 864-870.
17. DUTKA B.J. and K.K. KWAN (1977). - Confirmation of the single-step membrane filtration procedure for estimating Pseudomonas aeruginosa densities in water. Appl. Environ. Microbiol. 33: 240-245.
18. CRONE C.P. and G.H. TEE (1979). - Staphylococci in swimming pool water. J. Hyg. Camb. 73: 213-220.
19. RHAME F.S. (1979). - The ecology and epidemiology of Pseudomonas aeruginosa. In Pseudomonas aeruginosa, the Organism, Diseases it Causes, and their Treatment. L.D. Sabath Ed. Hans Huber Publishers, Bern, Switzerland.
20. FAVERO M.S., L.A. CARSON, W.W. BOND and N.J. PETERSEN (1971). - Pseudomonas aeruginosa: growth in distilled water from hospitals. Science 173: 836-838.
21. VASCONCELOS G.J. and R.G. SWARTZ (1976). - Survival of bacteria in sea-water using a diffusion chamber apparatus in situ. App. Environ. microbiol. 31: 913-920.

MICROBIOLOGICAL POLLUTION IN CULLERA BAY

by

Inmaculada AMOROS MUNOZ, José Luis ALONSO MOLINA
and
Inmaculada PERIS AGUIRRE
Instituto de Hidrologia y Medio Natural
E.T.S. de Ingenieros de Canales y Puertos
Universidad Politécnica de Valencia
B.P. 2012, Valencia (Espana)

Abstract

The Cullera bay is one of the most attractive touristic resorts on the Valencia coast, with estuarine characteristics.

The heterotrophic bacterial populations levels in Cullera coastal waters are influenced by the presence of the river Jucar.

Over the last years, many studies have been developed in order to know the incidence of pollution and the bacteriological quality of the water .

The microbial activity of recreational waters must be defined indirectly in terms of the occurrence of intestinal pathogens and population levels of opportunistic pathogens that can cause infections of the skin, eye, and mucus membranes.

The incidence of total coliforms, faecal coliforms, faecal streptococci, considered as microbial indicators in water, and fungi in sand, were investigated for a year, giving the FC:FS ratios and correlations between faecal indicators, in 27 different sampling stations.

*
* *

Introduction

Cullera bay, located 40 km south of Valencia on the Mediterranean coast, is a most important pleasure resort with a population increasing from 20,000 to 200,000 inhabitants during the summer.

In the present study, total coliforms, faecal coliforms and faecal streptococci and the presence of fungi and yeasts in sand were investigated.

Correlation coefficients between bacteriological parameters in sea-water samples were obtained to study the relationships between them and their level of significance. Kruskall-Wallis tests were calculated to check the homogeneity of pollution throughout the study.

FC: FS ratios were obtained to know the sources of pollution.

Materials and methods

- Samples. - 27 sampling sites were monitored: nine beach stations, two sewage disposal inputs. Their distribution in the area is shown in figure 1.

Frequency of sampling was monthly except in the summer.

- Bacteriological indicators: according to the Standard Methods for enumeration and isolation of indicators organisms:

- Presence of fungi and yeast: the pour plate technique in Saubouraud agar (Merck);

- Statistical analyses: correlation coefficients of Bravais-Pearson were calculated with the "Stat Enter Program" (Boalax Informatica).

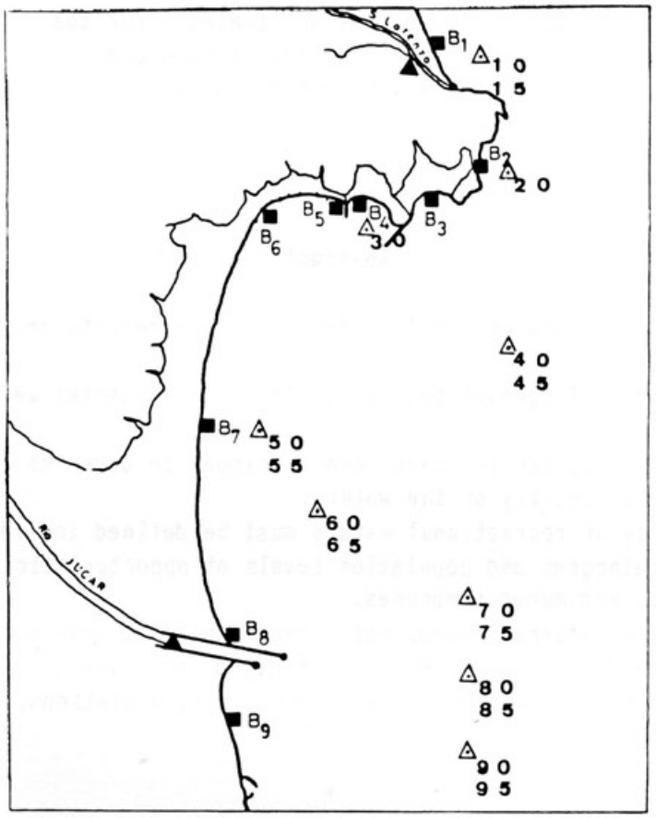


Figure 1. - Map of the monitored zone
■ Beach areas
△ off shore stations (0 surface, 5 depth)
▲ sewage effluents

Results and discussion

As expected, the bacteriological indicators in Cullera bay, were greatly influenced by the discharge of sewage effluents and polluted streams, principally in the area influenced by the discharge (stations 1, 7, 8 and 9).

Considerable pollution was caused by the river Jucar, where a number of domestic and irrrigation sewage effluents are discharged, and introduced into bay.

The polluted streams S. Lorenzo mostly receives irrigation waters so that its faecal pollution level is lower than in river Jucar.

The heavy load of pollution discharged into the receiving coastal waters streads across the surface sea-water, not affecting deep water, (lowest values were obtained in deep water).

The persistence of contamination at the surface may be due to the absence of water mass circulation stet depth, the wind being the principal disperser of pollutants, and the factor most responsible for the littoral processes (1).

Only one exception is noted during the month of July when there is an absence of wind which facilitates the dispersal of pollutants to the bottom of the sea-water.

PETRILLI et al. also found a decrease in pollution at 6 m depth on Italian coasts (2).

Conversely, DE MAEYER-CLEMPOEL recorded an increase in pollution at depth on the Belgian coast (3).

Bacteriological values at beaches remained low all the year, except the months of July and August, where a considerable increase in total coliforms occurred. Conversely. faecal coliforms and faecal streptococci values remained low, not exceeding the EEC Standards for bathing waters (4).

The greatest bacteriological counts were found in the beaches nearest to the river Jucar, station B_8 and B_9 (with a max. value of 1,609 total coliforms/100 ml) and to the San Lorenzo stream station B_1 due to the influence of these sewage flow; and at stations B_2 due to the effluent of an inefficient treatment plant.

These facts verify the necessity of an effective treatment of waste waters to protect receiving coastal waters from pollution risks. The presence of faecal organisms in bathing waters constitutes a human hazard, because of the probability of finding pathogens. BRISOU and DENIS (5) indicated that a number of coliforms higher than 200/100 ml increased the probability of finding Salmonella in 70-98 per cent of waters and in 50-80 per cent of sediments.

Correlation coefficients obtained with bacteriological data (Table 1) show the close relationship that exists between concentrations of total coliforms, faecal coliforms and faecal streptococci. The highest relationship was found between total coliforms and faecal coliforms (r = 0.8). Conversely PETRILLI (6) and HUGUES (7) found the highest correlation between faecal coliforms and faecal streptococci, whereas SAYLER (8) found a higly significant correlation for the microbiological variable, but the highest correlation was found for total coliforms and faecal streptococci. However, CARNEY (9) found detectable differences between correlation coefficients in similar locations. No correlation was observed for indicator organism at some stations and a strong correlation was observed at others.

Table 1
Regression Coefficient (r), significance level and confidence level by Fisher

	r	Significance level	confidence level		
TC-TF	0.8	1 %	0.81	0.89	0.93
TC-FS	0.55	1 %	0.32	0.55	0.71
FC-FS	0.56	1 %	0.29	0.56	0.73

Kruskall-Wallis tests to see the variability of pollution in beach areas, showed that those with a great degree of pollution variability and monthly significantly different values were: at stations

B3, B4, B5, B6, B7, which presented very slight bacteriological pollution, except in the summer months, where occasional pollution occurred. Conversely, stations B9, B8 and B2 showed a homogeneous pollution, due to a continuous sewage discharge from the two effluents. LODEIRO (10) also found significant differences in faecal pollution at some monitored beaches in La Coruna (Spain).

FC: FS ratios (11) were calculated in beach areas, showing that at six beaches (B1, B2, B4, B7, B8, and B9), FC: FS ratios greater than 4.0 were obtained. This result could indicate recent pollution. In the same way, FUJIOKA (12) found that in marine waters faecal coliforms were much more sensitive to sunlight inactivation than were faecal streptococci, and so the high ratio of FC: FS in domestic sewage might be maintained for a few hours after the sewage has been discharged to the marine environment. At offshore stations, FC: FS ratios were not significant due to the low values of faecal streptococci found.

References

1. ANONIMO (1983). - Informe sobre los problemas de la bahia de Cullera y en particular los planteados por la proliferacion de algas rojas filamentosas. No publicado. Universidad Politecnica. Vol. 1.
2. PETRILLI F., S. DE FLORA, L. LEMORI (1975). - Pollution of coastal waters in Italy. Bacteriological and virological investigations. Marine pollution and Marine Waste disposal pp 411-421.
3. Yde M., S. DE MAEYER-CLEMPOEL et Z. DARTEVELLE (1980). - La pollution fécale en mer du Nord au large d'Ostende (Belgique). Revue Internationale d'Océanographie Médicale 59.
4. E.E.C. European Communities (1976). - Council Directive of 8 December 1975 concerning the quality of bathing waters. Off. Journal of European Communities 19. L31/1L-31/7.
5. BRISOU J.F. at F.A. DENIS (1978). - Hygiène de l'environnement maritine. Masson. Paris.
6. PETRILLI et al. (1980). - Microbiological evaluations of coastal waters quality in the Tyrrhenian sea. Programme in Water Technology. 12. 129-136.
7. HUGUES B., J.R. LEFEVRE, M. PLISSIER and A. CINI (1981). - Distribution of viral and bacteriological densities in sea-water near a coastal discharge of treated domestic sewage. Zentralblatt fur Backteriologie, Mikrobiologie und Hygiene. 173/6, 507-516.
8. SAYLER G.S., J.D. NELSON, A. JUSTICE and R.R. COLWELL (1975). - Distribution and significance of faecal indicators organisms in the Upper Chesapeake Bay. Applied Microbiology. 30. 625-638.
9. CARNEY J., C. CARTY, and R. COLWELL (1975). - Seasonal occurrence and distribution of microbial indicators and pathogens in Rhode river of Chesapeake Bay. Applied Microbiology.
10. LODEIRO L., C.M. FERREIROS y M.T. CRIADO. - Analisis de la variabilidad de la contaminacion fecal en las playas de la Coruna. Congreso Nacional de Microbiologia.
11. GELDREICH E.E. and B. KENNER (1969). - Concepts of faecal streptococci in stream pollution. Journal of Water Pollution Control Federation 41: 336-352.
12. FUJIOKA R.S., H.H. HASHIMOTO, E.B. SIWAK and R.H.F. YOUNG (1981). - Effect of sunlight on survival of indicator bacteria in sea-water. Applied and Environmental Microbiology 41: 690-696.

THE QUALITY OF COASTAL WATERS IN THE GREATER ATHENS AREA

by

S. SOTIRACOPOULOS, D. SPALA
and E. VALIANTZA
Environmental pollution control project - Athens
147, Patission Street, GR-112 51 Athens (Greece)

Abstract

With the aim of defining the pollution levels of bathing sea-water and specifying the requirements for the rational design of a new liquid disposal system, a sampling programme was established by the Environmental Pollution Control Project - Athens in 1975.

The sampling programme for the quality of coastal waters included densely populated areas, summer resorts, and where extensive shipping traffic, discharges of waste water and other pollutional inputs take place. Remote areas which are neither visited by large numbers of holidays-makers, nor subject to any pollution loads, as well as heavily polluted areas, where bathing is prohibited are also included to provide background and comparative information.

Our data indicate that all bathing waters, with a few exceptions where the waters are intermittently polluted, are of acceptable quality according to the Greek Sanitary Regulation (E1B 221/1965 i.e. the mean value during the bathing season is less than 1,000 total coliforms per 100 ml, of sea-water). The same result is obtained, in a different way, by the E.E.C. Directive 76/160 1976.

*
* *

Introduction

With the aim of defining the pollution levels of bathing sea-water and specifying the requirements for the rational design of a new liquid waste disposal system, a sampling programme was established by the Environmental Pollution Control Project - Athens in 1975.

The sampling programme for the quality coastal waters (Fig. 1, table I) includes densely populated areas, summer resorts, and where extensive shipping traffic, discharges of waste water and other pollutional inputs take place. Remote areas with are neither visited by large numbers of holiday-makers nor subject to any pollution loads, as well as heavily polluted areas, where bathing is prohibited, are also included to provide background and comparative information.

The sampling stations are classified in table 1 according to use.

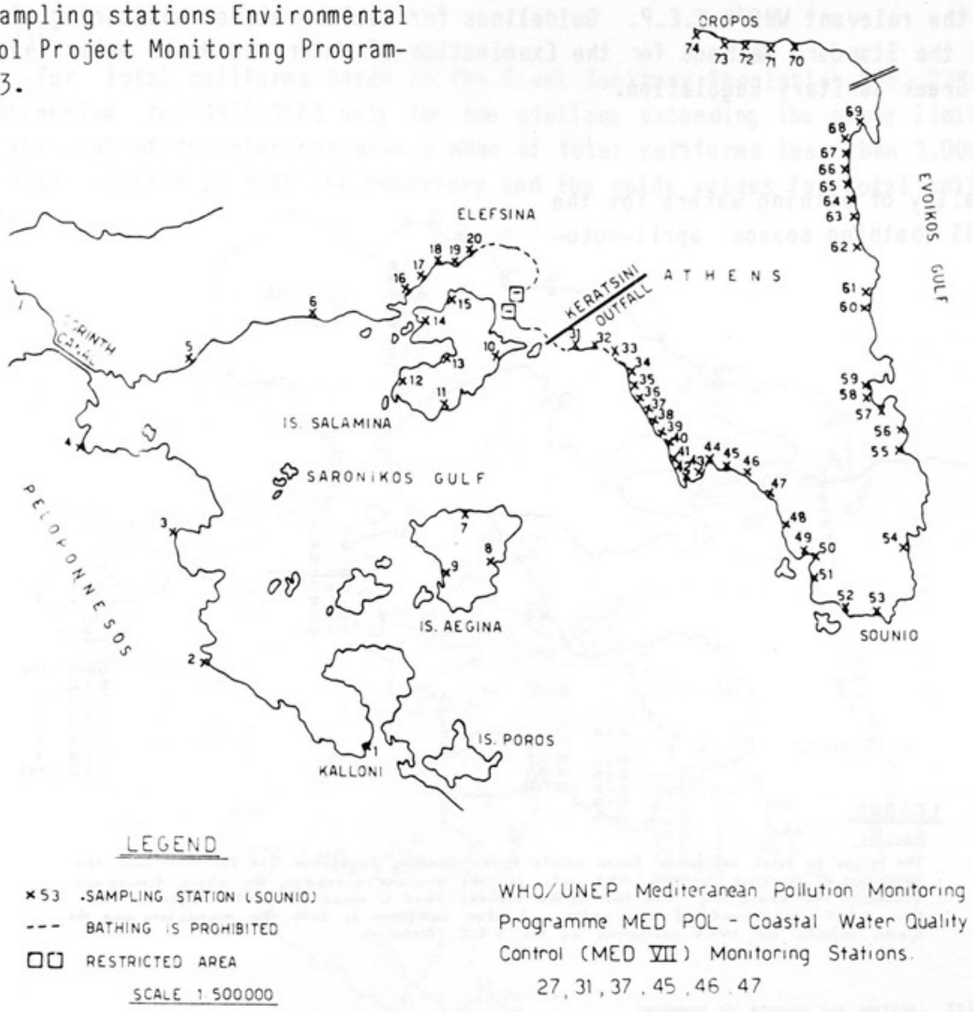
Type A: Organized beach with all conveniences supervised by either the Hellenic Tourist Organization or the Municipality (admission fee).

Type B: Natural open beach of high aesthetic level where large numbers of bathers are observed.

No	Name of Station	Type	No	Name of Station	Type
1	Kalloni	C	37	Alimos Beach MED VII	C
2	Epidavros	C	38	Agios Cosmas	A
3	Corfos	C	39	Glyfada Asteria Beach	A
4	Loutra Or.Elenis	B	40	Voula Beach A	A
5	Ag.Theodori	C	41	Voula Beach B	A
6	Kinetta	B	42	Kavouri	B
	ISLAND OF AEGINA		43	Vouliagmeni Astir Beach	A
7	Souval a	C	44	Vouliagmeni Beach	A
8	Agia Marina	B	45	Varkiza Beach MED VII	A
9	4th Kilometer	B	46	Kiani Akti MED VII	B
	ISLAND OF SALAMINA		47	Lagonisi MED VII	B
10	Selinia	C	48	Saronida	B
11	Kakia Vigla	C	49	Anavyssos	B
12	Kanakia	B	50	Fokca	B
13	Eantio	C	51	Arsida	B
14	Agios Georgios	C	52	Legraina	B
15	Psilli Ammos	C	53	Sounio	B
16	Nea Peramos	C	54	Lavrio	D
17	Akti Papaioannou	C	55	Daskalio	B
18	Piraiki-Patraiki	C	56	Kakia Thalassa	B
19	Loutropyrgos	C	57	Avlaki A	A
20	30th kilometer	C	58	Avlaki B	B
21	Elefsina Shipyards	D	59	Porto Rafti	B
22	Cement "TITAN"	D	60	Agios Spyridonas	B
23	Elefsina Beach	D	61	Loutsa A	B
24	Elefsina A	D	62	Loutsa B	B
25	Aspropyrgos	D	63	Rafina	B
26	Skaramagas	D	64	Mati	B
27	Perama Beach MED VII	C	65	Zoumberi	B
28	Keratsini	D	66	Agios Andreas	B
29	Akti Themistocleous	D	67	Nea Makri	B
30	Freattys Beach	A	68	Marathonas GC	B
31	Votsalakia Beach MED VII	A	69	Marathonas	B
32	Microlimano	D	70	Marathonas Schinias	B
33	Flisvos	C	71	Ag.Apostoli	B
34	Batis	B	72	Oropos A	B
35	Edem	C	73	Oropos B	B
36	Alimos (EOT)	A	74	Oropos C	D

Table I. - Sampling stations ; type of beach.

Figure 1. - Sampling stations Environmental Pollution Control Project Monitoring Programme for 1976-1983.



Type C: Beach where bathing is not prohibited and is traditionally practised by a number of bathers.
Type D: Bathing is prohibited.

The Greek Sanitary Regulation E1b 221/1965(1) classifies natural waters with respect to disignated uses. According to the current regulations bathing is permitted in all waters where the mean value of total coliforms is less than 1,000 organisms per 100 ml of water. Bathing is prohibited in all ports, areas where ships are permanently anchored and within 200 metres on either side of discharges. A prefectorial decision issued each year before the bathing season begins informs the public about areas where bathing is prohibited.

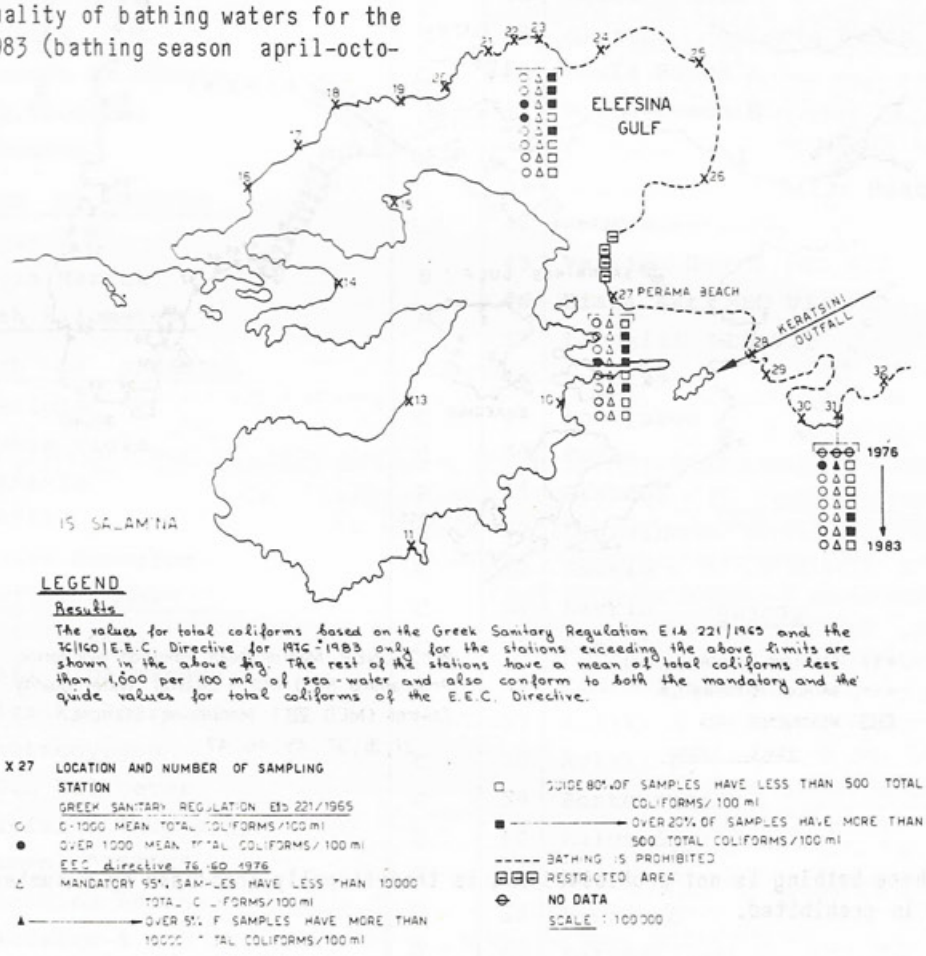
The E.E.C. Directive 76/160/1976 (2) prescribes that 95 per cent of bathing water samples must have coliform counts below the mandatory level of 10,000 per 100 ml water. Under the more stringent quideline level the requirement is that 80 per cent of samples shall have less than 500 coliforms per 100 ml of sea-water.

Methodology

At each station, once a month for most, two to five samples were collected 20 to 30 cm below the surface of the water and at about 1.2 to 1.5 metres depth (about chest level). The density of total coliforms was estimated by the most probable number (MPN) and the membrane filtration techniques as

described in the relevant WHO/U.N.E.P. Guidelines for health-related monitoring of coastal water quality (3) and the Standard Methods for the Examination of Water and Waste water (4), which has been adopted by the Greek Sanitary Regulation.

Figure 2. - Quality of bathing waters for the years 1976-1983 (bathing season april-october).



For systematic beach surveillance, a scheme which took into account the prevailing conditions at the sampling stations was developed in order to secure uniform criteria in examining the visual appearance of beaches. These observations include:

Shore

1) Trash
   a) Household, b) beach, c) sea
2) Seaweeds
3) Tar

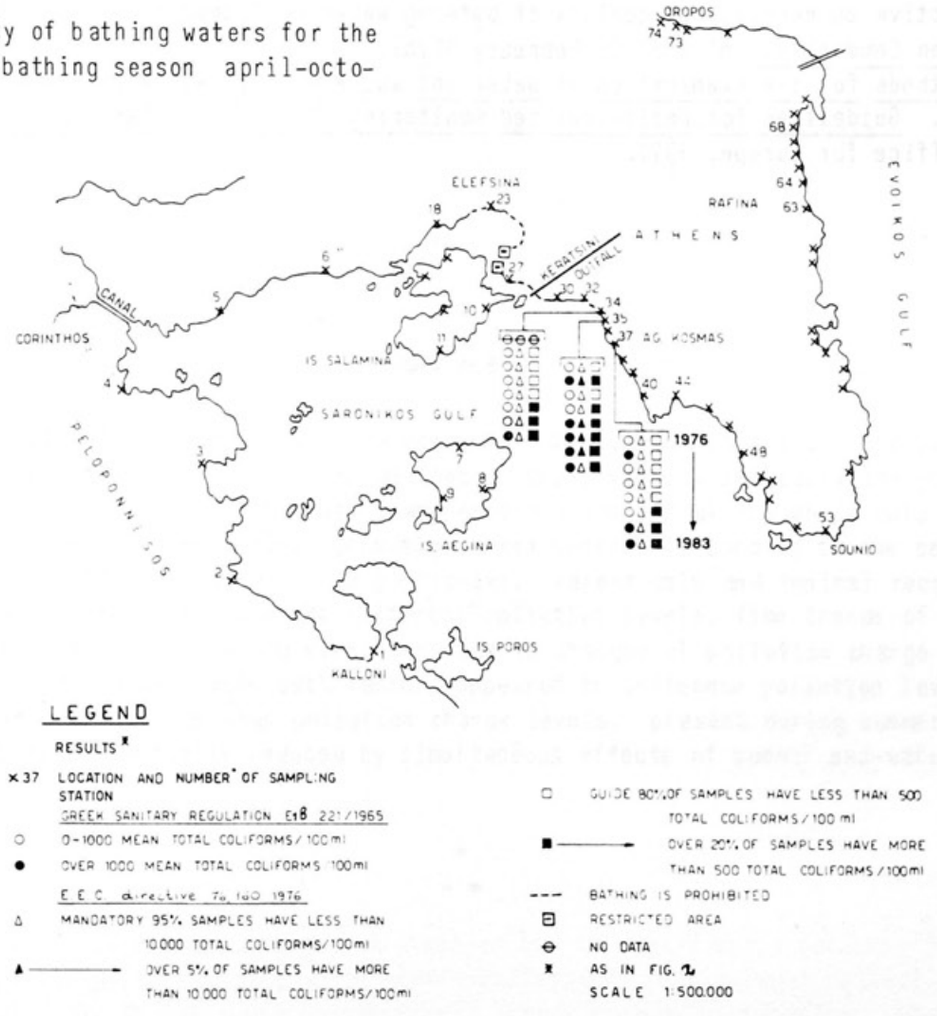
Sea

1) Foating materials
   a) Debris b) tar c) oil d) plastics
2) Blooms
3) Fish kills

Results

The values for total coliforms based on the Greek Sanitary Regulation E1b 221/1965 and the 76/160/E.E.C. Directive for 1976-1983 only for the stations exceeding the above limits are shown in Fig (2 and 3). The rest of the stations have a mean of total coliforms less than 1,000 per 100 ml of sea-water and also conform to both the mandatory and the guide values for total coliforms of the E.E.C. Directive.

Figure 3. - Quality of bathing waters for the years 1976-1983 (bathing season april-october).



Discussion

Our data indicate that all bathing waters with a few exceptions where the waters are intermittently polluted are of acceptable quality according to the Greak Sanitary Regulations (E1b 221/1965 i.e. the mean value during the bathing season is less than 1,000 total coliforms per 100 ml of sea-water).

The same result is obtained in a different way according to the E.E.C DIrective 76/160 1976.

Each year, before the bathing season begins, the Committee for the Sanitary Control of Coastal and Riverside Waters evaluates the suitability for bathing of coastal waters. This evaluation is based on Sanitary Regulations, laboratory examinations of previous years, and local investigations of the conditions prevailing.

Acknowledgements

We thank the staff of the Environmental Pollution Control Project - Athens, for their technical assistance.

References

1. Sanitary Regulation E1b 221/65 and the relative amendments published in the Official Government Gazette (1965, 1974).
2. Council directive concerning the quality of bathing water (8 December 1975). - Official Journal of the European Community., n° L 31 (5 February 1976).
3. Standard methods for the examination of water and waste-water, 14th ed. APHA - AWWA - WPCF, 1975.
4. WHO/U.N.E.P. Guidelines for health-related monitoring of coastal water quality. Copenhagen, WHO, Regional Office for Europe, 1977.

TIME TRENDS ANALYSI[OF SEA-WATER POLLUTION INDICATOR LEVELS IN SELECTED ITALIAN COASTAL AREAS

by

L. VILLA*, F.A. AULICINO*, G. DONATI*
M. GABRIELE**, A. PICCIONI**, L. VOLTERRA*
and G.A. ZAPPONI**
* Laboratorio di Igiene del Territorio
** Laboratorio di Tossicologia Comparata ed Ecotossicologia
Istituto Superiore di Sanita, Viale Regina Elena 299
00161 Roma (Italy)

Abstract

A selected set of data concerning sea-water microbiological pollution indicator levels, as measured in a representative group of italian coastal areas was statistically analysed to identify water pollution variation causes. The data examined are a sub-set of the whole data collected according to Italian regulations concerning recreational sea control (according to the corresponding EEC directive and the WHO/UNEP guidelines). In particular, recent data and typical recreational coastal areas were considered, including sites at different pollution levels. Time trends of pollution levels were examined, separately considering variations due to changes of pollution charge and those attributable to climate and season. Both such factors appeared to influence pollution levels significantly. In particular, effects of high pollution charge levels, present during summer holiday periods sometimes appeared to be partially reduced by simultaneous effects of summer sea-water conditions.

*
* *

Introduction

As is well known, the reproducibility of sea-water microbiological pollution measurements is relatively limited. Many causes have been indicated as responsible for the variability of Total Coliform (TC), Faecal Coliform (FC) and Faecal Streptococci (FS) levels. Several meteorological factors, such as winds, rainfall amount, temperature level and solar radiation, together with surface currents and sea status, have been indicated to exert a consistent influence on the above-mentioned sea-water microbiological pollution indicators (C.B.B.C, 1959: VILLA et al., 1981).

Human population represents the most relevant source of sea-water microbiological pollution. Due to the remarkable fluctuations in the number residents, which characterize recreational seaside resorts, microbiological pollution charges in recreational sea-waters are susceptible to consistent change during the different seasons and the week (WHO/W. Poll. 72.10).

Lastly, the variability of microbiological pollution measurements due to both sampling and laboratory determination, represents a significant part of overall variability (GELDREICH, 1975). This paper reports a study aimed at characterizing time variations of microbiological pollution indicator levels.



Fig. 1. Selected Italian districts (near eight cities) from which the 115 sampling points were chosen.

Materials and methods

A set of 115 sampling sites (Fig. 1) was selected among those periodically sampled and controlled according to Italian regulations. Statistical analysis of data was carried out using log-transformation of data, suitable to produce normal (Gaussian) distribution of TC, FC and FS data (GELDREICH, 1975). The log-transformed data distribution appeared to be consistent with the normal statistical distribution hypothesis (KOLMOGOROV-SMIRNOV test; SIEGEL, 1956). Two-way analysis of the variance method was used to separate site-variability from time-variability.

Results

In fig. 2 the histograms of whole values measured from January 1980 to September 1980 are shown for the nine months.

The number of values exceeding 100 FC/100 ml of water, which is relatively high in January, February and March, appears to decrease in April and May. After May, FC values appear to increase slighty with a maximum in August. The corresponding time trend is shown in fig. 3, where points

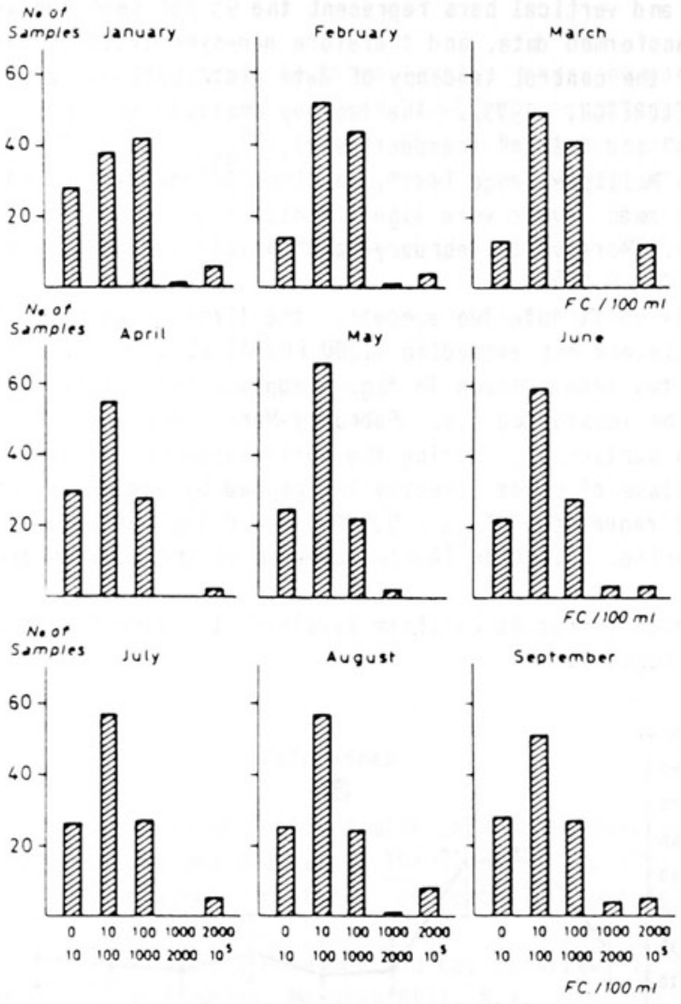


Fig. 2. Distribution histogram of the entire lot of values measured from January 1980 to September 1980.

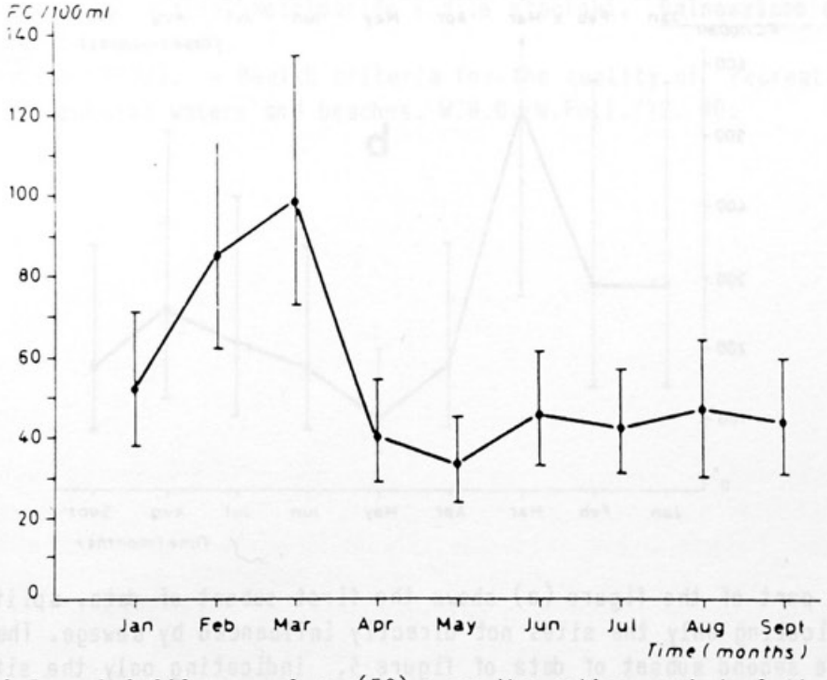


Fig. 3. Time trend of Faecal Coliforms values (FC) over the entire period of time. Points represent geometric means and vertical bars the 95 per cent confidence limits.

represent geometric means and vertical bars represent the 95 per cent confidence limits of geometric means (computed on log-transformed data, and therefore non-symmetrical). Geometric means were adopted as reliable indicators of the central tendency of data distribution, due to the assessed log-normal distribution of data (GELDREICH, 1975). The two-way analysis of variance indicated a significant difference between "months" and "sites" (respectively), $F^{8}_{912} = 5.41$, $P < 0.005$; $F^{114}_{912} = 8.02$, ($P < 0.005$). The Duncan's "New Multiple Range Test", applied to analysis of variance data, allowed us to assess that February-March mean levels were significantly higher than the mean levels other months ($P < 0.05$) (SENTER J., 1969). Moreover, February-March levels values proved to be more than twice as high as April-May values ($P < 0.01$).

If the set of sites is split into two subsets, the first of which including sites not directly influenced by sewage (FC levels not exceeding 1,000 FC/100 ml of sea-water) and the second including the remaining sites, the two trends shown in fig. 4 appear. For the first class of sites (Fig. 4 a), two classes of levels may be identified i.e. February-March levels and the remainder, significantly different ($P < 0.01$). In particular, during the April-September period no significant differences appear. As far as the class of sites directly influenced by sewage is concerned (Fig. 4 b), a statistically significant regression ($F^{1}_{87} = 5.590$, $P < 0.025$) of pollution levels versus time was found in the May-August period. Pollution levels appeared to increase according to the time function: $(FC) = 102\ t^{0.67}$.
(where (FC) = geometric mean of Faecal Coliform levels, t = time (months), t = 1, April; t = 2, June; t = 3, July; t = 4, August).

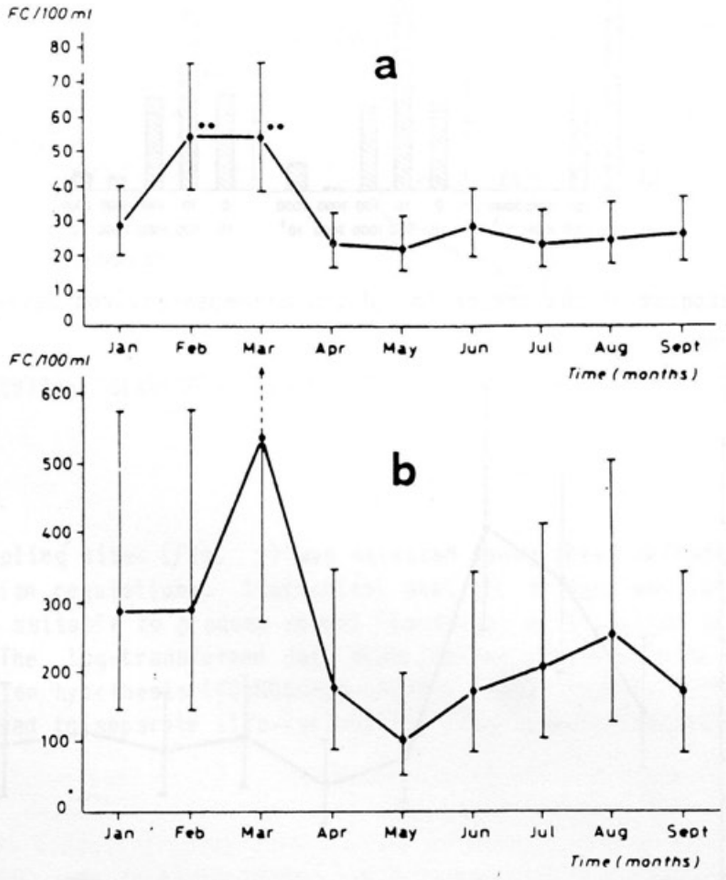


Fig. 4. The upper part of the figure (a) shows the first subset of data, split from the data set of figure 3, indicating only the sites not directly influenced by sewage. The lower part of figure (b) shows the second subset of data of figure 3, indicating only the sites with FC exceeding 1,000 FC/100 ml.

Conclusion

The analysis of time behaviour of Faecal Coliform levels, measured monthly in 1980 in a set of 115 sampling sites (including non-contaminated areas,low-level contaminated areas and medium-level contaminated areas) indicated the February-March mean FC levels to be significantly higher than the ones measured in April-September. This difference appeared more evident at sites not directly influenced by sewage. Moreover, for this class of site, during the April-September period, the FC levels appeared practicaly constant (geometric mean values ranging from 22 to 27 FC/100 ml of sea-water).

The overall variability of levels, as measured at sites directly influenced by sewage, is noticeably higher than that of levels measured in lowly contaminated sites. Moreover, at sites directly influenced by sewage, FC levels appeared to increase continuously during the period from April to August. This behaviour. not evident for low-level contaminated sites, may be ascribed to the increasing pollution charge due to the increasing number of residents in recreational seaside resorts during the swimming period.

Therefore, two factors may be hypothesized to influence overall microbiological pollution phenomena: an increase in the pollution load during summer, and some meteorological factor(s), liable to reduce pollution levels in low-contamination sites, during late spring and summer. These meteorological factors may be hypothesized to be U.V. solars rays and/or temperature, as well as the sea conditions during this period (C.B.B.C., 1959: VILLA et al., 1981).

References

Committee on Bathing Beach Contamination of Public Health Laboratory Service (1959). - Sewage contamination of coastal waters in England and Wales, The Hygiene, 57, 435.

GELDREICH E.E. (1975). - Microbiological criteria concepts for coastal bathing waters, Ocean Management, 3, 225.

SENTER J. (1969). - Analysis of data, Scott, Foresmon and Co. Glenview, Illinois.

SIEGEL S. (1956). - Non-parametric statistics, Mc-Graw Hill, N.Y.

VILLA L. and L. VOLTERRA (1981). - La variazione degli indici microbici di inquinamento delle acque marine in rapporto alle condizioni meteomarine e alle stagioni, Balneazione e qualità microbiologiche delle acque, Edimex, Roma.

World Health Organization (1972). - Health criteria for the quality of recreational waters with special reference to coastal waters and beaches, W.H.O./W.Poll./72. 10.

THE OCCURRENCE OF INDICATOR MICRO-ORGANISMS IN THE COASTAL MEDITERRANEAN WATER OF ISRAEL ALONG BATHING BEACHES

by

FATTAL B., OLEVSKY-PELEG E., PELEG I.
AGURSKY T. and SHUVAL H.I.
The Environmental Health Laboratory
SchoO of Public Health and Community Medicine
The Hebrew University of Jerusalem Hadassah Medical School
P.O.B. 1172, Jerusalem (Israel)

Abstract

The concentrations of indicator micro-organisms in coastal sea-water of three bathing beaches in Tel-Aviv and the surrounding area were examined. Seventy-eight (78) water samples were taken, and tested within seven hours of sampling. The results show that the concentrations of indicator bacteria at all three beaches were within the allowable range recommended by WHO/UNEP. The bacterial indicators and their log mean concentrations (cfu (colony-forming units) per 100 ml) are as follows: faecal coliform, 46; enterococci (mE medium), 20; faecal streptococci (KF medium), 41; E. coli, 13; and E. coli + Klebsiella, 21. The concentration range of these bacteria at all three beaches was from 0 to 750 cfu/100 ml. High correlation was found between faecal coliform and E. coli ($r = 0.88$) ($p < 0.01$), and the lowest between E. coli + Klebsiella and enterococci ($r = 0.56$) ($p < 0.01$). For faecal coliform and enterococci a comparison was made between those tests performed within seven hours, and those performed after the sambles had been stored for 24 hours at 5 °C. It was found that only faecal coliform showed significant die-away ($p < 0.02$) after 24 hours.

The above results were obtained within the framework of a WHO/UNEP epidemiological study of the relationship between microbial quality of coastal sea-water and morbidity among swimmers.

*
* *

Introduction

The UNEP Long Term Pollution Monitoring and Research Programme, in co-operation with WHO, determined that there was a high priority need for an epidemiological study in order to evaluate the relationship between the bacterial content of coastal sea-water and swimmers' health. Within the framework of this project, three different beaches in Tel-Aviv and its vicinity were tested for various bacterial indicators. This paper presents only the preliminary results of the first part of the study, which describe the concentration levels of the following indicators: faecal coliform (FC), faecal streptococci (FS), enterococci (Ent), E. coli and E. coli + Klebsiella (E. coli + KL) in the sea-water at the three beaches, and the correlation between concentrations of these bacteria. WHO

standards for FC and FS exist, but, to date, no comparison has been made between the five different bacterial indicators (FC, FS, Ent, E. coli and E. coli + K1) for the purpose of establishing uniform standards for any given sea-water sample. For FC and Ent a comparison was made between those tests performed within seven hours after sampling, and those performed after the samples had been stored for 24 hours at 5 °C in order to determine the die-away rates of these bacteria.

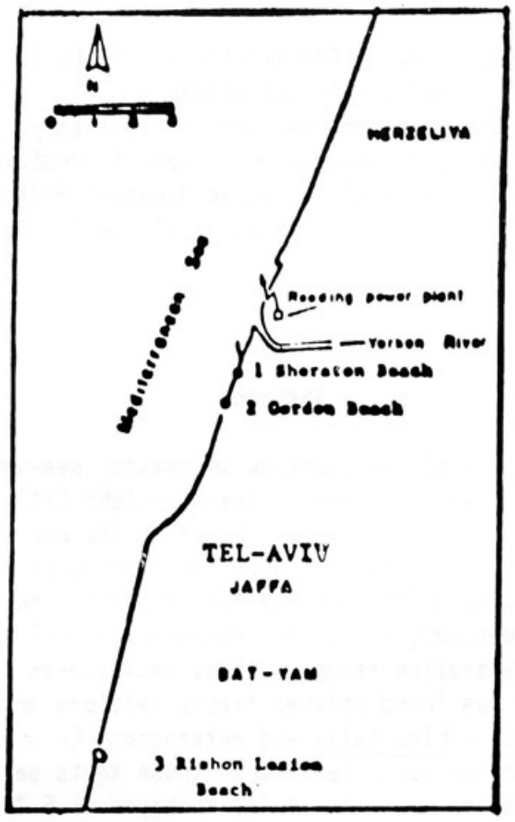


Figure 1. - The area under study in which samples were taken.

Materials and methods

a) Sampling. During the bathing season of May-August 1983, 78 samples were taken from three beaches (see fig. 1). Sheraton and Gordon beaches (Nos. 1 and 2) are located three km south, and Rishon leZion beach (n° 3) about 15 km south of the Reading power plant, where the untreated sewage of Tel-Aviv is discharged into the sea at a point 880 m from the seashore.

Samples were usually taken weekend (Friday-Sunday), generally twice a day: in the morning and in the afternoon. Composite samples (eight times 500 ml each, for a total volume of 4.0 l) were drawn out with a plastic container approximately ten cm below the water surface, in an area where most of the bathers were concentrated.

b) Procedures for bacterial determinations. The FC and FS concentrations were determined with reference methods recommended by WHO 6,7. E. coli concentrations, as well as E. coli + K1, were tested using the mTEC method(DUFOUR and CABELLI, 1975; DUFOUR et al., 1981) and the Ent concentration was determined according to LEVIN et al. 1975.

	Gordon		Sheraton		Rishon leZion		All beaches	
Log mean	117		38		16		46	
Standard deviation	4.2		6.3		4.5		5.9	
Standard error	.14		.24		.17		.44	
Maximum	640		650		169		650	
Minimum	1		1		2		1	
Frequency distribution								
	#	%	#	%	#	%	#	%
0-10	2	7	3	18	8	36	13	19
11-100	7	24	9	53	11	50	27	40
101-1000	20	69	5	29	3	14	28	41
No. of samples	29		17		22		68	

F value (3 beaches) = 10.04 (P=0.002)

Table 1. - Fecal coliforms - water surface (cfu/100 ml).

	Gordon		Sheraton		Rishon leZion		All beaches	
Log mean	67		49		19		41	
Standard deviation	3.3		3.3		3.4		3.7	
Standard error	0.10		0.13		0.13		0.19	
Maximum	500		710		270		710	
Minimum	7		5		3		3	
Frequency Distribution								
	#	%	#	%	#	%	#	%
0-10	2	6	2	9	8	33	12	15
11-100	17	52	13	62	14	59	44	57
101-1000	14	42	6	29	2	8	22	28
Number of samples	33		21		24		78	

F value (3 beaches) = 6.62 (P=0.001)

Table 2. - Fecal streptococci - water surface (cfu/100 ml).

	Gordon		Sheraton		Rishon leZion		All beaches	
Log mean	41		15		10		20	
Standard deviation	3.3		12.0		7.0		6.7	
Standard error	.10		.31		.22		.29	
Maximum	450		410		400		450	
Minimum	1		0		0		0	
Frequency Distribution								
	#	%	#	%	#	%	#	%
0-10	3	9	5	25	12	50	20	26
11-100	25	76	11	55	9	38	45	58
101-1000	5	15	4	20	3	12	12	16
No. of samples	33		20		24		77	

F value (3 beaches) = 4.59 (P = 0.013)

Table 3. - Enterococci - water surface (cfu/1000 ml).

c) Meteorological and sea conditions. No significant differences in pH, water temperature or wave height were found for the three beaches studied. The mean pH was 8.1, and mean water temperature about 26 °C. Wave heights ranged from 65 cm in Gordon, and 67 cm in Sheraton, to 81 cm in Rishon leZion. It should be noted that sampling was performed on different days at the Rishon leZion beach than at the Sheraton and Gordon beaches, whereas samples of the latter two beaches were generally collected on the same day. This explains the fact that the wave heights were almost identical on the Sheraton and Gordon beaches. The wind direction was mainly west, and occasionally north-west on all three beaches.

	Gordon	Sheraton	Rishon leZion	All beaches
Log mean	30 (49)	9 (21)	5 (6)	13 (12)
Standard deviation	3.5 (3.5)	10.6 (4.0)	7.3 (7.7)	7.2 (5.9)
Standard error	.11 (.11)	.16 (.31)	.24 (.23)	.33 (.40)
Maximum	599 (750)	133 (216)	183 (193)	559 (750)
Minimum	0 (0)	1 (1)	0 (0)	0 (0)
Frequency distribution				
	# %	# %	# %	# %
0-10	7 (4) 21 (12)	8 (3) 42 (17)	14 (14) 58 (58)	29 (21) 38 (28)
11-100	22 (20) 67 (61)	8(12) 42 (66)	9 (9) 38 (38)	39 (41) 51 (55)
101-1000	4 (9) 12 (27)	3 (3) 16 (17)	1 (1) 4 (4)	81 (13) 11 (17)
No. of samples	33 (33)	19 (18)	24 (24)	76 (75)

F value (3 beaches) (E.coli) = 6.90 (P = 0.002)
F value (3 beaches) (E.coli+Klebsiella) = 12.17 (P=0.00001)
* shown in parantheses

Table 4. - E. coli and E. coli and Klebsiella* - water surface (cfu/1000 ml).

Results

Tables 1-4 show the log mean, standard deviations and standard error, maximum and minimum cfu/100 ml, the number of samples, and the frequency distribution for FC, FS, Ent, E. coli and E. coli + K1, for each of the three beaches studied, and for all three beaches together. These tables show that the concentrations for all the bacteria were the highest for the Gordon beach when compared with the Sheraton and Rishon leZion beaches. The Rishon leZion beach consistently exhibited the lowest bacterial concentration. The frequency distribution also shows a higher proportion of 101-1,000 cfu/100 ml for the Gordon and Sheraton beaches, as compared to the Rishon leZion beach, for all bacteria. The differences between the bacterial concentrations of the three study beaches were found to be highly significant ($P < 0.01$). However, all the bacterial concentrations found in this study fall within UNEP/WHO standards of water quality.

Table 5 shows the Pearson correlation coefficients, with their significance levels, for all bacterial concentrations at the three beaches together. In every case, the correlations were high, positive and significant at $P < 0.01$. The lowest correlation was found between E. coli + K1 and Ent ($r = .56$), and the highest correlation was between E. coli and E. coli + K1 ($r = .95$) (as expected, since E. coli is included within the E. coli + K1 measurement), as well as between E. coli and FC ($r = .88$)

	Fecal coliform	Fecal streptococci	Entero-cocci	E. coli + Klebsiella
Fecal streptococci	.71 (70)*			
Enterococci	.74 (67)	.80 (76)		
E. coli + Klebsiella	.83 (67)	.62 (76)	.56 (72)	
E. coli	.88 (67)	.65 (75)	.61 (72)	.95 (75)

1 Zero concentrations are omitted from the figures in this table. However, no significant differences were found whether or not 0 concentrations were included
2 The correlations were significant at $p<0.01$ level in all cases
* No. of cases in parentheses

Table 5. - Pearson correlation coefficients matrix between the logarithms of bacteria counts found in all beaches togethers 1, 2.

Table 6 presents a comparison of two tests for bacterial indicators (i.e. FC and Ent). The first test was carried out within seven hours of sampling, and the second after storage for 24 hours at 5° C. This table shows log means, standard deviations and standard errors, minimum and maximum cfu/100 ml, numbers of samples, and percentage of die-away. A T-test demonstrates the significant decrease (at $p < .02$) of the second test performed for FC as compared to the first test (log means of 52 and 76 cfu/100 ml). Thus, the FC die-away was 32 per cent . It is worth mentioning that of the 19 pairs, 15 (79 per cent) showed a decrease of 10 per cent or more in the levels of FC. No significant differences were found between the two tests fo Ent (means 26 and 22 cfu/100 ml), which represent 15 per cent bacterial die-away. However, of the 22 pairs, 13 (59 per cent) showed a decrease of 10 per cent or more in the levels of Ent.

	Fecal coliform tested		Enterococci tested	
	same day	after 24hr	same day	after 24hr
Log mean	76	52(32%)*	26	22(15%)*
Standard deviation	3.8	3.9	3.4	3.3
Standard error	.16	.17	.13	.12
Minimum	3	6	2	2
Maximum	557	694	184	160
No. of samples	19	19	22	22
	T value = 2.6 (P=0.02)		T value = 1.7 (P=0.10)	

* percent of die-away

Table 6. - Comparison of bacterial indicators (cfu/100 ml) tested on the same day of sampling and after being stored 24hr at 5° C.

Conclusions

1. The results of this study, regarding the beaches near Tel-Aviv, correlated well with those of FATTAL et al. (1983) for FC and FS.

2. All bacterial indicators tested at the three beaches were within the UNEP/WHO Interim Guidelines for bathing water quality.

3. Rishon leZion beach, located 15 km south of the Tel-Aviv sewage plant, showed the lowest concentrations of each of the bacterial indicators tested, when compared to the other two beaches, which are situated close to the sewage plant.

4. E. coli was found in the lowest mean concentration of the five bacterial indicators at each of the three beaches, and FC showed the highest mean concentration.

5. A high positive correlation was found between E. coli and FC ($r = 0.88$), $P < 0.01$, and the lowest correlation was found between E. coli + Kl and Ent ($r = 0.56$) $P < 0.01$.

6. FC and Ent concentrations were compared on the same day of sampling, and after storage for 24 hours at 5 °C. It was found that only the FC indicator shows significant die-away ($P < 0.02$) after 24 hours, as compared to the results of sampling after seven hours.

7. At a later stage, we will be able to determine which indicator is best suited for determination of the health risks to swimmers associated with sea-water pollution at bathing beaches. CABELLI (1979) notes that the best indicators for this type of correlation are E. coli and Ent.

Acknowledgements

We wish to express our appreciation and thanks to WHO and the UNEP Coordinated Mediterranean Pollution Monitoring and Research Programme, who have made this study possible. We would also like to thank Ms Ora Grafstein and Miriam Hornoff for their editorial assistance.

References

1. CABELLI V.J. (1979). -Hazards to health associated with swimming in sewage polluted sea-water. Israel Ecological Society, Proc. of 10th Scientific Conference, Sde Boker.
2. DUFOUR A.P. and V.J. CABELLI (1975). - A membrane filter procedure for enumerating the component genera of the coliform group in sea-water. Appl. Microbiol. 29: 826-833.
3. DUFOUR A.P., E.R. STRICKLAND and V.J. CABELLI (1981). - Membrane filter method for enumerating Escherichia coli. Appl. Environ. Microbiol 41(5): 1152-1158.
4. FATTAL B., R.J. VASL, E. KATZENELSON and H.I. SHUVAL (1983). - Survival of bacterial indicator organisms and enteric viruses in the Mediterranean coastal waters off Tel-Aviv. Water Res. 17(4): 397-402.
5. LEVIN M.A., G.R. FISHER and V.J. CABELLI (1975). - Membrane filter technique for enumeration of enterococci in marine waters. Appl. Microbiol. 30: 66-71.
6. WHO - Reference Methods for Marine Pollution Studies n° 2. UNEP. (1982). - Determination of faecal coliforms in sea-water by the membrane filtration culture method.
7. WHO - Reference Methods for Marine Pollution Studies N° 4. UNEP (1982). - Determination of faecal streptococci in sea-water by the membrane filtration culture method.

ANALYSE COMPAREE DE LA RESISTANCE AUX ANTIBIOTIQUES DES ENTEROBACTERIES ISOLEES DE L'EAU ET DU CRABE PACHYGRAPSUS MARMORATUS LE LONG DU LITTORAL DE MONASTIR, TUNISIE

par

A. BAKROUF-FEDHILA (1), M. JEDDI (1)
et M.J. GAUTHIER (2)
(1) Faculté de Médecine Dentaire
Rue F. Bourguiba 5019, Monastir, Tunisie
(2) I.N.S.E.R.M., Unité 40
1, avenue Jean Lorrain, 06300, Nice, France

Résumé

Cette étude a porté sur 385 souches bactériennes appartenant aux entérobactéries isolées de l'eau de mer et du crabe P. marmoratus. La résistance de ces souches a été testée par rapport aux antibiotiques suivants: ampicilline, gentamycine, kanamycine, streptomycine, tétracycline, chloramphénicol et sulfamides forts.

Les résultats montrent que les entérobactéries isolées de l'eau de mer étaient globalement plus résistantes que celles provenant des animaux. Parmi les diverses espèces étudiées, E. coli était la plus fréquente et la plus résistante aux inhibiteurs sélectionnés. Ce résultat est discuté par rapport aux données de la littérature et d'un point de vue sanitaire.

Abstract

385 bacterial strains belonging to Enterobacteriaceae were isolated from sea-water and crabs P. marmoratus collected along the Monastir shore. Their resistance to eight antibiotics was screened. Results showed a higher resistance within the water borne group. E. coli was the most frequent and resistant species. Sanitary aspects of these findings are discussed and compared with litterature data.

*
* *

Introduction

De nombreux travaux ont analysé la résistance aux agents antimicrobiens des bactéries telluriques rejetées en mer par les eaux usées domestiques. Diverses données ont également été publiées concernant la répartition de cette résistance chez ces mêmes bactéries hébergées par des organismes marins, le plus souvent des mollusques lamellibranches utilisés en alimentation humaine.

Le présent travail visait à étudier comparativement l'extension de l'antibio-résistance chez de nombreuses souches isolées de l'eau de mer et de crabes vivant dans cette eau, au niveau de stations contaminées par des effluents domestiques, dont une partie provenait d'installations hospitalières.

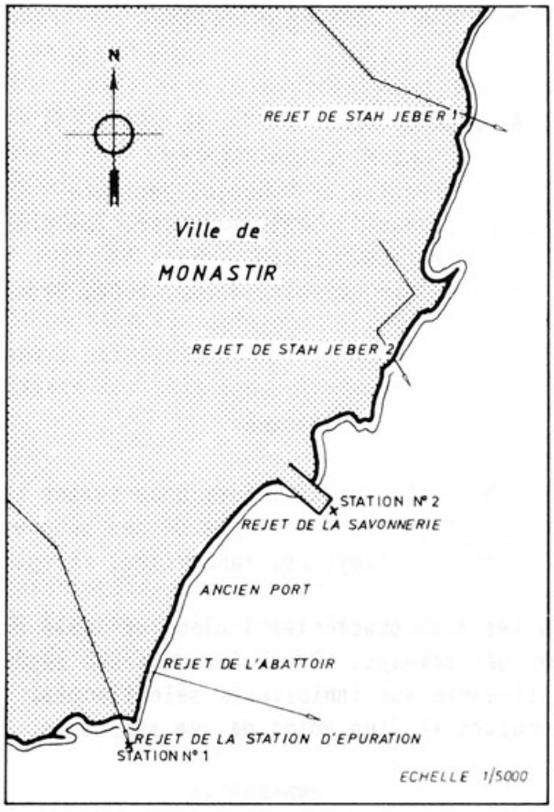


Figure 1
Zone d'étude et position des stations
de prélèvement d'eau et de crabes

Matériel et méthodes

Zone d'étude et stations de prélèvements :

Ce travail a été réalisé sur le littoral de Monastir, ville tunisienne située au bord de la Méditerranée. La côte Sud-Est de cette ville est basse et reçoit les rejets de la plupart des canaux d'eaux usées ; elle présente donc une forte concentration en matières organiques et en microorganismes.

Les prélèvements (crabes et eau) ont été faits à dix mètres du canal de la station d'épuration vers le large (station n° 1) et à dix mètres au large du débouché du canal de la savonnerie (station n° 2) (Fig. 1).

Analyses bactériologiques :

Les isolements de souches ont été réalisés à partir d'échantillons d'eau et de broyats de crabes.

L'ensemencement a été fait sur gélose lactosée au bromo-crésol pourpre. Les colonies présentant des caractères d'entérobactéries ont été isolées et identifiées par les méthodes traditionnelles. L'antibiogramme a été réalisé sur milieu de Mueller-Hinton, à l'aide de disques préchargés des antibiotiques suivants (Institut Pasteur Productions) : ampicilline (A, 25 ug/disque), streptomycine (S, 30 ug), kanamycine (K, 30 ug), gentamycine (G, 30 ug), chloramphénicol (C, 30 ug), tétracycline (T, 30 ug) et sulfamides forts (SU, 200 ug). La lecture de ces tests a été faite après 24 heures d'incubation à 37° C.

Résultats

Les résultats des tests d'identification, donnés pour 100 nl d'eau ou 10 nl de broyat de crabe, sont présentés dans le tableau n° 1.

La fréquence des caractères de résistance à un ou plusieurs antibiotiques, pour l'ensemble des souches isolées au cours de ce travail, figure dans le tableau n° 2.

Les tableaux n° 3, 4, 5, 6, 7, et 8 présentent les résultats de l'analyse des caractères de résistance aux sept antibiotiques sélectionnés, des souches identifiées aux genres ou espèces suivants (respectivement) : E. coli, Klebsiella, Proteus mirabilis, Proteus morgani, Citrobacter et Enterobacter.

Discussion

Sur les 385 souches d'entérobactéries testées, plus des deux tiers (72 %) se sont avérées résitantes à un antibiotique ou plus. Ces souches présentaient cependant une multirésistance moins importante que celles qui ont été isolées de matières fécales au cours d'une enquête faite en Tunisie par ZRIBI (1978) en milieu rural. En effet, 40 % des souches isolées de l'eau et 52 % de celles qui provenaient de crabes ont montré une résistance à un antibiotique au moins (Tableau n°2), alors que 64 % des souches isolées au cours de l'enquête en question présentaient une telle résistance à ces mêmes antibiotiques.

Par ailleurs, la résistance des souches isolées au cours de ce travail variait également selon le genre et dépendait de la nature de l'antibiotique considéré.

Ainsi, les souches appartenant à l'espèce E. coli (Tableaux n° 1 et 9) étaient à la fois les plus abondantes et les plus résitantes. Une telle conclusion a déjà été apportée par d'autres auteurs, lors d'études portant sur la résistance des entérobactéries présentes dans les eaux usées, les eaux marines et les coquillages (BORDERON et BORDERON, 1974 ; COOKE, 1976 ; THIRKELL et BLANKSON, 1981). Les Klebsiella, bien que moins abondantes, étaient aussi résitantes que E. coli. Les autres entérobactéries étaient sensibles ou résistantes à un antibiotique seulement, dans la plupart des cas.

Si l'on considère la répartition de la résistance des souches selon les antibiotiques (Tableau n° 10), on constate une plus forte résistance pour la tétracycline et l'ampicilline, ce qui est en accord avec les observations de ZRIBI pour la flore intestinale humaine locale. Aucune résistance n'a été observée pour la kanamycine. La résistance au chloramphénicol et aux sulfamides forts était toujours associée à une autre résistance dans le cas de E. coli, mais elle pouvait être simple pour les autres genres ou espèces.

Quinze antibiotypes différents ont pu être isolés, dont 4 présentaient une résistance simple, 5 une résistance à 2 antibiotiques, 5 une résistance à 3 antibiotiques, 2 une résistance à 4 antibiotiques et 1 seul une résistance à 5 antibiotiques.

Station n°	Genres ou espèces						
	E. coli	*Klebsiella*	*Citro-bacter*	*Entero-bacter*	*Proteus morgani*	*Proteus mirabilis*	Total
1 -eau	27	36	2	4	0	1	70
2 -eau	70	21	1	0	2	6	100
1 -crabe	60	12	3	1	6	13	95
2 -crabe	97	9	6	2	5	1	120
Total	254	78	12	7	13	21	385

Tableau n° 1. - Nombre de souches d'entérobactéries isolées à partir de chaque prélèvement, classées par genres ou espèces

Nombre de souches résistantes à :	Dans l'eau		Dans les crabes	
	nombre	%	nombre	%
1 antibiotique	68	40	111	51
2 antibiotiques	14	8	52	24
3 antibiotiques	15	8	4	1
4 antibiotiques	8	4	0	0
5 antibiotiques	4	2	0	0
Total des résistants	109	64 %	167	76 %

Tableau n° 2. - Fréquence de la résistance à un ou plusieurs antibiotiques de l'ensemble des souches isolées, sans distinction taxonomique

Antibiotiques	Eaux		Crabes	
	Station 1	Station 2	Station 1	Station 2
T	5	12	10	21
A	3	11	9	21
S	3	8	9	19
A + T	2	4	8	15
S + A	1	4	6	14
S + A + T	2	4	1	1
SU + A + T	1	3	1	0
SU + T + C	0	2	0	0
S + SU + T	0	2	0	1
A + S + C + T	1	2	0	0
A + S + SU + T	2	2	0	0
A + T + S + SU + C	1	1	0	0
Total résistants	21 (80%)	55 (78%)	44 (73%)	92 (94%)
Total sensibles	6 (20%)	15 (22%)	16 (27%)	5 (6%)

Tableau n° 3. - Répartition des souches d'*E. coli* selon leur résistance à un ou plusieurs des antibiotiques sélectionnés

L'un des aspects les plus intéressants de ces résultats paraît être la différence entre le nombre des souches résistantes dans l'eau et les crabes, et leur polyrésistance. En effet, les souches résistantes isolées de l'eau étaient moins nombreuses que celles qui provenaient des crabes, mais elles présentaient une polyrésistance plus étendue. Ceci est en accord avec les observations de NIEMI et coll. (1983), selon lesquelles la fréquence de la polyrésistance des bactéries de différen-

tes eaux naturelles n'était pas toujours élevée dans les mêmes échantillons où la résistance était elle-même importante. En outre, la résistance des E. coli isolés au cours de ce travail était plus forte que celle des autres entérobactéries, contrairement aux données de BEN REJEB (1977) qui montraient une plus faible résistance de cette espèce par rapport aux autres représentants du groupe, dans une population isolée en milieu hospitalier local.

Il semble donc y avoir un certain remaniement de la résistance globale de la population bactérienne entérique aux antibiotiques, du milieu tellurique au milieu marin, puis de celui-ci aux

Antibiotiques	Orgine des souches			
	eau-station 1	eau-station 2	crabe-station 1	crabe-station 2
A	11	9	0	5
A + T	2	3	2	1
A + G	0	1	0	0
A + SU	0	3	0	0
A + S	1	1	1	2
A + C	1	2	1	1
A + C + G	0	1	0	0
A + T + S + G + SU	0	1	0	0
Total résistants	15 (40%)	21 (100%)	4 (30%)	9 (10%)
Total sensibles	21 (60%)	0 (0%)	8 (70%)	0 (0%)

Tableau n° 4. - Répartition des souches appartenant au genre Klebsiella selon leur résistance à un ou plusieurs antibiotiques

Antibiotiques	Origine des souches			
	eau-station	eau-station 2	crabes-station 1	crabe-station 2
T	1	2	4	0
A + T + C + S + SU	0	1	0	0
Total résistants	1 (100%)	3 (50%)	4 (17%)	0 (0%)
Total sensibles	0 (0%)	3 (50%)	9 (83%)	1 (100%)

Tableau n° 5. - Répartition des souches appartenant à l'espèce Proteus mirabilis selon leur résistance à un ou plusieurs antibiotiques

Antibiotiques	Origine des souches			
	eau-station 1	eau-station 2	crabe-station 1	crabe-station 2
T	0	0	2	0
S	0	1	0	0
SU	0	0	1	0
Total résistants	0	1 (50%)	3 (50%)	0 (0%)
Total sensibles	0	1 (50%)	3 (50%)	5 (100%)

Tableau n° 6. - Répartition des souches appartenant à l'espèce Proteus morgani selon leur résistance à un ou plusieurs antibiotiques

Antibiotiques	Origine des souches			
	eau-station 1	eau-station 2	crabe-station 1	crabe-station 2
A	1	0	1	1
T	0	0	0	1
A + S	0	0	0	1
Total résistants	1 (50%)	0 (0%)	1 (30%)	3 (50%)
Total sensibles	1 (50%)	1 (100%)	2 (70%)	3 (50%)

Tableau n° 7. - Répartition des souches appartenant au genre Citrobacter selon leur résistance à un ou plusieurs antibiotiques

organismes qui le peuplent. La différence qui a été observée entre les souches d'E. coli provenant de l'eau de mer et celles qui ont été isolées en milieu hospitalier local peut vraisemblablement s'expliquer par une variabilité temporelle de cette résistance (les deux études n'ayant pas été réalisées

Antibiotiques	Origine des souches			
	eau-station 1	eau-station 2	crabe-station 1	crabe-station 2
A	1	0	0	1
S	0	0	0	1
Total résistants	1 (25%)	0	0	2 (100%)
Total sensibles	3 (75%)	0	1 (100%)	0 (0%)

Tableau n° 8. - Répartition des souches appartenant au genre Enterobacter selon leur résistance à un ou plusieurs antibiotiques

simultanément) et par la diversité des sources de contamination bactérienne, une partie des entérobactéries présentes dans les eaux usées n'ayant pas une origine humaine. Une augmentation de la résistance à l'ampicilline chez E. coli de l'amont vers l'aval d'un émissaire d'eaux usées a été décrite par THIRKELL et BLANKSON (1981). Par contre, la modification quantitative et qualitative de la résistance des souches entériques entre l'eau et les crabes suggère une évolution du pool des marqueurs de résistance au cours du transit de ces germes en mer et dans les organismes animaux,

Espèce ou genre	E.coli	Klebsiella	Enterobacter	Citrobacter	Proteus morgani	Proteus mirabilis
% résistants	76	75	43	22	33	26

Tableau n° 9. - Répartition des pourcentages de résistance à 1 antibiotique au moins selon les genres ou espèces

ainsi qu'une sélection des souches dans ce milieu biologique. On sait que les entérobactéries peuvent transférer leurs plasmides de résistance aux antibiotiques par conjugaison "in situ" (ALTHERR et KASWECK, 1982). Par ailleurs, en accord avec MORGAN et coll. (1976), ces résultats montrent que

Origine des souches :	Antibiotiques						
	T	A	SU	S	C	K	G
eaux	66 (38%)	84 (50%)	27 (15%)	43 (25%)	18 (10%)	0	1 (0,5%)
crabes	88 (41%)	91 (42%)	3 (1%)	52 (22%)	2 (1%)	0	0
Total	154(40%)	175(46%)	30 (7%)	95 (24%)	20 (6%)	0	1 (0,2%)

Tableau n° 10. - Nombre et pourcentage des souches résistantes à chacun des antibiotiques testés (résistance simple ou croisée).

les organismes marins contaminés peuvent modifier qualitativement la dissémination de l'antibiorésistance bactérienne du milieu marin à l'homme, en hébergeant une microflore dont les caractères de résistance peuvent être sensiblement différents de ceux de la flore ambiante.

Références

ALTHERR R.M. et K.L. KASWECK (1982). - In situ studies with membrane diffusion chambers of antibiotic resistance transfer in Escherichia coli. Appl. Environ. Microbiol., 44 : 838-843.

BEN REJED (1977). - Etude de la résistance aux antibiotiques de différentes souches bactériennes isolées à l'hôpital Charles Nicolle en 1975 et 1976. Thèse de Médecine, Tunis.

BORDERON E. et J.C. BORDERON (1974). - Etude de la proportion d'E. coli résistants aux antibiotiques des eaux d'adduction d'Indre et Loire. Med. Mal. Infect., 4 : 545-549.

COOKE M.B. (1976). - Antibiotic resistance among coliform and fecal coliform bacteria isolated from sewage, sea-water and marine shellfish. Antimicrob. Agents Chemother., 9 : 879-884.

MORGAN R.C., P. GUERRY et R.R. COLWELL (1976). - Antibiotic resistant bacteria in Chesapeake Bay. Chesapeake Sci., 17 : 216-219.

NIEMI M., M. SIBAKOV et S. NIEMELA (1983). - Antibiotic resistance among different species of fecal coliforms isolated from water samples. Appl. Environ. Microbiol., 45 : 79-83.

THIRKELL D. et M. BLANKSON (1981). - The speciation of coliform genera from above and below a sewer outfall and their susceptibility to antimicrobial agents. Ant. van Leeuw, 47 : 133-145.

ZRIBI M. (1978). - Résistance aux antibiotiques des entérobactéries de la flore intestinale du nomade à Nasrallah (Tunisie). Thèse de doctorat, Université Paul Sabatier, Toulouse.

ETUDE SUR LES FACTEURS REGISSANT LA QUALITE SANITAIRE ET LA SURVIE DES CLOVISSES (PALOURDES) TAPES DECUSSATUS DESTINEES A L'EXPORTATION

par

S. JEKOV et A. CHADLI
Institut Pasteur de Tunis
13, place Pasteur - Tunis - Belvédère, B.P. 74 (Tunisie)

Résumé

Une surveillance continue de la qualité bactériologique de l'eau de mer et des Clovisses "prêtes à exporter" est menée au parc conchylicole de Thyna-Sfax.

Simultanément sont effectuées des investigations portant sur certains paramètres physiques, propres au milieu marin (température, oxygène dissous), sur la dynamique de la mer, afin d'élucider dans un aspect écologique leurs répercussions sur la survie des Clovisses, après qu'elles soient retirées des plans d'eau de stabulation.

Des recommandations destinées à protéger cette survie sont élaborées. De nouvelles techniques d'épuration sont également mises au point et dûment expérimentées au parc conchylicole de Thyna.

*
* *

Dans le cadre du Plan d'Action pour la Méditerranée/PNUE nous avons entrepris la surveillance de la qualité sanitaire des eaux des parcs conchylicoles de Tunisie, dont la production est destinée à l'exportation. Parallèlement, nous avons procédé à l'échantillonnage des livraisons de Clovisses vivantes au moment de leur présentation à la douane de l'aéroport de Tunis, afin d'établir leur conformité aux normes bactériologiques en vigueur dans les pays importateurs.

Nous avons également observé les méthodes d'épuration et de conditionnement utilisées au parc de Thyna, rendant possible l'exportation des Clovisses même pendant les mois les plus chauds de l'année.

Ces méthodes de type empirique, résultats d'observations et d'épreuve effectuées pendant trois décennies, étaient conçues et appliquées par Ali MESGHANI, le gérant du parc.

Matériel et méthodes

De janvier 1983 à juin 1984, nous avons prélevé mensuellement 350 échantillons d'eau de mer, couvrant une bordure littorale de 15 km, au sud de Sfax, englobant les plans d'eau de stabulation de 95 % des Clovisses exportées.

Ces échantillons ont été soumis à la détermination du NPP/100 ml des Coliformes totaux, des Escherichia coli, des Stréptocoques fécaux, du NTG/ml/37° C.

Du 20 avril 1983 à mai 1984, nous avons examiné 396 échantillons de coquillages dont 242 étaient prélevés sur des livraisons destinées à l'Italie et constituées de 1.000 à 2.300 kg de Clovisses vivantes, provenant du parc de Thyna. Leur analyse comportait outre les paramètres sus-mentionnés, la recherche des germes pathogènes.

Les résultats concernant les eaux sont présentés au tableau 1, ceux portant les Clovisses au tableau 2 (la recherche de germes pathogènes s'est avérée négative).

Tableau 1.
Nombre le plus probable de coliformes

COLIFORMES TOTAUX		ESCHERICHIA COLI	
Nb échantillons	NPP 100 ml	Nb échantillons	NPP/100 ml
325	<2	342	<2
8	2	7	2
6	4	1	4
5	9		
4	23		
1	43		
1	75		

Discussions

En ce qui concerne la présence d' E. Coli, ces résultats révèlent que :

- l'eau de mer est constament propre à la stabulation des Clovisses.
- 95,04 % des Clovisses examinées sont propres à la vente directe aux consommateurs et 4,95 % après une épuration obligatoire. La législation italienne (Leggi 27 Aprile, 1978) admet l'importation de Clovisses présentant un NPP limité à 39 E. Coli, donc 100 % des livraisons de Clovisses exportées étaient conformes à la loi.

Tableau 2
Nombre le plus probable d'Escherichia Coli
par gramme de broyat de Clovisses

Nb échantillons	NPP/g	Nb échantillons	NPP/g	Nb échantillons	NPP/g
98	<0,2	1	1,2	5	3,3
46	0,2	3	1,3	1	4,9
7	0,4	1	1,4	1	7
22	0,5	4	1,7	4	7,9
1	0,6	0	2,1	1	9,4
3	0,7	3	2,2	1	18
17	0,8	7	2,3	2	22
1	0,9	0	2,6	1	24
10	1,1	1	2,7	1	35

Les Clovisses se vendent vivantes : la protection de leur survie est le principe qui régit tous les procédés de leur production et de leur commercialisation.

Les conditions de vie, propres au milieu d'épuration, différent de celles des niches écologiques. Les Clovisses sont privées de la vase et du sable du fond de la mer. Progressivement elles maigrissent et si la température de l'eau est élevée et l'oxygène dissous réduit, cet état du milieu se traduit par une baisse de la résistance vitale. De telles Clovisses lors du conditionnement et d'un transport prolongé manifestent déjà différents taux de mortalité indicatifs de leur capacité vitale.

Afin de réduire les facteurs défavorables à la survie des Clovisses propres au milieu d'épuration, nous avons modifié certains procédés de la pratique traditionnelle pour la préparation des Clovisses à exporter. Au lieu de suspendre dans l'eau de mer les sacs contenant les Clovisses à épurer accrochés part des cordes fixées aux pilons de "tables" immobiles des parcs de stabulation, selon notre procédé, les sacs immergés dans l'eau sont accrochés sur les bords d'un radeau spécialement conçu, qu'on peut disloquer comme une "table mobile" et ancrer dans des endroits des plans d'eau où la température, l'oxygène dissous, la présence de courant d'eau et d'autres facteurs hydrobiologiques, préalablement étudiés se sont avérés les plus propices à la survie des Clovisses.

Sur un autre radeau "atelier" les Clovisses apportées des comptoirs sont triées, lavées abondamment (en profitant de l'eau pure du large) placées dans les sacs et transférées ultérieurement sur le radeau "table mobile" afin d'être immédiatement immergées dans les plans d'eau de stabulation.

Il existe encore au parc de Thyna un secteur où les Clovisses ne sont pas récoltées. Ce secteur joue le rôle, peut-être d'un couvoir traversé par un courant d'eau de mer qui côtoie la berge vers le sud. Ce courant emporte les naissains des Clovisses vers de nouveaux gîtes dans de meilleures conditions naturelles.

Rappelons que dans le parc de Thyna, dès 1943, les petites Clovisses, séparées lors du triage, sont réimplantées dans des secteurs repérés du parc. Une grande partie des Clovisses s'adapte aux nouveaux gîtes et dans l'espace de 2 ans grossissent et atteignent des dimensions permettant leur récolte.

Cette pratique peut être considérée comme un élevage de Clovisses qui précède de quelques décennies ce qu'on est en train d'expérimenter, ces dernières années. dans d'autres pays producteurs de Clovisses.

RESULTATS PRELIMINAIRES DU CONTROLE DE L'APPORT DE QUELQUES POLLUANTS DANS LA MER MEDITERRANEE

par

H. ABOUZAID*, et A. FOUTLANE**
* Laboratoire de Contrôle de Qualité des Eaux
Office National de l'Eau Potable
Rabat (Maroc)
** Service de Contrôle de Pollution
Laboratoire de Contrôle de Qualité des Eaux
Office National de l'Eau Potable
Rabat (Maroc)

Introduction

Dans le cadre du programme à long terme de surveillance continue et de recherche sur la pollution de la Méditerranée Phase II, le Laboratoire Central de l'Office National de l'Eau Potable, contrôle l'apport de quelques polluants amenés en Méditerranée, sur la côte Nord du Maroc, par les deux bassins versants de la Moulouya et du Rio Martil. Le contrôle intervient au niveau des deux stations d'échantillonnage situées à proximité de l'embouchure des Oueds Moulouya et Martil dans la Méditerranée. La station sur l'Oued Moulouya se trouve en aval de la station hydrométrique de Méchra Safsaf et après confluence avec l'Oued Zegzel (station n° 2). Celle sur l'Oued Martil se situe à l'aval immédiat de la ville de Tétouan et de la station hydrométrique de Toretta (station n° 1).

Matériel et méthodes

Les échantillons sont effectués avec une fréquence mensuelle. Le prélèvement, le transfert et la conservation des échantillons destinés à l'examen bactériologique ainsi que les techniques utilisées pour la recherche et la numération des germes indicateurs de pollution fécale sont conformes aux recommandations de l'O.M.S. relatives à la méthode de la fermentation en tubes multiples pour les coliformes Escherichia coli et les streptocoques fécaux et à la méthode du milieu solide pour les clostridium sulfito réducteurs (1).

Les déterminations des paramètres DCO, DBO5, MES azote Kjeldahl et phosphore total sont effectuées selon les méthodes décrites dans les "Standard Methods for the examination of water and waste water" (2). Les détergents anioniques sont dosés par la méthode du bleu de méthylène.

Le dosage des métaux lourds (Zinc, Cadmium, Mercure, Plomb et Chrome) est fait par la technique d'absorption atomique.

Enfin les débits rapportés correspondent à des valeurs ponctuelles.

Résultats et discussion

Analyses bactériologiques

Les résultats d'analyse des paramètres de qualité bactériologique de l'eau au niveau des deux stations d'échantillonnage sont illustrés par le tableau 1.

Paramètres / Station	Date de prélèvement	Coliformes totaux	Escherichia Coli	Streptocoques fécaux	Clostridium sulfito-réducteur
Station 1 M. Tetouan.	17.6.83	$)1,1\ 10^3$	$)1,1\ 10^3$	0	-
	22.7.83	$4,6\ 10^3$	$1,5\ 10^3$	$9,0\ 10^1$	$)10^2$
	26.8.83	$2,4\ 10^3$	$2,4\ 10^3$	$4,0\ 10^1$	$)10^2$
	27.9.83	$)1,1\ 10^7$	$)1,1\ 10^7$	$2,4\ 10^5$	$)10^2$
	13.10.83	$)1,1\ 10^5$	$)1,1\ 10^5$	$4,3\ 10^4$	$)10^2$
	9.11.83	$1,1\ 10^7$	$2,4\ 10^6$	$7,5\ 10^4$	0
	8.12.83	$)1,1\ 10^6$	$4,6\ 10^4$	$1,1\ 10^5$	$)10^2$
	10.1.84	$4,6\ 10^5$	$4,6\ 10^5$	$1,5\ 10^5$	$)10^2$
	7.2.84	$)1,1\ 10^7$	$)1,1\ 10^7$	$1,1\ 10^5$	$)10^2$
	8.3.84	$)1,1\ 10^7$	$)1,1\ 10^7$	$)1,1\ 10^7$	$)10^2$
	11.4.84	$)1,1\ 10^5$	0	0	$)10^2$
	3.5.84	$2,4\ 10^4$	$2,4\ 10^4$	$2,4\ 10^4$	0
Station 2 M. Berkane.	7.7.83	$)1,1\ 10^3$	$4,6\ 10^2$	$7,5\ 10^1$	$)10^2$
	31.8.83	$1,1\ 10^4$	$1,1\ 10^4$	$4,3\ 10^2$	$)10^2$
	15.9.83	$4,6\ 10^3$	$2,4\ 10^3$	1	$)10^2$
	21.10.83	0	0	$2,3\ 10^1$	0
	29.11.83	$4,6\ 10^3$	$4,6\ 10^3$	$1,2\ 10^3$	0
	27.12.83	$2,4\ 10^3$	$2,4\ 10^3$	$2,8\ 10^2$	0
	25.1.84	$4,6\ 10^3$	$1,1\ 10^3$	$4,6\ 10^2$	$2,5\ 10^2$
	29.2.84	$2,4\ 10^4$	$1,1\ 10^3$	$2,4\ 10^2$	0
	26.4.84	$2,4\ 10^3$	$2,4\ 10^3$	$2,4\ 10^2$	0

Tableau 1
Résultats des paramètres
de qualité bactériologique
dans 100 ml d'échantillon.

L'évolution des résultats des germes indicateurs de pollution fécale montre des variations intenses dans le temps au niveau des deux stations avec une acuité relative du degré de pollution au point situé en aval du bassin Rio Martil. Dans la station 1, il ressort que les concentrations en coliformes totaux varient de $2,4.10^3$ à des teneurs supérieures à 10^7 germes dans 100 ml d'échantillon. L'abondance de coliformes totaux et d'Escherichia Coli non quantifiée (avec le signe supérieur), malgré l'importance des degrés de dilution effectués, rend peu interprétables les résultats obtenus.

Ce degré élevé de pollution observé dans la station 1 peut être attribué au rejet des eaux usées domestiques de la ville de Tetouan directement dans l'Oued Martil.

A l'instar des germes coliformes totaux et d'Escherichia Coli les streptocoques fécaux sont beaucoup plus abondants dans la station 1 que dans la station 2 ; c'est ainsi qu'au niveau de la station 1, les concentrations en streptocoques fécaux varient de 0 à des teneurs supérieures à 10^5 germes/100ml d'échantillon tandis qu'au niveau de la station 2 l'intervalle correspondant est de 1 à 10^3 germes/100 ml.

L'abondance des germes anaérobies sporulés - Clostridium-sulfito- réducteurs a rendu difficile

leur détermination quantitative au niveau des 2 stations, après 24 heures d'incubation, en effet, tout le milieu de culture vire au noir par suite de la formation abondante de sulfure de fer dans les tubes.

Analyses physico-chimiques

Au moment de la rédaction de ce rapport, les débits moyens quotidiens nous permettant de déterminer les apports solides journaliers au niveau des 2 stations, n'étaient pas disponibles. Aussi nous limiterons-nous dans ce cadre, à déterminer les flux instantanés pour quelques paramètres de qualité par le biais des débits ponctuels qui nous sont communiqués par l'Administration de l'Hydraulique. Ces déterminations font l'objet du tableau 2.

	FLUX INSTATANES EN g/s											
	Date de prélèvement	Débit instantané m3/s	DBO_5	Oxydabilité (DCO)	N.Kjeldahl	PO_4 total	M.E.S totales	Zn	Cd	Pb	Hg	Cr
Station 1 Ma - Tetouan.	17.6.83	0,345	1,0	-	1,4	0,90	5,5	0	0	0,002	-	0
	26.8.83	0,180	3,6	(5,2)*	0,65	0,57	8,8	0	0	0	0	0
	27.9.83	0,147	2,2	(3,5)*	0,58	0,77	7,5	0,01	0	0,001	0	0
	13.10.83	0,147	-	0,40	0,60	-	-	0,02	0	0,001	0	0
	9.11.83	0,506	3,0	3,4	0,93	2,1	310	0	0	0,004	0	0,002
	8.12.83	1,740	64	(97)*	-	-	-	0,09	0,004	0,012	0,004	0,006
	10.1.84	13,89	83	(140)*	15	10,3	1000	0,28	0,03	0,06	0,03	0,11
	7.2.84	3,695	19	11	6,5	6,5	33	-	-	-	-	-
	8.3.84	5,084	25	20	7,9	4,3	50	-	-	-	-	-
	11.4.84	5,524	14	-	5,4	3,0	-	-	-	-	-	-
Station 2 M - Berkane.	17.7.83	5,395	6,5	17	6,2	-	183	-	-	-	-	-
	31.8.83	5,695	23	19	3,6	1,1	220	0,57	0,005	0,057	-	0,012
	15.9.83	5,678	12	23	3,5	0,91	182	0,51	0,004	0,054	-	0,010
	21.10.83	5,731	31	17	9,9	-	230	-	0,023	0	-	0,014
	29.11.83	5,695	29	14	0,91	0,97	274	0,60	0,001	0,024	0,001	0,030
	27.12.83	5,661	14	7,9	-	-	74	0	0,001	0,010	0,005	0,006
	25.1.84	5,645	23	58	9,2	1,1	310	0,20	0,014	0,017	0,003	0,034
	29.2.84	5,70	2,8	14,2	1,9	0,40	380	0,30	0,018	0,025	0,005	0,043
	26.4.84	5,695	40	16	2,0	1,1	125	0,23	-	-	-	-

* Ces valeurs correspondent à la DCO.

Tableau 2

Flux instantanés de certains paramètres de qualité durant la période d'observation.

Notons d'emblée que les flux instantanés des matières dissoutes et en suspension sont très faibles pour les deux stations pendant toute la période d'observation.

L'évolution de la température du pH et de l'oxygène dissous au niveau des 2 stations fait l'objet de la figure 1.

Les températures de l'eau dans les 2 stations restent voisines : elles varient de 20 à 27°C à l'étiage et atteignent 14°C durant l'hiver.

Les valeurs de pH enregistrées sont également voisines pour les deux points : elles se situent dans l'intervalle 7,5 à 8,5.

Les résultats d'analyse d'oxygène dissous montrent par contre une différence nette entre les 2 stations, à la station 1 les teneurs sont faibles : les valeurs relevées durant 1983 et début 1984 sont au-dessous de 5 mg/l, ces faibles valeurs sont probablement dues à l'influence des eaux usées de la ville de Tétouan qui atteignent directement l'Oued Martil tandis que les taux enregistrés pendant le printemps 1984 sont acceptables (5 à 9 mg/l). Cette meilleure oxygénation pourrait être due à l'effet des pluies tombées dans la région pendant la même période (débit de l'Oued plus élevé).

A la station 2, les eaux sont mieux oxygénées avec des valeurs situées dans l'intervalle de 7 à 9 mg/l.

L'évolution des paramètres d'intérêt biologique DBO5 et DCO montre des variations relativement faibles dans le temps. Au niveau des 2 stations 1 et 2, si l'on excepte les valeurs 20, 15 et 40 mg/l de DBO5 enregistrées respectivement durant les mois d'août, septembre et décembre 1983 au point n° 1, la teneur des matières organiques biodégradables est faible, les DBO5 étant inférieures à 5 mg/l. Au

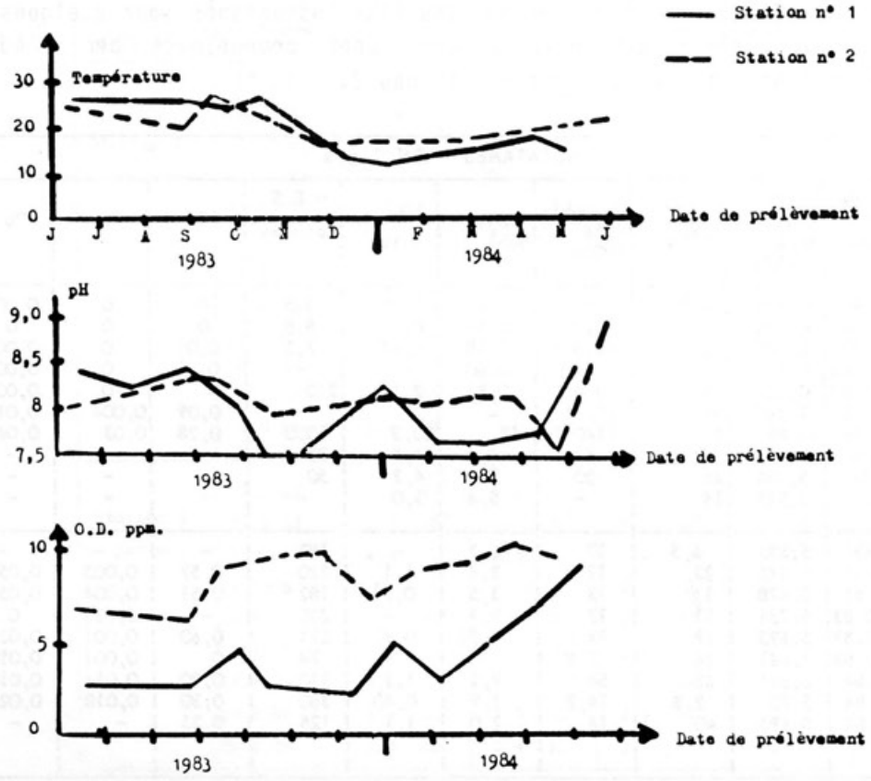


Fig. 1. -Evolution de la température du pH et de l'oxygène dissous dans les stations 1 et 2 entre juin 1983 et mai 1984.

niveau de la DCO, la différence est cependant sensible entre les deux stations de prélèvements. A la station 2 la totalité des échantillons prélevés accuse des valeurs d'oxydabilité inférieures à 5 mg/l d'oxygène consommé avec une moyenne de 3mg/l. Au niveau de la station 1, les valeurs des oxydabilités obtenues oscillent entre 2 et 7 mg/l avec une moyenne de 3 mg/l en oxygène consommé, mais dans 45 % des échantillons (les plus chargés en matières organiques) la technique du bichromate est utilisée pour l'évaluation des matières organiques.

Les valeurs mesurées de DCO varient entre 10 et 56 mg/l avec une moyenne de 30 mg/l d'oxygène.

Au point 1 les matières en suspension totales varient entre 10 et 60 mg/l avec des valeurs exceptionnelles de 600 et 300 mg/l après les pluies de novembre 1983 et mai 1984. Les valeurs trouvées à la station 2, se situent entre 5 et 70 mg/l. Ainsi les apports instantanés en matières solides engendrés par le bassin versant du Rio Martil sont disparates ; ils varient entre 5 et 1000 g/s.

Au niveau du bassin Moulouya, ces apports sont assez réguliers, ils oscillent entre 180 et 300 g/s de matières en suspension.

Les éléments nutritifs montrent des différences sensibles au niveau des 2 stations. Dans la station 1, les concentrations de phosphore total varient entre 0,5 et 5,2 mg/l de PO_4 avec une

moyenne de 2,1 mg/l. L'intervalle de variation des teneurs en azote Kjeldahl (total) au même point est de 0,8 à 4 mg/l de N avec une moyenne de 2,4 mg/l. Au point 2, les quantités des éléments nutritifs sont relativement faibles ; les phosphates totaux accusent des quantités stables voisines de 0,2 mg/l. L'azote varie entre 0,1 et 1,7 mg/l N avec une moyenne de 0,8 mg/l. Les charges instantanées les plus importantes en phosphates totaux proviennent de l'Oued Martil où les résultats enregistrés montrent des valeurs comprises entre 0,60 et 10 g/s en PO_4. La grille des charges dans le cas de la Moulouya est de 0,40 à 1 g/s de PO_4.

Les flux relatifs à l'azote total montrent un comportement similaire à celui du phosphore total. Les charges enregistrées au niveau de la station 1 dépassant celles du point 2 : c'est ainsi que les apports au point 1 varient entre 0,60 et 15 g/s d'azote total en N. Au point 2, le domaine de variation des apports en azote est de 0,9 - 10 g/s en N.

Il ressort de la détermination des détergents anioniques que ces composés sont présents en quantités relativement uniformes au niveau de la station 2, les teneurs variant de 0 à 0,75 mg/l. Par contre, dans la station 1 les résultats sont disparates ; ils varient de 0,2 à 8 mg/l. Enfin, les teneurs des cinq métaux (Zn, Cd, Hg, Pb, Cr) déterminées dans ce cadre sont très faibles pour les 2 stations. Les quatres métaux (Cd, Hg, Pb, Cr) montrent des concentrations voisines d'1 µg/l. Par contre, l'élément zinc est présent à des quantités variables de 0 à 100 µg/l. De ces concentrations instantanées résultent des apports voisins du milligramme/s pour les quatre éléments (Cd, Hg, Pb, Cr) et inférieurs à 600 mg/s pour l'élément zinc au niveau des 2 points de prélèvement.

Conclusion

Durant la campagne de prélèvement pour analyses, il s'est avéré que des points de vue bactériologique et biologique, les eaux de la station d'échantillonnage sur l'Oued Martil sont relativement plus polluées que celles du point de prélèvement situé en aval du bassin de l'Oued Moulouya. L'apport en éléments nutritifs engendré par le bassin du Rio Martil est également plus intense. Néanmoins les résultats d'analyses montrent qu'au moment des prélèvements il n'existe pas de pollution perceptible due aux micropolluants inorganiques contrôlés.

Des études ultérieures permettront de mieux suivre l'évolution de la qualité des eaux et par conséquent de contrôler l'apport des polluants dans la Méditerranée par les bassins versants du Rio Martil et Moulouya.

Références

(1) O.M.S. - Normes internationales pour l'eau de boisson, 3e édition 1972.

(2) Standard Methods for the examination of Water and wastewater 15 th - edition 1980 APHA - AWWA - WPCF.

INCIDENCE D'UNE REDUCTION DE REJETS POLLUANTS SUR LES PEUPLEMENTS BENTHIQUES DE L'ETANG DE BERRE*

par

G. STORA et G. BELLAN
Station Marine d'Endoume C.N.R.S. LA 41
rue batterie des Lions
13007 Marseille (France)

Résumé

Depuis plus de dix ans, des aménagements importants ont été réalisés dans l'étang de Berre visant à réduire les rejets d'altéragènes par les usines et les villes environnantes. Ces aménagements ont abouti dès 1980 à une réduction de la charge polluante rejetée de près de 90 % .

L'étude réalisée en octobre 1983 sur la distribution et la composition des peuplements benthiques de l'étang de Berre n'a pas permis de mettre en évidence une évolution particulière de ces derniers. Tout comme ce qui avait été observé de 1972 à 1976 le peuplement lagunaire euryhalin et eurytherme présent dans l'étang reste limité à un mince liseré côtier et ne subsiste pas à des profondeurs supérieures à 5 m.

Le maintien de ce peuplement LEE dégradé démontre bien que le déséquilibre de l'écosystème benthique de l'étang n'a pas pour vecteur principal la charge polluante du milieu mais les rejets erratiques d'eaux douces d'une usine hydro-électrique dans le nord de l'étang et son incidence directe ou indirecte sur les variations de salinité des eaux, les décharges de limon et les taux d'oxygène dissous.

Abstract

For more than ten years, considerable improvements aimed at reducing the influx of deleterious factors from neighbouring factories and cities have been carried out in the Berre lagoon. These developments have resulted in a nearly 90 % reduction in levels of pollutant influx from 1980 onwards. The study conducted in october 1983 on the distribution and composition of benthic populations of the Berre lagoon did not permit the demonstration of their specific evolution . As has been observed from 1972 to 1976, the lagunal euryhaline and eurythermic populations (LEE) present are limited to a thin coastal band and do not exist at depths greater than 5 m. The maintenance of this degraded LEE population demonstrated that the principal cause of the instability of the benthic ecosystem of the lagoon is not pollutant level in the environment but the erratic influx of fresh water from a hydroelectric factory in the north of the lagoon and their subsequent direct or indirect effect on the variations of environmental salinity, discharge of terraginous sediment and levels of dissolved oxygen.

---

* Etude réalisée avec l'aide du C.N.E.X.O. Contrat 83/7318

Introduction

Depuis 1966, la mise en service d'une usine hydro-électrique entraînant des rejets massifs et quasi permanents d'eaux douces dans l'étang de Berre, a profondement modifié les conditions du milieu. Plus particulièrement ces modifications ont eu des répercussions marquées sur la distribution et la composition des peuplements benthiques (FEBVRE 1968, BELLAN 1972, STORA 1976, BELLAN et STORA 1976).

Situé dans un secteur fortement industrialisé l'étang de Berre se caractérise par une charge polluante importante (ARNOUX et al. 1980, GIORGETTI 1981, BOUCHARD 1981) et depuis plus de 10 ans des aménagements importants ont été réalisés visant à réduire les rejets d'altéragènes par les usines et les villes environnantes. Ces aménagements entre autres ont abouti dès 1980 à une réduction de près de 90 % de la charge polluante rejetée (STANKIEVITCH 1983). La dernière cartographie détaillée des peuplements macrobenthiques de l'étang de Berre ayant été effectuée en 1977 (STORA et ARNOUX 1983), le présent travail avait pour but de mettre en évidence l'éventuelle incidence de la réduction de ces rejets polluants sur la distribution et la composition des peuplements macrobenthiques de l'étang.

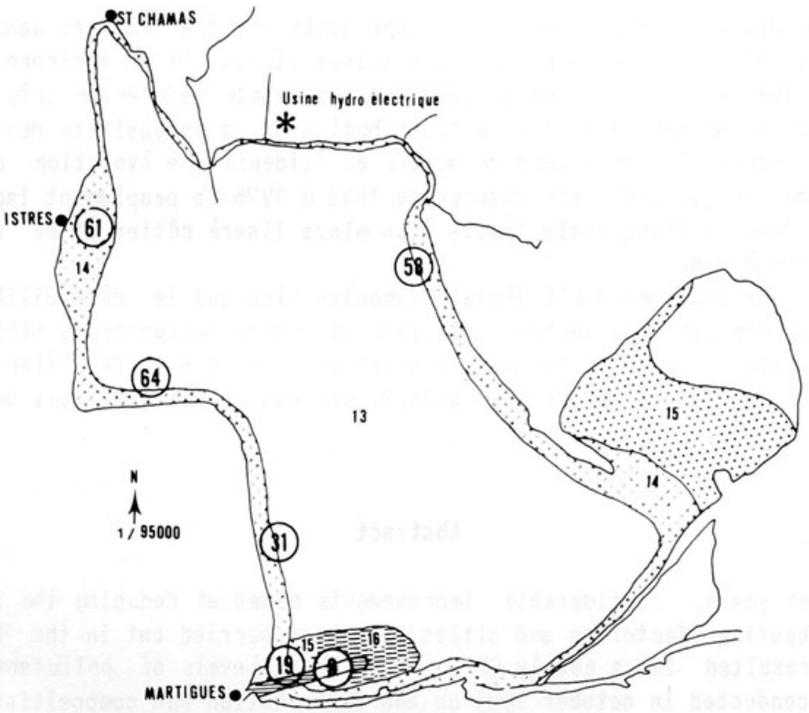


Fig. 1
Situation des peuplements benthiques de l'étang de Berre en octobre 1983. 1) Biocénose SVMC, 7) Zone de transition SVMC-LEE, 13) Zone azoïque, 14) Peuplement LEE, 15) Peuplement LEE très dégradé, 16) Zone très dégradée à Capitella capitata. ○ Stations étudiées.

Méthodologie

Les prélèvements ont été réalisés les 13 et 14 octobre 1983 dans l'étang de Berre et le 21 octobre dans l'étang de Vaine. Au total 41 stations ont été étudiées recouvrant l'ensemble des étangs. L'engin de prélèvement utilisé est une benne Orange Peel. A chaque station, 4,5 dm3 de sédiments sur une surface de 1/12 de m2 ont été prélevés. Cette surface et ce volume de sédiments correspondant au volume et à la surface minimum pour le peuplement lagunaire et euryhalin (LEE) de

l'étang (STORA 1982) ont été obtenus par la réunion d'1/4 de prélèvements de 4 coups de benne. Les sédiments prélevés ont été tamisés sur place sur un tamis de maille de 1.3 mm de côté et conservés dans une solution de formol à 10 %. Au laboratoire, l'ensemble des prélèvements ont été triés et tous les organismes vivants prélevés.

Résultats et discussion

Situation des peuplements benthiques en octobre 1983

a) Cartographie des peuplements (Fig. 1)

4 zones peuvent être mises en évidence où les peuplements sont variables qualitativement et quantitativement et adaptés à vivre en milieu marin ou saumâtre. Ces zones sont :

- la partie centrale de l'étang,
- la zone sud-ouest dans les fonds de 5 à 9 m,
- le chenal de l'étang de Berre,
- la bordure côtière jusqu'à 5 m de profondeur

1. La partie centrale de l'étang

L'étude des stations situées entre 5 m et le fond de l'étang, à l'exception du chenal de navigation et la zone sud-ouest, révèle une absence totale d'espèce macrobenthique. Alors que dans le sud les refus de tamis sont constitués de valves de Pélécypodes morts, la plupart des prélèvements dans le nord n'ont permis de ramener que de la vase pratiquement pure. Il faut cependant noter la présence de quelques larves de Chironomides dans les fonds de 6 m dans le nord de l'étang.

2. La zone sud-ouest

Dans ce secteur sous l'influence directe des courants de marées, seule la Polychète Capitella capitata a pu être récoltée dans des fonds de 8 m au nord du chenal de navigation. Dans les autres stations notamment dans les fonds de 5 à 7 m, seul un peuplement LEE extrêmement dégradé quasi inexistant se maintient.

3. Le chenal de navigation

Dans le chenal de navigation dragué à 9 m de profondeur, un peuplement référable à la biocénose des sables vaseux de mode calme (SVMC) se maintient. A plus de deux milles de Martigues en direction du centre de l'étang ce peuplement SVMC céde la place à un peuplement LEE dégradé qui disparaît très rapidement.

4. La bordure côtière jusqu'à 5 m de profondeur

Les prélèvements réalisés sur cette bordure côtière permettent de constater le maintien d'un peuplement plus ou moins dégradé se référant à la biocénose LEE. Les espèces caractéristiques exclusives de la biocénose LEE récoltées sur la bordure côtière sont Nereis succinea, Mercierella enigmatica, Hydrobia cf. ulvae, Brachidontes marioni, Cerastoderma glaucum, Balanus eburneus, Cyathura carinata, Sphaeroma hookeri et Corophium insidiosum. Le peuplement LEE de la bordure côtière se caractérise par un faciès à Balanus eburneus florissant. L'installation de cette espèce au moment des prélèvements est extrêmement récente dans la mesure où la plupart des individus se présentent sous forme juvénile. Il en est de même pour Cerastoderma glaucum.

Dans l'étang de Vaine seuls les fonds de la partie sud proche du seuil faisant communiquer l'étang de Vaine et l'étang de Berre présentent un peuplement "florissant". Dans le reste de l'étang le peuplement LEE est extrêmement dégradé et même inexistant dans les fonds de 3 m de la partie nord de l'étang.

a) Evolution spatio-temporelle.

Etude de la dynamique des peuplements benthiques de l'étang de Berre.

a) Evolution spatio-temporelle.

La distribution des peuplements benthiques de l'étang de Berre en 1972 (STORA 1976) (fig. 2) comparée à celle de 1983 ne permet pas de mettre en évidence une différence marquée. Le peuplement LEE reste limité à la bordure côtière et à l'exception de la zone sud-ouest et du chenal de navigation, la plus grande partie de l'étang ne présente aucun peuplement macrobenthique.

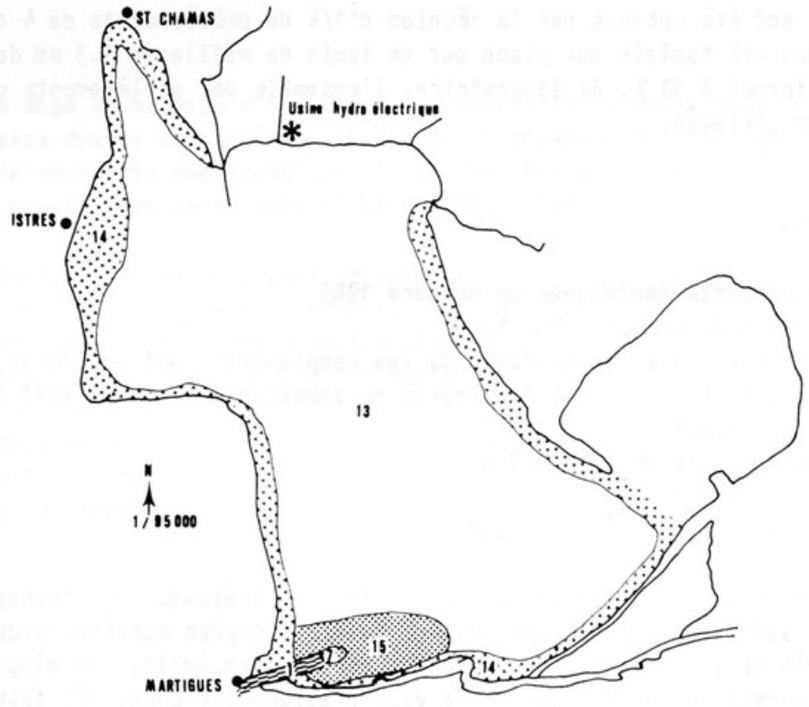


Fig. 2

Situation des peuplements benthiques de l'étang de Berre en 1972. 1) Biocénose SVMC, 7) Zone de transition SVMC-LEE, 13) Zone azoïque, 14) Peuplement LEE, 15) Peuplement LEE dégradé.

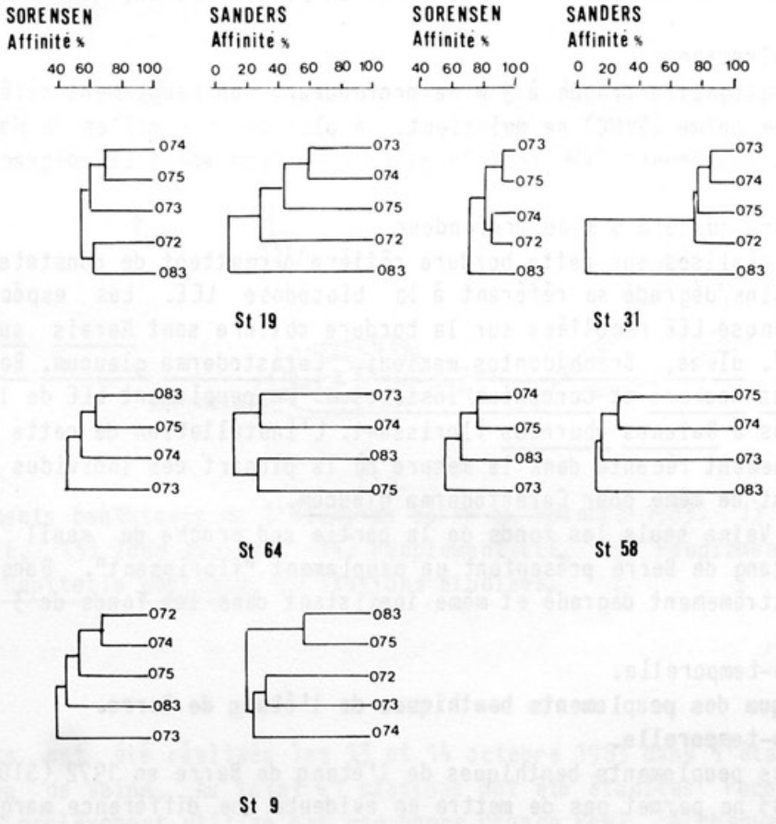


Fig. 3

Affinité au cours du temps du peuplement des différentes stations étudiées.

b) Evolution qualitative et quantitative des peuplements.

Cette étude comparative a été effectuée sur les stations 9, 19, 31, 64 et 58 (fig. 1) suivie régulièrement d'octobre 1972 à janvier 1976. (STORA 1982).

L'évolution tant qualitative que quantitative des peuplements de ces stations n'est absolument pas en relation avec les saisons mais directement sous la dépendance des conditions altérantes du milieu. Cependant les comparaisons ont été effectuées sur des prélèvements réalisés au mois d'octobre intégrant de ce fait une éventuelle action du facteur saisonnier. Il est bien évident qu'il ne s'agit pas en comparant les prélèvements de 1972 à 1976 à ceux de 1983 de mettre en évidence une évolution continue des peuplements mais de rechercher les similitudes ou les différences pouvant exister dans la composition des peuplements antérieurs ou actuels au sein d'une même station pour une période de prélèvements identique.

1. Peuplement du chenal

Le tableau 1 présente diverses composantes du peuplement présent à la station 9 aux cours des différentes années de prélèvements. Le nombre d'espèces et l'abondance du peuplement en 1983 est proche des valeurs obtenues en 1972 et 1974. Il en est de même pour l'indice de Shannon et l'équitabilité qui reste dans des valeurs moyennes par rapport aux autres années. La dominance des indicateurs biologiques de perturbation que sont les espèces indicatrices de pollution, de perturbation maximale, de richesse en matière organique ou à large répartition écologique, est élevée en 1983 dénotant un milieu perturbé mais correspond à une dominance de ces espèces habituellement trouvée à la station 9.

Les dendrogrammes établis à partir du coefficient de Sorensen (fig. 3) montrent une composition qualitative du peuplement qui reste assez semblable au cours des différentes années d'étude. Par contre le coefficient de Sanders met en évidence le regroupement particulier des prélèvements 1975-1983 et à un pourcentage d'affinité moindre les prélèvements des années 1972, 1973 et 1974. Le regroupement des prélèvements 1975-1983 tient plus particulièrement à la dominance élevée de Lumbrineris latreilli. Cette espèce indicatrice de perturbation qui apparaît pour la première fois comme espèce dominante en octobre 1975 occupe la même position en 1983.

Enfin, l'étude de l'évolution temporelle de la structure du peuplement de la station 9 par les diagrammes rangs-fréquences (fig. 4) montre que la courbe obtenue en 1983 est très proche de celles des peuplements en 1972 et 1974 correspondant à un état mature.

2. Peuplement de la bordure côtière

Pour les stations de la bordure côtière, l'étude du tableau 1 ne montre pas de différences significatives entre le nombre d'espèces récoltées dans les différentes stations en octobre 1983 par rapport aux autres années. Par contre à une exception près (station 58 en octobre 1974) c'est en octobre 1983 que l'on obtient une abondance maximale du peuplement LEE. Comme nous l'avons vu précédemment, cette abondance marquée tient à l'installation d'un faciès à Balanus eburneus présent sous une forme juvénile. En règle générale si la présence de faciès est relativement normale dans un peuplement LEE, leur dominance dépasse rarement 60 % du peuplement en place. Ces faciès, sauf dans les stations très perturbées, ont tendance à se surimposer à un peuplement LEE et non à l'écraser. Or si l'on considére l'abondance du peuplement de chaque station pour 4.5 dm3 de sédiment en l'absence de l'espèce dominante (tableau 1) on peut constater pour les stations du sud et notamment la station 31 l'extrême appauvrissement du peuplement en octobre 1983 par rapport aux autres années.

De même si la dominance des espèces indicatrices de perturbation maximale est peu élevée du fait de la prédominance de Balanus eburneus on peut constater que dans la plupart des stations on retrouve en octobre 1983 Nereis succinea, Hydrobia cf. ulvae et les larves de chironomides en position subdominantes traduisant un déséquilibre important du peuplement LEE.

L'incidence de ce faciès juvénile écrasant le peuplement se traduit cependant par des indices de diversité et une équitabilité extrêmement faible en octobre 1983. L'étude des dendrogrammes établis d'après le coefficient qualitatif de Sorensen (fig. 3) met en évidence une affinité importante du peuplement de chaque station au cours des différentes années de prélèvements.

Tableau 1

Evolution temporelle de différents paramètres das peuplements SVMC et LEE de l'étang de Berre.

STATION 9	Oct. 1972	Oct. 1973	Oct. 1974	Oct. 1975	Oct. 1983
Nbre d'espèces	19	8	26	11	21
Abondance	381	68	286	71	366
Shannon	2.55	1.74	3.51	1.96	2.86
Equitabilité	0.60	0.58	0.75	0.57	0.65
Dominance indic.					
Biol. perturbation	77.56	66.17	77.64	94.38	71.86

STATION 19	Oct. 1972	Oct. 1973	Oct 1974	Oct. 1975	Oct. 1983
Nbre d'espèces	14	7	10	10	12
Abondance	2676	1410	11789	4268	8239
Abondance sans sp D.	1143	880	6541	1420	848
Shannon	2.00	1.91	2.12	1.23	.71
Equitabilité	0.52	0.68	0.64	0.37	0.20
Dominance indic.					
Biol. pert. max. %	16.39	37.73	.95	5.81	2.77

STATION 31	Oct. 1972	Oct. 1973	Oct. 1974	Oct. 1975	Oct. 1983
Nbre d'espèces	7	6	7	7	6
Abondance	2091	2767	1311	1466	4688
Abondance sans sp D.	638	1657	713	703	46
Shannon	1.37	1.76	1.95	1.86	0.10
Equitabilité	0.49	0.68	0.69	0.66	0.04
Dominance indic.					
Biol. pert. max. %	30.12	58.63	52.13	32.40	0.77

STATION 64	Oct. 1973	Oct. 1974	Oct. 1975	Oct. 1983
Nbre d'espèces	8	11	7	9
Abondance	56	612	191	2015
Abondance sans sp D.	38	239	82	647
Shannon	2.51	1.72	1.91	1.44
Equitabilité	0.84	0.50	0.68	0.45
Dominance indic.				
Biol. pert. max. %	7.15	11.5	1.52	21.49

STATION 58	Oct. 1973	Oct. 1974	Oct. 1975	Oct. 1983
Nbre d'espèces	4	8	5	7
Abondance	30	1347	35	979
Abondance sans sp D.	12	547	12	122
Shannon	1.48	1.90	1.54	0.79
Equitabilité	.74	.63	.66	.28
Dominance indic.				
Biol. pert. max. %	3.33	14.11	17.14	10.93

Le pourcentage d'affinité est rarement inférieur à 50 % montrant la constance du peuplement dans le temps. Par contre, l'affinité du peuplement de chaque station au cours du temps, calculé par le coefficient quantitatif de Sanders est extrêmement faible du fait du développement au cours du temps de différents faciès. Seule la station 31 montre un regroupement marqué des années 1972, 1973, 1974 et 1975 du fait du maintien à cette station d'un faciès à Brachidontes marioni.

L'étude de l'évolution temporelle de la structure du peuplement LEE de chaque station par les diagrammes rangs, fréquences confirme en 1983 le caractère juvénile du peuplement en place. Ce caractère semble la règle pour les stations appauvries du nord de l'étang (stations 64 et 58), les courbes obtenues pour les différentes années étant proches de celles obtenues en 1983. Pour la station 19 et notamment la station 31 la destructuration du peuplement par rapport aux autres années est particulièrement importante.

Discussion

Les études réalisées in situ dans l'étang de Berre de 1972 à 1976 et in vitro sur des espèces caractéristiques de la biocénose LEE ont montré que la distribution et la dynamique des peuplements benthiques de l'étang de Berre sont influencées plus particulièrement par 4 facteurs principaux que sont les variations erratiques des rejets d'eaux douces et leur incidence directe sur la salinité, les décharges de limons, les faibles taux d'oxygène dissous en profondeur ainsi que la pollution chimique du milieu. (STORA 1982, STORA et ARNOUX 1983).

Les variations erratiques d'eaux douces

Les apports d'eaux douces dans l'étang de Berre sont extrêmement fluctuants. De ce fait les animaux sont soumis à des variations de salinité importantes. Beaucoup d'espèces ont leur optimum de développement dans des tranches de salinité basses ou élevées mais ne supportent pas de variations rapides et de fortes amplitudes.

Les apports de limons

Les eaux douces sont chargées en limons. L'action des ces limons est en règle générale limitée à la partie nord de l'étang mais par effet de chasse l'influence de ce facteur altéragène peut être marquée jusque dans la partie sud. C'est ainsi qu'au cours du deuxième semestre 1975 l'ensemble du peuplement LEE de la bordure côtière a été enfoui sous une importante couche de limon entraînant une mortalité extrêmement importante du peuplement.

L'oxygène dissous

L'extension du peuplement LEE au-delà de -5 m est limitée entre autres par les faibles taux d'oxygène dissous ou les conditions anoxiques temporaires régnant en profondeur. Lors de forts débits l'appauvrissement du milieu en oxygène dissous est directement lié à l'absence de mélange entre les eaux de surface et les eaux de fond. Pour des débits faibles l'augmentation de salinité favorise le développement d'une production primaire importante qui contribue lors de sa dégradation à l'appauvrissement du milieu en oxygène dissous. (MINAS 1973).

Les polluants

Toutes pollutions domestiques et industrielles confondues, les polluants sont apportés essentiellement par les eaux marines en provenance du golfe de Fos, par les affluents d'eaux douces naturels ou artificiels et par les rejets des villes et industries installées sur la bordure de l'étang.

En règle générale et sauf pour les faibles taux d'oxygène dissous, aucun des facteurs altéragènes n'est franchement limitant. Le plus souvent la mortalité ou l'absence de colonisation sont la résultante d'effets synergiques dus à la réunion de deux ou plusieurs conditions subcritiques.

Depuis près de dix ans un certain nombre de mesures ont été prises pour améliorer la situation de l'étang de Berre. Parmi ces mesures, la plus importante est sans nul doute la réduction de la charge polluante déversée. Etant donné la situation des peuplements benthiques en 1983 la résorption de plus de 90 % des rejets polluants au sein de l'étang ne semble pas avoir pour le moment une incidence marquée sur le devenir de l'écosystème benthique.

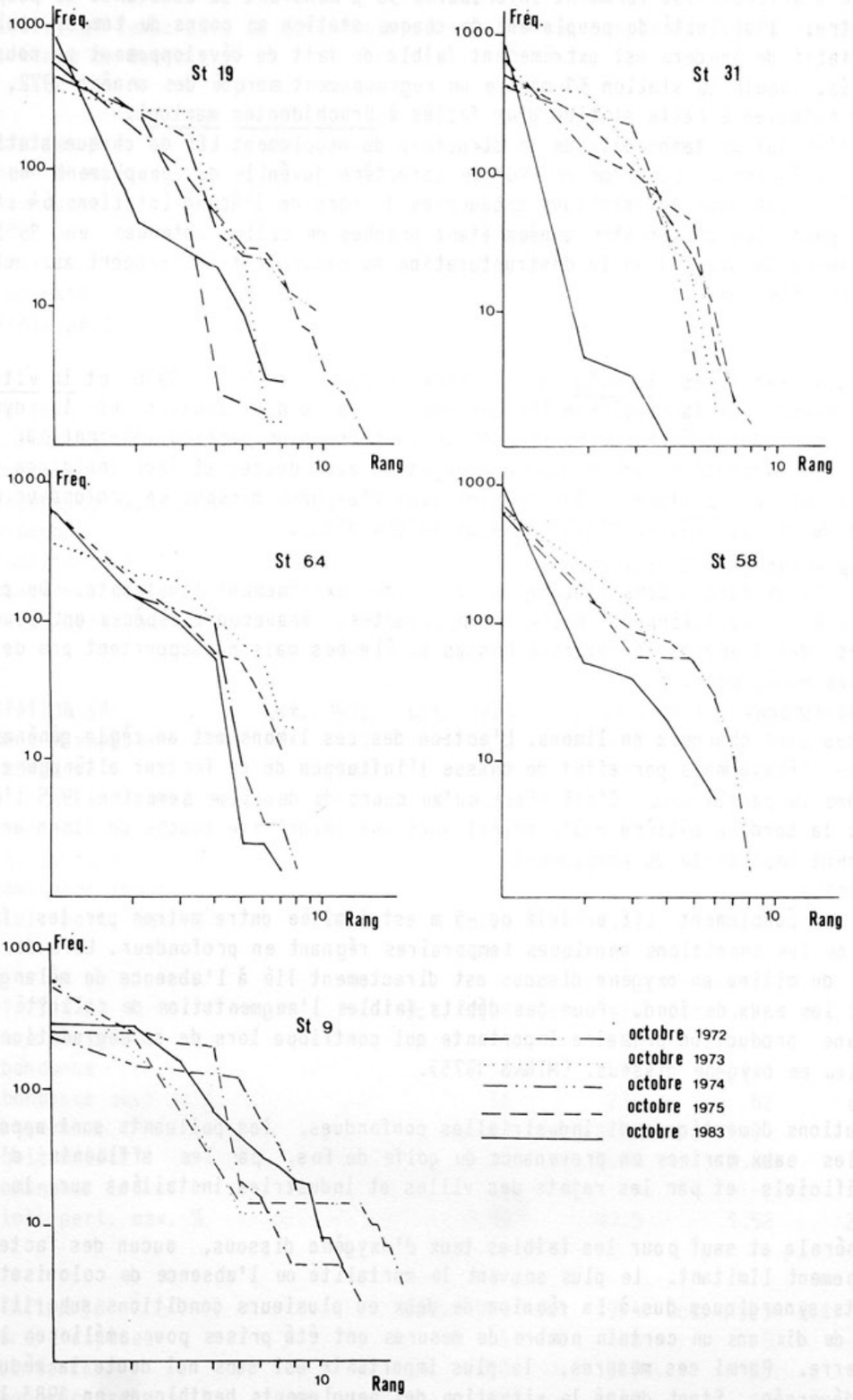


Fig. 4
Evolution temporelle des diagrammes rangs-fréquences.

Ceci en fait peut se comprendre par la prédominance marquée des effets synergiques entre altéragènes par rapport à l'action de concentrations de polluants sensu stricto dans la dégradation des peuplements de l'étang.

Pour une pollution chimique moins importante, le peuplement LEE du nord de l'étang s'est toujours présenté sous une forme appauvrie par rapport au sud plus pollué (ARNOUX et STORA 1979) du fait de l'instabilité des conditions du milieu liée aux décharges d'eaux douces. D'autre part si la réduction des rejets polluants peut avoir une incidence directe sur les concentrations de la phase liquide, les sédiments de l'étang constituent une formidable réserve à polluants pouvant, dans certaines conditions telle qu'une augmentation de salinité, être relargués dans le milieu.

Parmi les quatre grands facteurs altéragènes décrits précédemment, les trois premiers sont directement sous la dépendance des rejets d'eaux douces. Or si la création d'un nouveau bassin de décantation a pu limiter les dépôts de limon dans l'étang, les décharges d'eaux douces et leur incidence sur les variations de salinité et l'isolement de la couche profonde des années 1970 à 1983 sont restées les mêmes (CONTAT 1983). La continuité de ces rejets est sans nul doute responsable de la persistance d'un peuplement limite LEE sur un mince liseré côtier. Le peuplement en place en octobre 1983 est un peuplement juvénile postérieur à une forte mortalité qui a certainement son origine dans la variation des débits passant de 579.000.000 m3 en juin à 61.877.000 m3 en âout 1983. Une telle mortalité du peuplement LEE de la bordure côtière à la suite d'une chute brutale des rejets d'eaux douces a pu être observée en mars 1973 et en juillet 1974 (STORA 1982, STORA et ARNOUX 1983).

Conclusions

La comparaison de la distribution des peuplements benthiques de l'étang de Berre ainsi que leur composition ne permet pas de mettre en évidence une évolution particulière de ces peuplements en 1983 par rapport à 1972.

Ceux-ci sont limités sur un mince liseré côtier, dans le chenal de navigation et la zone sud-ouest sous l'influence des courants de marées. Le peuplement LEE de la bordure côtière reste le même, caractérisé par une richesse spécifique faible et un grand nombre d'individus lié à l'explosion monospécifique d'une ou quelques espèces trouvant des conditions optimales de vie. En règle générale, ces faciès temporaires ou permanents se surimposent au peuplement normal. En octobre 1983 un faciès à Balanus eburneus juvénile écrase le peuplement LEE traduisant un déséquilibre important de ce peuplement.

L'absence de toute évolution malgré les aménagements réalisés pour réduire la pollution chimique sensu stricto confirme le caractère perturbateur majeur du rejet des eaux douces dans la dégradation de l'écosystème benthique.

Références

A. ARNOUX, J.L. MONOT, P. BOUCHARD, C.B. AIRAUDO, (1980). - Evolution et bilan de la pollution des sédiments de l'étang de Berre. Ves J. Etud. Poll. : 433-446, Cagliari, C.I.E.S.M.

A. ARNOUX, G. STORA, (1979). - Distribution de quelques alteragènes présents dans l'étang de Berre : leur influence sur la répartition de la macrofaune benthique. Rapp. Comm. int. mer Médit., 25/26, 3 : 149-150.

G. BELLAN, (1972). - Effects of an artificial stream on marine communities. Mar. Poll. Bull., 3(5) : 74-77.

G. BELLAN, G. STORA, (1976). - Effet et conséquences d'un apport artificiel d'eaux douces sur les peuplements benthiques d'un étang marin. IIIe J. Etud. Poll. : 147-152, Split, C.I.E.S.M.

P. BOUCHARD, (1981). - Etude de l'état de la pollution des sédiments de l'étang de Berre. 1re partie: la pollution organique. Thèse univ. Pharmacie Marseille : 184 p.

H. CONTAT, (1983). - Impact du rejet d'eaux turbides par une centrale hydro-éléctrique. Cas de la centrale de Saint-Chamas. Diplôme d'Etat docteur en Pharmacie. Univ. Aix-Marseille : 131 p.

J. FEBVRE, (1968). - Etude bionomique des substrats meubles de l'étang de Berre. Rec. Trav. St. mar. Endoume, 44 : 297-355.

C. GIORGETTI, (1981). - Etude de l'état de la pollution des sédiments de l'étang de Berre. 2 la pollution inorganique. Thèse Univ. Pharmacie Marseille : 149 p.

M. MINAS, (1973). - Sur la synthèse et la dégradation de la matière organique dans l'écosystème de l'étang de Berre. Dynamique et bilans. Rapports avec le régime hydrologique. Thèse Doctorat. Univ. Aix-Marseille II : 340 p.

A. STANKIEVITCH, (1983). - Traitement des effluents d'une raffinerie de pétrole. Diplôme d'état Docteur en Pharmacie. Univ. Aix-Marseille : 47 p.

G. STORA, (1976). - Evolution des peuplements benthiques d'un étang marin soumis à un effluent d'eaux douces. Bull. Ecol., 7(3) : 275-282.

G. STORA, (1982). - Recherches de bionomie descriptive et expérimentale (in vivo, in vitro) dans quelques biotopes littoraux soumis à des variations naturelles ou artificielles des conditions du milieu (notamment dans l'étang de Berre et le golfe de Fos). Thèse Doctorat. Univ. Aix-Marseille II : 327 p. + annexes.

G. STORA, A. ARNOUX, (1983). - Effects of large freshwater diversions on benthos of a mediterranean lagoon. Estuaries 6 (2) : 115-125.

ETUDE HISTORIQUE ET EVALUATION ACTUELLE DE L'IMPACT DE L'EUTROPHISATION SUR L'ECOSYSTEME DE LA LAGUNE DE TUNIS

par

J. ZAOUALI et S. BAETEN
Institut National Agronomique de Tunisie
43, avenue Charles Nicolle
1002 Tunis (Tunisie)

Résumé

La progression des facteurs d'eutrophisation dans la lagune de Tunis est étudiée à la lumière des données historiques accumulées depuis le début du premier millénaire et des résultats des travaux scientifiques réalisés dans le domaine des communautés benthiques depuis le début du XXe siècle.

Il est montré que l'impact de l'eutrophisation urbaine (ville de Tunis) a joué un rôle important dès la moitié du deuxième millénaire. A l'heure actuelle, en dépit d'énormes efforts d'aménagement, la situation de l'écosystème lagunaire étudiée par le biais de l'analyse factorielle des correspondances (stations - espèces animales benthiques) reste très critique.

*
* *

Introduction

La lagune de Tunis qui sépare la ville de Tunis de la mer et de Carthage (Fig. 1) a depuis toujours subi les contrecoups de la proximité de ces grands centres urbains. Les documents historiques traitant de la lagune sont donc très anciens (-150, Polybe, in de Folard, 1730) : mais si les premiers écrits sont, en raison de l'importance stratégique de la zone de Carthage, relatifs à des faits d'armes, ceux qui suivent nous fournissent, le temps passant, des indications de plus en plus nombreuses et précises sur la population du lac.

Données historiques

L'évolution de la pollution

Une revue des nombreux ouvrages se repportant à Tunis et à sa lagune nous a permis de recueillir les témoignages suivants : 1300, ABOULFEDA, "dans la partie du lac qui touche Tunis se rassemblent les immondices de la ville" ; 1470, ADORNE (in Brunschvig, 1936), "en été le lac pue étrangement" 1625, GRANCHAMP, "le lac est un étang de peu de profondeur à eau morte et sale" ; 1727, SHAW, "le

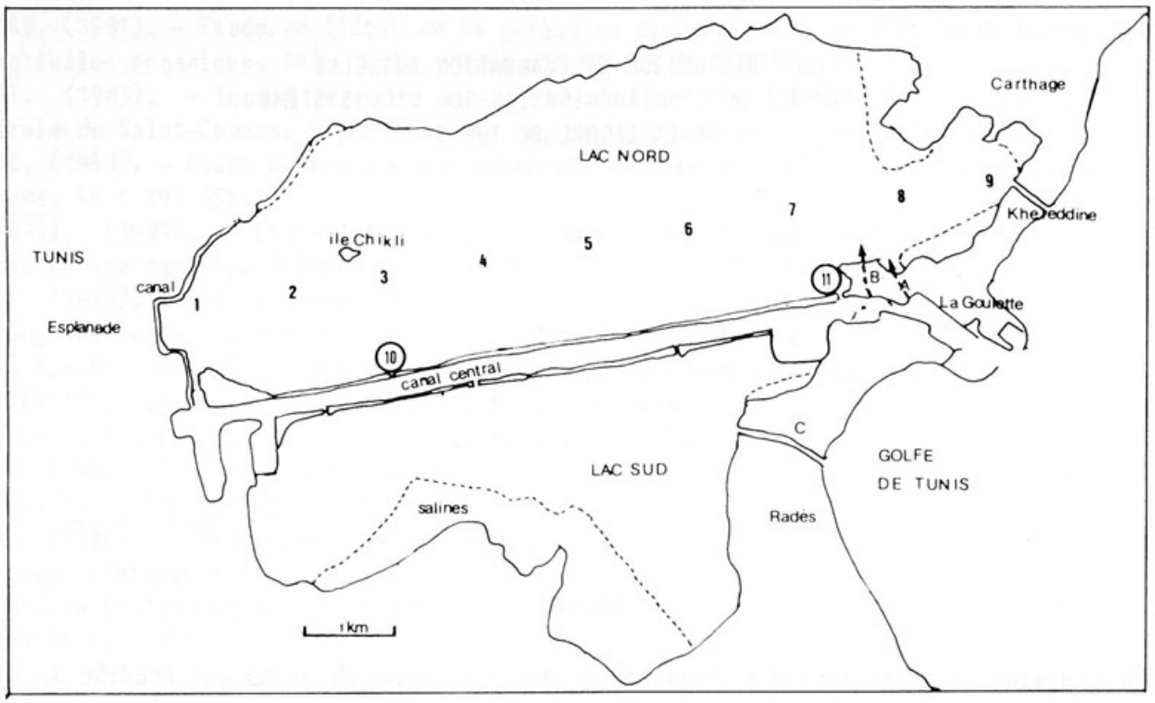


Fig. 1
Le lac de Tunis : en pointillés : parties isolées:
flèches : rejets des eaux de refroidissement des usines :
A, B : usines thermo-électriques en fonctionnement
C : usine thermo-électrique en cours d'installation
chiffres encerclés : chenaux 10 (de Chikly) et 11
1 à 9 : stations de prélèvements.

lac est presqu'entièrement fort bas car il reçoit toutes les immondices de la ville... il est sur les berges sec et puant" : 1783, DESFONTAINES (in PEYSONNEL et DESFONTAINES, 1786) "le lac est rempli d'immondices... il répand une odeur infecte en été" : 1801, FRANCK (1816), "l'hydrogène sulfuré a son dégagement favorisé par les vents du sud de sorte qu'il règne dans plusieurs endroits de la ville de Tunis une puanteur insupportable... les vents d'est ont l'inconvénient de porter sur la ville les exhalaisons fétides de ce cloaque ; 1853, PELLISSIER, "il y a près de Tunis une vase noire et infecte dont les horribles exhalaisons répandent dans l'atmosphère une insupportable puanteur" ; 1861, GUERIN, "le lac n'a jamais été profond, des sondages ont prouvé qu'il n'a jamais dépassé 1.70 m... la ville de Tunis ne cesse de déverser dans ce bassin ainsi que dans un réservoir commun toutes les immondices de ses kandaks (=égouts)... le lac s'est peu à peu encombré d'une vase fétide qui s'accumule insensiblement et qui finira à la longue par le combler si on n'y remédie pas... on remarque ça et là un grand nombre de bas-fonds qui, à l'époque des grandes chaleurs, exhalent des miasmes pestilentiels".

Devant un tel état de chose, depuis toujours, des solutions d'assainissement ont été envisagées. C'est ainsi que, dès le VIIIe siècle (EL BEKRI, 1068) le canal à la mer, qui vraisemblablement existait déjà mais qui s'était envasé amenant un assèchement partiel du plan d'eau, est recreusé et prolongé par un premier canal central en direction de la ville de Tunis. Par la suite furent entrepris d'autres travaux tant au niveau de la Goulette que de Khéreddine (lac nord) et de Radès (lac sud). Le plus important de ces ouvrages ayant été, en 1885, le creusement du canal central actuel

reliant les ports de la Goulette et de Tunis et partageant de façon définitive la lagune en deux parties. Sur ce canal furent successivement creusés des chenaux annexes dont l'emplacement et le nombre subirent de constantes modifications... De plus, furent construites au niveau de la Goulette, deux centrales thermo-électriques prenant leurs eaux de refroidissement dans le canal central et les rejetant dans la partie sud-est du lac nord.

L'évolution des peuplements benthiques

Complétant les renseignements fournis par les ouvrages historiques, les observations des voyageurs naturalistes, dès le début du XVIIIe siècle et les travaux scientifiques, depuis le début du XIXe siècle, permettent d'esquisser un premier schéma de l'évolution de la lagune au niveau benthique.

a) Les peuplements végétaux

En 1724, PEYSONNEL (1786) signale la présence "d'Acetabulum et de Fucus" (qui serait Posidonia oceanica). HELDT (1929) donne une carte de la végétation du lac nord (à cette époque, il communique avec le canal central par deux chenaux, l'un au niveau du port de Tunis, l'autre au niveau de la Goulette, il n'y a pas d'ouverture directe sur la mer). Sur cette carte, les Posidonies signalées deux siècles plus tôt (elles ont bien existé car elles furent retrouvées en sondage) ont disparu : elles ont été remplacées par des Zostères (parties centrale et orientale, colonisées de plus par des Entéromorphes). Les Acétabulaires persistent encore dans la partie centrale ; enfin, les Ulves occupent la partie occidentale du lac proche de la ville. MOLINIER et PICARD (1954) signalent la pénétration de Caulerpa prolifera dans la partie est du lac (en 1952, le canal de Khéreddine a été recreusé) : les Ulves occupent non seulement la partie orientale de la lagune mais encore ont gagné du terrain en direction de la zone centrale, les Acétabulaires ont disparu (envasement, enrichissement en matière organique, diminution de l'éclairement). ZAOUALI (1974) montre de nouvelles mutations : dans la partie orientale du lac nord Caulerpa prolifera a été remplacée par Gracilaria confervoïdes, les Ulves ont envahi près des 2/3 du lac. Dans la zone occidentale le peuplement végétal est dominé par les Entéromorphes alors que les Chaetomorphes se mêlent aux Ulves dans la partie centrale : quelques Zostères persistent dans la région la plus proche du canal de Khéreddine. ZAOUALI et BAETEN (1983) indiquent qu'en 1981, dans la partie occidentale, les Ulves ont disparu (très forte turbidité), elles ont, par contre, progressé en direction de la zone orientale : dans la partie centrale du lac la stagnation amène la pullulation des Chaetomorphes : dans la partie orientale Gracilaria est toujours abondante alors que les Zostères ont été complètement éliminées.

b) les peuplements animaux

Leur évolution est plus difficile à étudier car les documents sont, en la matière, peu nombreux. BRUNN (1940) donne quelques résultats qualitatifs et quantitatifs pour la région proche du port de la Goulette : ceux-ci nous permettent de noter la présence des éléments dominants de la biocénose L.E.E. (lagunaire euryhaline et eurytherme). Les espèces les plus abondantes sont des Bivalves : en premier lieu, Cardium edule (Cerastoderma glaucum) suivi par Syndesmia tenuis : viennent ensuite les Crustacés, ce sont dans l'ordre d'abondance : Idothea viridis, Gammarus (locusta?), Sphaeroma serratum. VUILLEMIN (1952) traite des récifs du Serpulien Mercierella enigmatica (Ficopomatus enigmaticus) et signale qu'ils ont été découverts en 1944 par HELDT. Depuis quand sont-ils installés ? Il est difficile de le préciser. Néanmoins l'ensemble des travaux qui leur sont consacrés permettent de penser que leur évolution n'a été nette qu'à partir du moment où les courants dans le lac ont été réactivés, notamment par le creusement du chenal dit de Chikly (chenal n° 10). Leur évolution est telle qu'en 1953 HELDT craint un comblement du lac par les "micro-atolls" formés par ce Serpulien. La progression des récifs gagne inexorablement du terrain d'ouest en est comme l'attestent les travaux ultérieurs (équipe RUDIS, 1967 ; ZAOUALI, 1974 : ZAOUALI et BAETEN, 1983). Cet envahissement matérialise un enrichissement croissant en matière organique et va de pair, comme nous l'avons noté, avec une large extension de la flore dite "nitrophile" et l'apparition (HELDT, 1929), puis le renouvellement de plus en plus fréquent des crises dystrophiques estivales se traduisant par le développement d'eaux rouges bactériennes.

Les travaux d'assainissement actuels et étude de leur impact.

Plus de 40 rapports techniques (5.000 pages environ) furent écrits sur l'eutrophisation de la lagune, le premier (Société Hydrocure) datant de 1950. Mais ce ne fut qu'aux alentours de 1980 que démarrèrent les travaux d'aménagement du canal de l'Esplanade appelé à détourner les eaux usées de la ville ainsi que les eaux pluviales dont la forte teneur en sels nutritifs a été mise en évidence (BELKHIR, 1980). Ce canal, qui longe la partie du lac nord vers Tunis, récupère les eaux usées et les eaux pluviales pour les rejeter directement dans le canal central au niveau du port de Tunis : ces eaux ainsi récupérées ne peuvent plus, en principe, retourner dans le lac, le chenal 10 ayant été équipé par des écluses semi-automatiques qui ne s'ouvrent que sous la poussée des eaux sortant de la lagune.

Matériel et méthodes

Afin de vérifier les résultats sur l'évolution de la faune benthique des mesures d'assainissement entreprises, nous avons effectué dans le lac nord deux séries de prélèvements : la première avant l'achèvement du canal de l'Esplanade en 1980-81, l'autre en 1983-84.

Les stations échantillonnées pendant les périodes automnales et hivernales correspondent à une radiale centrale (Fig. 1) joignant Tunis au canal de Khéreddine. Elles sont au nombre de 9, mais, dans tous les cas les stations les plus proches de la ville (1 et 2) se sont révélées azoïques au niveau des espèces animales benthiques (vase noire gluante).

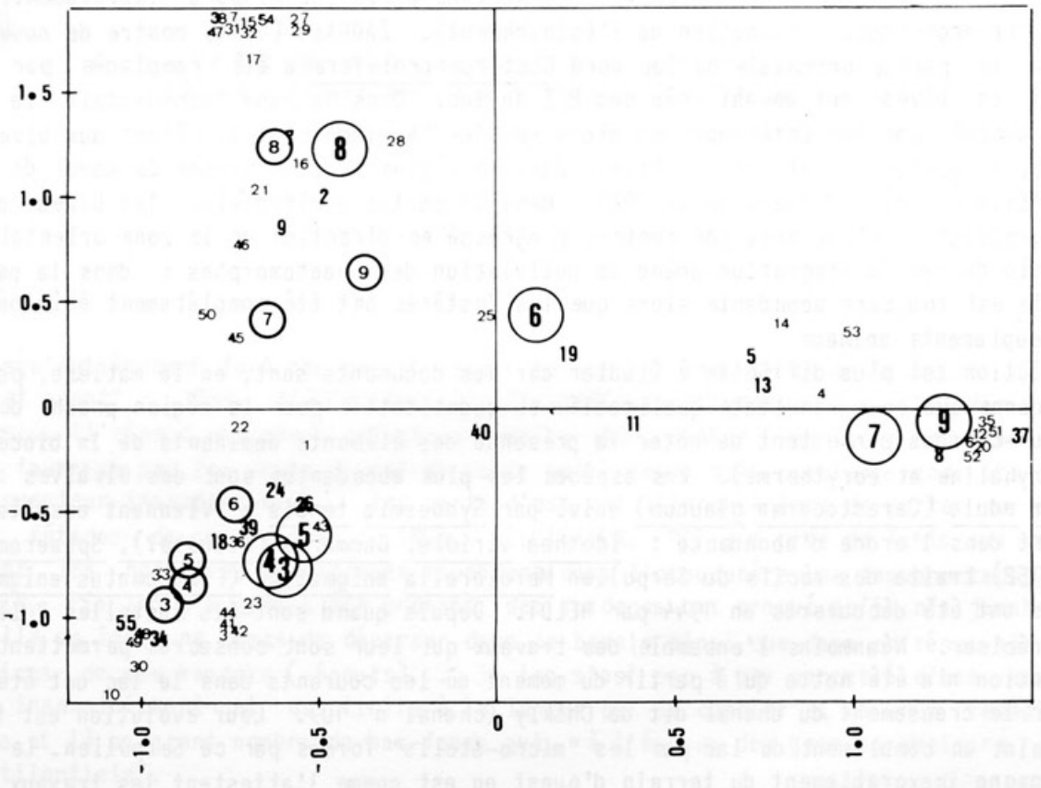


Fig. 2
Analyse des correspondances : axes 1 et 2 :
grands cercles : stations (campagne (1980-81)
petits cercles : stations (campagne 1983-84)
chiffres gras : espèces de poids supérieur à 3 %

A fin de comparaison, nous avons choisi de traiter simultanément les résultats de ces deux campagnes par la méthode de l'analyse factorielle des correspondances (stations-espèces). Cette façon de procéder, nous offre, en effet, la possibilité de fixer sur un même graphe l'ensemble de l'information fournie et ainsi nous permet de matérialiser la position "bionomique" des stations échantillonnées pendant l'une et l'autre période.

Le tableau de contingence des données ainsi obtenu compte 55 lignes représentant chacune une espèce benthique (code : annexe 1) et 14 colonnes correspondant aux 7 stations prises en compte dans chaque série de prélèvements. Chaque cellule du tableau contient, en conséquence, l'effectif total de l'espèce correspondant à la ligne dans la station considérée pour l'une ou pour l'autre période étudiée. Sur le graphe (fig. 2) les stations sont numérotées de 3 à 9 (grand cercle) pour la première série de prélèvements et de 3 à 9 (petit cercle) pour la seconde.

Les prélèvements benthiques ont été faits à partir d'une barque à l'aide d'une drague type Rallier du Baty tirée à chaque station pendant un temps identique à vitesse uniforme.

Résultats

Les résultats, reportés sur le graphe, montrent que le pourcentage d'inertie (ou de variance expliquée) du premier axe est de 29 % et celui du second de 19 %, soit près de 50 % au total. Il est donc permis de considérer qu'ils représentent la plus grande part de l'information fournie par l'analyse (pourcentages par la méthode du "bâton brisé" : 24,46-16,77 %).

L'étude de la distribution des espèces va nous permettre de les qualifier. Pour l'axe 1, les espèces de poids le plus fort (caractères gras) montrent un net regroupement vers la gauche du graphe, soit dans l'ordre : 18 (Hydrobia ventrosa), 7 (Corophium volutator), 24 (Cerastoderma glaucum), 39 (Ficopomatus enigmaticus), 55 (Chironomes), espèces caractéristiques des milieux lagunaires eutrophisés. A l'inverse, dans la partie droite du graphe nous trouvons : 8 (Corophium insiduosum), 13 (Leptocherus pilosus), 5 (Sphaeroma serratum), espèces lagunaires ayant néanmoins un préférendum pour les zones "marinisées". Ce premier axe peut donc être défini comme un axe de "pollution" (partie gauche du graphe) ou de "marinisation" (partie droite).

Pour le second axe l'identification est un peu plus difficile, d'autant plus que la répartition du nuage des points présente une forme parabolique nous permettant de penser qu'il existe entre les deux premiers facteurs une liaison non linéaire dite effet Guttman (BLANC et LAUREC, 1976). Nous essaierons néanmoins de l'identifier. L'observation des points regroupés dans la partie haute du graphe montrant une dominance d'espèces en relation avec un milieu marqué par un certain hydrodynamisme (vice-versa dans la partie basse) : nous qualifierons donc cet axe d'axe d'hydrodynamisme, faible dans la partie basse du graphe, plus fort dans la partie haute. L'effet Guttman, quant à lui, montrerait que les espèces situées au sommet de la parabole (partie haute du graphe) seraient vraisemblablement les mieux adaptées à l'ensemble des conditions du milieu matérialisées par les axes 1 et 2, alors que les espèces situées à gauche et à droite du graphe, seraient caractéristiques du facteur considéré.

Discussion

Dans ce contexte l'étude de la position bionomique des stations nous fournit les indications suivantes : (1) pour la série de prélèvements de la campagne 1980-81, les stations 3, 4 et 5 sont situées dans la zone polluée de courant atténué ; la station 6 occupe une position intermédiaire ; la station 8 est reportée dans la zone de pollution sous influence des courants (rejets des usines thermo-électriques) ; les stations 7 et 9 sont dans la zone de pollution minimale sous légère influence marine (chenal 11 et canal de Khéreddine) ; (2) pour la série de prélèvements de la campagne 1983-1984, les stations 3, 4 et 5 ont gagné du terrain en direction du pôle d'eutrophisation maximale: la station 6 a rejoint les précedentes : elle a donc très fortement rétrogradé (hydrodynamisme très atténué et pollution beaucoup plus accentuée) la station 8 n'a pas bougé ; même influence des rejets des usines proches : les stations 7 et 9 ont rejoint la station 8, elles restent sous l'influence des courants, mais leur taux de pollution a nettement augmenté.

En définitive, en nous permettant de reporter sur un même graphe les positions biocénotiques des différentes parties du lac nord de Tunis à deux années d'intervalle, cette étude nous fournit un moyen de montrer, à l'inverse de ce que l'on aurait pu attendre, la forte avancée de l'eutrophisation dans le lac.

En d'autres termes, il est donc possible de considérer que, malgré les très importants travaux entrepris pour bonifier le lac, les résultats n'ont pas été à ce jour, et de loin, à la hauteur des espérances. C'est ainsi, qu'en 1983, dès le mois de mai apparaissaient les premières "eaux rouges" et, qu'au mois de juillet de la même année, une crise dystrophique débutait dans la partie orientale du lac nord, se traduisant par la mort de plus de 100 tonnes de poissons : qu'enfin, à la mi-mars 1984 les eaux rouges s'amorçaient sur presque toute la surface du lac nord et envahissaient toute la partie centrale du lac sud !

Des explications peuvent être fournies à cet état de chose.

Les eaux détournées par le canal de l'Esplanade, eaux plus légères que celles du canal central, ne s'enfoncent pas. Elles constituent donc une lame superficielle qui va pouvoir repénétrer dans la lagune au niveau des chenaux sans avoir subi le phénomène du lagunage naturel qui se faisait autrefois dans la zone occidentale du lac (KELLY et al., 1977). En effet, au niveau du chenal 10, les vannes à sens unique qui ont été installées sont très souvent coincées en position ouverte : de ce fait, elles permettent la réintroduction quasi immédiate de l'eau détournée par le canal de l'Esplanade.

Au niveau du chenal de Khéreddine, le présence d'une deuxième vanne semi-automatique ne s'ouvrant que sous la poussée des eaux marines, a, en empêchant la sortie de l'eau du lac, contribué à la formation d'un énorme bouchon de vase qui gêne la pénètration en force du courant marin. Il en résulte une stagnation beaucoup plus grande du plan d'eau.

Les récifs de Ficopomatus enigmaticus qui, il y a encore 3 ans, couvraient une très large surface du lac et filtraient une importante quantité de matériel organique, n'ont pas disparu, mais en raison de l'atténuation des courants, sont presque tous morts. Ils jouent donc encore un rôle de barrière mais ne permettent plus le recyclage d'une grande partie de la matière organique, ce qui amène une prolifération accrue des algues "nitrophiles".

Conclusion

Cette étude nous a permis de prouver que la pollution dans la lagune de Tunis est un phénomène très ancien : l'eutrophisation du milieu étant formellement attestée depuis le XVe siècle.

L'application de l'analyse factorielle des correspondances au contrôle de l'évolution des peuplements benthiques semble constituer un outil intéressant qui nous a permis :

- de qualifier et de quantifier les contraintes subies par les peuplements :
- de reporter sur un même graphe leur évolution temporelle et ainsi de mesurer leur réponse en fonction des modifications du milieu.

C'est ainsi que nous avons pu montrer :

- que les deux principaux facteurs régissant le peuplement du lac nord sont : 1 - l'eutrophisation : 2 - l'hydrodynamisme :
- que le problème du recul de l'eutrophisation dans le lac n'a pas encore été résolu par les mesures de bonification prises, bien au contraire.

Bien que les lagunes - milieux sélectifs très sensibles - possèdent un pouvoir régulateur naturel élevé, il nous paraît donc dangereux, voire inutile, d'esquisser le rééquilibrage d'un biotope en ne tenant compte, comme cela a été fait dans la lagune de Tunis, que des seules données physico-chimiques, sans se préoccuper des réponses biologiques de l'écosystème ainsi perturbé. Par ailleurs, il est utopique de consacrer un énorme effort financier pour épurer le seul lac nord, la lagune formant un tout englobant le lac nord, le canal central, le lac sud. Comme le soulignent à juste titre BELKHIR et HADJ ALI SALEM (1983), dans le lac sud "l'observation sur de grandes étendues d'eau

de faible salinité, très turbide et de couleur brun marron, indique bien la présence des eaux usées du grand Tunis" (en provenance du canal central). De plus le lac sud est encore l'objet d'importants bouleversements dont on ne peut évaluer l'impact futur sur l'ensemble de l'écosystème : creusement d'un grand port dans sa partie nord-est, construction d'une troisième centrale thermo-électrique au niveau du canal de Radès.

ANNEXE 1

Listes des espèces récoltées
dans le lac nord de Tunis

1. Gammarus aequicauda
2. Gammarus insensibilis
3. Idothea baltica (d)
4. Idothea viridis
5. Sphaeroma serratum (d)
6. Sphaeroma ephippium (d)
7. Corophium volutator
8. Corophium insiduosum (d)
9. Balanus amphytrite
10. Microdeutopus gryllotalpa (d)
11. Erichtonius difformis (d)
12. Lyanassa plumosa
13. Leptocherus pilosus
14. Paraceis sculpta
15. Melita palmata (a)
16. Cymadusa hirsuta (a)
17. Brachynotus sexdentatus (a)
18. Hydrobia ventrosa
19. Amycla corniculum
20. Monodonta turbiformis (d)
21. Cyclonassa neritea
22. Tricolia pulla (a)
23. Pirenella conica (d)
24. Cerastoderma glaucum
25. Loripes lacteus (d)
26. Syndesmia tenuis
27. Gastrana fragilis (d)
28. Tapes decussatus
29. Tapes aureus (d)
30. Bittium reticulatum (a)
31. Cerithium vulgatum (a)
32. Gibbula varia (a)
33. Haminea hydatis (a)
34. Conopeum seurati
35. Bugula stolonifera (d)
36. Bowerbankia gracilis
37. Zoobothryon verticillatum (d)
38. Cryptosulla palesiana (a)
39. Ficopomatus enigmaticus
40. Hydroides dianthus (d)
41. Scolelolepis fuliginosa (d)
42. Vermiliopsis striaticeps (d)
43. Nereis diversicolor (d)
44. Polydora caeca
45. Hydroides elegans (a)
46. Hydroides norvegica (a)
47. Amphytrite rubra (a)
48. Capitella capitata (a)
49. Polyphtalus pictus
50. Harmothoe sp. (a)
51. Anemonia contarinii (d)
52. Amphipholis squamata (d)
53. PLANAIRES N.D. (D)
54. Ciona intestinalis (a)
55. Chironomes n.d. (a)

(d) espèce disparue en 1983-84
(a) espèce apparue en 1983-84

Références

ABOULFEDA, (1306). - (traduit par J.T. REINAUD, 1883). - Géographie d'Aboulfeda. Imprimerie nationale (Paris), 2 (1), 327 p. 2 (2), 320 p.

M. BELKHIR , (1980). - Eutrophisation du lac de Tunis : études physico-chimiques et biomasses phytoplanctoniques et macro-algales. Thèse spécialité (Biologie marine) Faculté des Sciences de Tunis, 194 p. (ronéo)

M. BELKHIR et M. HADJ ALI SALEM, (1983). - Notions d'hydrologie comparée dans le lac de Tunis et le golfe de Tunis. Bulletin de l'Institut Scientifique et Technique, d'Océanographie et des Pêches, Salammbô, 10, 5-26.

F. BLANC et A. LAUREC, (1976). - De l'heuristique au thaumaturgique en traitement des données d'écologie marine. Cahiers ORSTOM, 14 101-107.

R. BRUNSCHVIG, (1936). - Deux récits de voyages inédits en Afrique du Nord au XVe siècle : voyage d'ABDELBASIT à Tunis en 1462 : voyage d'ADORNE à Tunis en 1470. Larose (Paris), 269 p.

A.F. BRUNN, (1940). - Etudes quantitatives sur la faune du lac de Tunis et du golfe de Tunis dans la région de Salammbô.Bulletin de la Station Océanographique de Salammbô, 4à, 20 p.

EL BEKRI, (1068). - (traduit par Mac GUCKLIN de Slane, 1913). - Description de l'Afrique septentrionale. A. Jourdan (Alger), 405 p.

Ch. (de) FOLARD, (1730). - Histoire de Polybe (traduit du grec par V. THUILLIER). P. Gaudouin (Paris), 2, 558 p.

L. FRANCK et J.J. MARCEL, (1816). - Histoire de la Régence de Tunis. Bouslama (fac simile, Tunis), 228 p., 28 pl., 1 c.

P. GRANCHAMP, (1625). - (édité en 1938). - Une mission délicate en Barbarie au XVIIe siècle. Aloccio (Tunis), 58 p.

V. GUERIN, (1861). - Voyage archéologique dans la Régence de Tunis. Plon (Paris), 1, 438 p., 2, 395 p.

J. HELDT, (1929). - Le lac de Tunis (partie nord) : résultats de pêches au filet fin. Bulletin de la Station Océanographique de Salammbô, 11, 1-16:

J. HELDT, (1953). - Mercierella enigmatica Fauvel et le comblement éventuel du lac de Tunis. Bulletin de la Station Océanographique de Salammbô, 33, 6 p., 2 pl.

M.G. KELLY et al., (1977). - Research on the eutrophication of the lake of Tunis, background and recommendations. Institut Scientifique et Technique, d'Océanographie et des Pêches, Salammbô, 17 p.

R. MOLINIER et J. PICARD, (1954). - Eléments de bionomie marine sur les côtes de Tunisie. Bulletin de la Station Océanographique de Salammbô, 48, 47 p.

E. PELLISSIER, (1853). - Description de la Régence de Tunis Bouslama (fac. simile, Tunis), 455 p., 1 c.

J.A. PEYSONNEL, (1724) et A. DESFONTAINES, (1783-1786). - (publié par Hureau de la Malle, 1838). - Voyage dans les Régences de Tunis et d'Alger. Gide (Paris, 1, 485 p., 2, 385 p.

RUDIS (équipe R.U.D.I.S., Yougoslavie, 1967). - Lacs de Tunis nord et sud : assainissement. Archives du Ministère de l'Agriculture (Tunis).

M.D. SHAW, (1743). - Voyages de Monsieur SHAW dans plusieurs provinces de Barbarie et du Levant. J. NEAULME (La Haye), 1, 414 p.

Société Hydrocure, (1950). - Etude de l'évacuation à la mer des eaux usées d'égout (recherches bactériologiques). Rapport du Ministère de l'Agriculture (Tunis), ronéo.

S. VUILLEMIN, (1952). - Les récifs à Mercierella enigmatica Fauvel. Bulletin de la Société des Sciences naturelles de Tunisie, 5, 209-211.

J. ZAOUALI, (1974). - Les peuplements malacologiques dans les biocénoses lagunaires tunisiennes. Etude de l'espèce pionnière Cerastoderma glaucum Poiret (1789). Thèse de Doctorat d'Etat, Université de Caen, 345 p., ronéo.

J. ZAOUALI et S. BAETEN, (1983). - Impact de l'eutrophisation dans la lagune de Tunis (partie nord) : analyse des correspondances. Rapports de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée, 28 (6), 327-332.

N.B. - Ce travail a été conduit en dehors du programme Med-Pol.

ACTION DES POLLUANTS SUR LES COMMUNICATIONS CHIMIQUES, INTERORGANISMES ET INTERPOPULATIONS, AU SEIN D'UN ECOSYSTEME MARIN

par

Maurice FONTAINE
Institut Océonographique,
195, rue St Jacques, Paris (France)

Introduction

Cet exposé serait prématuré si son auteur avait la prétention de le présenter comme la mise au point d'un ensemble cohérent de résultats importants obtenus dans les divers domaines évoqués. Mais, si l'on veut bien admettre que la science n'est pas faite seulement d'une somme de connaissances et de leurs interprétations, mais aussi de la prise de conscience de ce qu'on ignore, qu'il importe de connaître et qui apparaît à la portée de nos investigations aujourd'hui ou demain, alors je crois que peut se justifier le choix du thème précité. Le point de départ en est le rôle, en biologie marine, absolument primordial, et probablement très ancien des communications chimiques intra et interspécifiques qui assurent le maintien de la vie (l'exemple des gamones, qui ne sont en somme que des phéromones (p) de gamètes, en témoigne) et aussi le maintien de certaines formes très complexes de la vie qui sont représentés par des écosystèmes plus ou moins bien en équilibre.

Il y a déjà longtemps, l'attention a été attirée (FONTAINE, 1970) sur le fait que de nombreux polluants présentent vraisemblablement des capacités d'interaction avec certaines autres substances chimiques qui sont les agents assurant les communications chimiques évoquées dans le titre de cet article, agents qui ont reçu des noms très variés (ectohormones, métabolites externes, télergones, substances ectocrines, écomones (e), télémédiateurs chimiques) et parmi lesquels on a discerné plusieurs catégories (allomones, kairomones, p., exoactones, endoactones). Nous n'emploierons que le terme général d'e. dû à FLORKIN et celui de p. pour désigner les e. intra-spécifiques.

AUBERT et al., 1972, ont donné des exemples de l'action de plusieurs polluants chimiques sur la fonction antibiotique d'une Diatomée d'une part et de bactéries marines d'autre part, et aussi (PESANDO & AUBERT, 1975) sur un inhibiteur de croissance d'une Diatomée marine produit par un Péridinien et ils ont proposé certaines modalités d'action (AUBERT et al., 1975). Cette action peut se situer à divers niveaux des cycles de ces e. . Nous allons envisager ceux-ci successivement, et si nous donnons quelques exemples pour justifier notre point de vue, nous n'avons jamais la prétention d'être exhaustif.

Nous ne citerons que les e. dont on connaît, de façon plus ou moins approfondie, la nature chimique et tenterons de montrer, en premier lieu, la variété des voies métaboliques que peuvent toucher les très divers polluants.

Les bactéries marines peuvent attirer des prédateurs dans des conditions oligotrophiques par la libération d'A.M.P. cyclique. Des Dinoflagellés peuvent secréter des e. de nature protéique, l'une accroissant la synthèse des caroténoïdes chez une Diatomée, l'autre favorisant la libération par celle-ci d'un antibiotique vis-à-vis des bactéries (fraction nucléosidique), activité antibiotique potentialisée par la sécrétion d'acides gras protégés de la photooxydation par la synthèse accrue de pigments caroténoïdes (AUBERT M. et al., 1980-1981). Un antagonisme entre bactéries marines et certaines bactéries telluriques serait dû à un lipopolyoside (GAUTHIER, 1970).

Les gamètes femelles des algues brunes émettent des p. qui attirent les gamètes mâles, p. dont certaines ont été identifiées (ectocarpène et dihydroectocarpène, fucoserratène notamment) et qui appartiennent au groupe des hydrocarbures (BARBIER, 1972).

L'anémone de mer Anthopleura elegantissima, à la suite d'une attaque, émet une p. de défense, l'anthopleurine qui est un polypeptide de P.M.5.208. Chez la gorgone Plexaura homomalla, c'est une prostaglandine 15 S PGA_2 qui représente une e. la protégeant des prédateurs (GERHART, 1984). L'Octopus vulgaris assure sa défense non seulement par un jet d'encre noire et la fuite mais aussi par la présence dans ce jet liquide d'orthoquinones qui perturbent la fonction olfactive du prédateur. Parfois, ce sont les prédateurs eux-mêmes qui libèrent dans le milieu des e. incitant à la fuite des proies recherchées. Tels sont les esters de choline, produits par les glandes hypobranchiales des Gastéropodes prédateurs et qui chassent d'autres Mollusques. L'étoile de mer Marthasterias glacialis cause une violente réaction de fuite chez certains Mollusques et les corps responsables sont des saponines et des glucosides stéroïdiques.

Les larves de Balanus balanoïdes se fixent sur des surfaces où les adultes ont préalablement déposé un mélange attractif complexe formé de mucopolysaccharides et de protéines associées à des acides nucléiques. Pour la plupart des Crustacés Décapodes Brachyoures, l'attraction sexuelle du mâle par la femelle se produit lors de la mue et est attribuable soit à l'hormone de mue elle-même, l'ecdysterone (KITTREDGE et al., 1971), soit à un métabolite de ce stéroïde (FONTAINE, 1983).

De nombreux Invertébrés et Vertébrés marins émettent des flavines et des ptérines (FONTAINE et al., 1963 : MONZIKOFF & GAILL, 1973 ; MONZIKOFF, 1977) dont certaines au moins doivent se comporter comme e. (FONTAINE et al., 1978, 1982).

Chez les poissons, la nature chimique des e. apparaît aussi variée, p. ex. l'e. responsable de l'attraction du gamète mâle par le gamète femelle, celles responsables de la découverte de la proie, certaines e. réglant le comportement social sont de nature protéique : amines primaires, secondaires ou tertiaires, aminoacides simples ou substitués, estérifiés, méthylés ..., peptides (FONTAINE, 1981). D'autres e. émises par les crustacés et poissons femelles et guidant vers elles les mâles apparaissent de nature stéroïdique. Donc les e. émises par les animaux marins sont de structure chimique très variée et la diversité chimique elle-même des polluants permet de supposer que certains d'entre eux puissent agir sur la biosynthèse, la libération, l'état des e. dans le milieu aussi bien que sur la sensibilité des organes sensoriels qui reçoivent leurs messages et sur les p. de conduction de l'influx nerveux et d'intégration qui suivent.

Voyons, en premier lieu, la biosynthèse des e. :

de nombreux polluants, notamment des métaux, agissent sur les enzymes digestives qui sont nécessaires à l'assimilation des aliments, c'est-à-dire à l'apport des métabolites indispensables à la biosynthèse des e. Cette biosynthèse elle-même peut être perturbée directement par l'action des polluants sur des enzymes impliqués dans le métabolisme de certaines e. . Chez les sporophytes d'une algue brune (Laminaria saccharina) en culture, il a été constaté par KREMER & MARKHAM (1982) que de très faibles additions de Cd dans le milieu inhibent une ou plusieurs étapes de la biosynthèse des protéines. D'AMELIO et al., 1974, ont mis en évidence l'effet inhibiteur du plomb sur la synthèse des protéines chez les Crustacés et les poissons. VIARENGO et al., 1980 a et b, ont montré que le cuivre, à de très faibles concentrations, non seulement inhibe l'absorption par la moule Mytilus galloprovincialis des amino-acides présents dans l'environnement mais réduit aussi considérablement l'incorporation des amino-acides marqués dans les protéines, c'est-à-dire le taux global de synthèse de celles-ci alors que certaines protéines particulières liant le Cu sont induites dans les branchies. Les deux métaux précités doivent donc avoir une répercussion sur la biosynthèse de certaines e. . Le Cadmium pénétrant dans les organismes planctoniques se lie d'abord à des protéines et perturbe de nombreuses fonctions enzymatiques, les perturbations connues étant rassemblées par PREVOT (1980) à l'occasion d'une étude sur un Dinoflagellé marin Prorocentrum micans. Chez les poissons Téléostéens Tautogolabrus adspersus (GOULD & KAROLUS, 1974) et Pseudopleuronectes americanus (GOULD, 1977) le cadmium à doses subléthales affecte l'efficacité de métallo-enzymes et d'enzymes, efficacité qui dépend de mécanismes allostériques. GUPTA et al., (1983) observent que divers composés phénoliques stimulent

l'activité de la transaminase oxalacétique glutamique et de la transaminase glutamique pyruvique dans divers tissus de Téléostéens, ce qui suggère l'éventualité d'une conversion accrue des amino-acides en céto-acides ou vice versa et de toute façon une perturbation de métabolisme protéique. Sur les Téléostéens, l'intoxication par un herbicide, le basaline, à concentrations subléthales, inhibe la phosphatase acide, la deshydrogènase lactique et la transaminase pyruvique glutamique (RASHATWAR & ILYAS, 1983). JACKIM et al., (1970) et JACKIM (1973), ont souligné l'importance en général du rôle des métaux polluants dans l'inactivation de nombreuses enzymes chez les poissons.

Des agressions chimiques que représentent les polluants, interviennent non seulement sur l'activité des enzymes normales, mais sur la synthèse de nouvelles protèines, p. ex., la C.R.P. (C. reactive protein), (WINKELHAKE et al., 1983). et l'oxydase à fonction mixte. D'aussi profondes modifications métaboliques doivent intervenir sur la biosynthèse de certaines e.

Il est très probable que divers polluants puissent perturber aussi la synthèse des e. de nature stéroïdique. P. ex. le Cadmium (SANGALANG & O'HALLORAN, 1972), les P.C.B. (FREEMAN & IDLER, 1975) altèrent la synthèse des stéroïdes au niveau des testicules des Salmonidés. Le Cadmium et l'Aroclor (P.C.B) perturbent le métabolisme stéroïdien de la morue. Les eaux urbaines traitées altérent le métabolisme hépatique du 4 androstene-3, 17-dione chez la truite arc-en-ciel (HANSSON, 1981). Des polluants très divers (Méthylmercure, Cadmium, Arsenic, Selenium et un P.C.B. (Aroclor 1254) troublent la biosynthèse des hormones stéroïdiennes en augmentant le taux de cortisol et de testostérone (sauf le Se et l'Aroclor) et en diminuant la synthèse de corticostérone chez le phoque gris (FREEMAN & SANGALANG, 1977). Il n'est guère douteux que la biosynthèse des e. stéroïdiques soit également touchée par ces polluants.

Il faut aussi prendre en considération le fait que certains polluants sont tranformés par voie métabolique et excrétés sous la forme de substances susceptibles d'intervenir de façon différente des premières, soit sur les e. présentes dans le milieu ambiant, soit sur les récepteurs de celles-ci. On peut donner comme exemple le fait que le pétrole ingéré par les moules apparaît dans les fèces ou pseudofèces, mais sous une composition différente de celle polluant les eaux. Il contient des fractions plus lourdes et plus largement aromatiques, cette dérive des hydrocarbures aliphatiques vers des alcanes plus lourdes et des isoprenoïdes pouvant influencer l'action du polluant, notamment sur les organes sensoriels (MIRONOV, 1979).

La libération de certaines e. se fait très probablement au niveau :

a) de la membrane cellulaire pour de nombreux organismes microplanctoniques. La structure de celle-ci et son fonctionnement peuvent être altérés par de nombreux polluants.

b) de la peau. C'est le cas de certaines e. qui assurent les communications interindividus (HARA, 1973) : c'est le cas aussi très vraisemblablement d'acides aminés et de peptides qui déclenchent le comportement d'alimentation et très nombreux sont les polluants qui altèrent la structure de l'épithélium cutané. De plus, multiples sont ceux qui sans modifier la dite structure déclenchent une hypersécrétion de mucus qui est d'ailleurs considérée par certains auteurs comme un mécanisme de défense, soit qu'il agglutine les particules en suspension (JOERGENSEN, 1981), soit qu'il lie certains polluants, métaux en particulier (HOWEL, 1982). C'est ainsi que les floculats d'hydroxyde de fer déclenchent une telle réaction chez la Moule (WINTER, 1970). LOCK et VAN OVERBEEKE (1981) ont montré l'influence du chlorure mercurique et du chlorure de méthylmercure sur l'hypersécrétion de mucus chez un Téléostéen, et il est fort probable que la sécrétion de certaines e. est liée à la sécrétion du mucus.

c) du rein. RICHARDS (1974) a montré que certaines communications interindividus peuvent se faire par l'urine. Or, nombreux sont les polluants qui altèrent la fonction rénale notamment les métaux qui s'accumulent dans certians reins d'Invertébrés, ceux qui produisent une nécrose de l'épithélium tubulaire rénal chez les Téléostéens (VOGEL , 1959) qui entraînent une dégénérescence de certains tissus du rein ou qui modifient certaines activités enzymatiques de cet organe (DUBALE & AWASTHI, 1982 - CHATTERJEE et al, 1982 - SHAFFI, 1982).

d) des branchies : nous n'avons pas connaissance de travaux mettant en évidence de flux sortant d'e., mais le fait n'est pas à exclure étant donné l'excrétion par les branchies d'amines variés et le rôle

d'e. de certaines d'entre elles. Or, on sait que de nombreux polluants modifient non seulement la fonction respiratoire, mais aussi les différents échanges entre le sang et le milieu ambiant (action du Cu sur la régulation ionique de la Plie (Platichthys flesus) (STAGG, 1981) et sur la réduction de l'activité Na K ATPase de ces branchies (STAGG & SHUTTLEWORTH, 1982), action pertubatrice sur l'ionorégulation de la même espèce, par les effluents de l'industrie du dioxide de Titane (LARSSON et al., 1980), action perturbatrice du Zn sur l'épithélium branchial de la truite arc-en-ciel (SKIDMORE & TOVEL, 1972), action du Cadmium sur les structures des organelles et en particulier des mitochondries des branchies de la crevette Palaemon serratus (PAPATHANASSIOU & KING, 1983). Les eaux acides altèrent aussi certains flux ioniques au niveau des branchies des Téléostéens (FROMM, 1980, MC WILLIAMS, 1980). Il est donc probable que divers polluants peuvent agir sur cette voie de libération de certaines e.

e) de l'orifice génital : c'est le cas d'e. déclenchant le comportement reproducteur et issues probablement des organes génitaux ou de leurs annexes (FONTAINE M., 1981). Or. l'hormonogénèse au sein de ceux-ci, et par suite très probablement, l'écomonogénèse qui leur sont liées sont perturbées par divers polluants, p. ex. le Zn (SPERANZA et al., 1977). les hydrocarbures (LOPEZ et al., 1981).

Action des polluants sur les e. dans le milieu:

On ne peut omettre une action directe des polluants sur les e. dans les eaux marines. Le cas le plus fréquent est sans doute celui d'une complexation. C'est ainsi que nous avons montré que la riboflavine exerce à de très faibles concentrations une action attractive sur les Civelles ou les jeunes Saumons (FONTAINE et al., 1982) sous certaines conditions précisées dans ce travail. Les travaux d'ALBERT (1953) entre autres ayant montré la grande "avidité" des flavines vis-à-vis de plusieurs métaux à l'état de traces. nous nous sommes demandés si certains métaux polluants fréquents au voisinage des estuaires, en se fixant sur la riboflavine dissoute ne pourraient inhiber son action sur le comportement Des mélanges équimoléculaires de riboflavine (aux concentrations actives sur les animaux expérimentés) et soit de $Cu\ Cl_2\ 2\ H_2O$, soit de $Zn\ Cl_2$, soit de $Zn\ SO_4\ 7\ H_2O$ soit de $Ni\ SO_4\ 6\ H_2O$, à doses non toxiques pour l'organisme considéré, révèlent soit la disparition, soit la nette diminution de l'action attractive de la riboflavine (FONTAINE et al., 1982). Les concentrations de sels métalliques mis en oeuvre sont en deçà de diverses concentrations fréquemment observées dans certains milieux pollués et seules, n'exercent aucune action apparente sur le comportement des Civelles. Elles sont aussi nettement plus faibles que celles altérant la structure des papilles chémoréceptrices de certains Poissons. Nous supposons donc que c'est un phénomène de chelation s'établissant entre certains métaux et certaines e. - non seulement flavines, ptérines, mais aussi e. de nature protidique - qui fait perdre à celles-ci leur caractère de télémédiateur chimique. Ce fait est à rapprocher de l'inactivation par le Zn de la p. sexuelle de Brachydanio rerio (BLOOM et al., 1978). Des interactions hydrophobes peuvent se produire aussi entre les organohalogènés et les protéines et altérer l'action des e. Il est sûr qu'un champ beaucoup plus large est à explorer en ce qui concerne les réactions des diverses e. présentes dans les eaux marines avec des polluants eux-mêmes de natures chimiques très variées.

Il faut noter aussi que certains polluants à action trophique (nitrates, phosphates) peuvent entraîner des blooms de certaines populations planctoniques et par suite une augmentation d'e. produites vraisemblablement par ces populations. D'autres polluants peuvent altérer les parois cellulaires et libérer brutalement une quantité importante d'e. (cas des pesticides agissant sur Asterionella japonica (AUBERT et al., 1972).

Signalons enfin que les transformations photochimiques des hydrocarbures ou des terpènes peuvent donner naissance à de nouvelles substances susceptibles d'agir sur certaines e.

Action sur les récepteurs:

Certains polluants, d'une structure chimique de même nature que celle d'une e. peuvent mimer l'action de celle-ci sur l'organe sensoriel cible. C'est ce qu'ont montré DERENBACH & GEREK (1980), en étudiant l'action de différents hydrocarbures en cause dans certaines pollutions sur l'attraction de gamètes mâles de Fucus serratus. L'huile Diesel, la plus efficace de tous les produits essayés ne

l'était toutefois qu'à des concentrations qui étaient sensiblement supérieures à celles du fucoserratène (e. naturelle), la sensibilité passant d'une concentration de 10^{-9}M pour celui-ci à 10^{-7}M pour le polluant considéré. Mais, il faut aussi prendre en considération le fait que des hydrocarbures ingérés peuvent se retrouver sous des formes différentes dans les fèces et pseudofèces de certains organismes, comme nous l'avons vu plus haut, et exercer sur les chémorécepteurs une action bloquante ou stimulante que n'exerçaient pas les produits initiaux, avant tout processus métabolique.

Il est probable que de nombreuses pollutions, urbaines en particulier, peuvent contenir des substances protéiques, peptides, acides aminés qui peuvent mimer les actions de certaines e. D'autre part, de nombreux polluants altèrent structure et fonction des organes olfactifs ou gustatifs. Des travaux comme ceux de BARDACH et al. (1965) ont montré à quelle faible concentration (0,5 p.p.m. dans l'eau ambiante) certains détergents lèsent les bourgeons du goût d'un Téléostéen. A des concentrations encore plus faibles, il peuvent altérer la perméabilité des membranes. Différents métaux peuvent aussi affecter les épithéliums gustatifs ou olfactifs des poissons et rendre inefficace une p. d'attraction sexuelle (GARDNER & LA ROCHE, 1973), (BLOOM et al., 1978). Plus récemment, BODAMMER (1981) a décrit les lésions observées dans les appareils olfactifs des larves de Pseudopleuronectes américanus et de Melanogrammus aeglefinus à la suite d'immersion dans des eaux polluées par de faibles doses de cuivre.

D'après KUHNHOLD (1970), si les larves de divers poissons marins n'évitent pas les hydrocarbures dont elles ne semblent pas percevoir les effets qu'elles manifestent cependant sur les téguments, c'est que les chémorécepteurs sont bloqués dès le premier contact. Il se peut aussi que certains polluants, par la voie de leur action sur la biosynthèse ou la libération des hormones sexuelles stéroïdes diminuent la sélectivité du récepteur (FONTAINE, 1981).

Phénomènes de conduction de l'influx nerveux et d'intégration de stimulus sensoriels:

Il est sûr que l'action de certains polluants s'exerce sur la transmission du stimulus reçu par l'organe sensoriel et sur les mécanismes d'intégration au niveau des centres. Déjà en 1969, ANDERSON & PETERSON avaient montré que le poisson Téléostéen montre, en présence de D.D.T., des modifications du comportement qui doivent s'expliquer par une action de ce pesticide sur le système nerveux à deux niveaux au moins (moelle épinière et toit optique). Ces constatations ont reçu leur explication quand on eut montré une action inhibitrice de plusieurs pesticides organophosphorés et organochlorés, carbamates, sur l'activité acétylcholinéstérasique, activité dont on sait qu'elle est essentielle pour la conduction de l'influx nerveux. SCHNEIDER (1975) suppose qu'ils exercent leur action neurotoxique en se dissolvant dans la phase lipidique de la membrane axonique dont ils diminuent la fluidité, affectant ainsi certaines transformations allostériques.

Les polluants agissent aussi sur les phénomènes d'intégration par diverses voies. On sait depuis longtemps que le méthylmercure rendu célèbre par la maladie de Minamata exerce une action neurotoxique chez l'Homme et des animaux marins variés. La recherche de ses mécanismes d'action a fait l'objet de nombreuses publications. Dès 1977, les travaux de CHANG et de SYVERSEN ont montré leur complexité - je retiendrai ici :

a) l'action freinatrice sur la synthèse des protéines ou l'action de dénaturation sur celle-ci. Or, on sait maintenant que le recueil, la transformation et la transmission d'informations reposent sur la fermeture et l'ouverture de canaux ioniques, ceux-ci étant constitués par des protéines spécifiques, présentes dans les membranes des neurones. Tel est un mécanisme par lequel le méthylmercure peut intervenir dans les phénomènes d'intégration.

b) l'action perturbatrice sur le métabolisme du RNA Or, par cette voie, ce polluant peut altérer la mémorisation de l'organisme vis-à-vis d'une ou d'un mélange d'e. En effet, on sait que le saumon (S.) revient à sa rivière natale grâce à un phénomène d'"imprinting" qui se déroule à une étape précise de son cycle biologique, la smoltification. Or, l'"imprinting du S., par une e. artificielle, la morpholine, s'accompagne d'une augmentation du RNA du cerveau (RAPPOPORT & DAGINAWALA, 1968). Donc, tout polluant qui, comme le mercure, perturbe le métabolisme du RNA, doit compromettre les mécanismes d'imprégnation et de mémorisation qui sont essentiels dans ce type de communication chimique qui est certainement très répandu dans le milieu marin. Plus récement, OMATA et al. (1982) montrent que

l'action du méthylmercure s'exerce sans doute par une action perturbatrice sur les enzymes synthétisant ou dégradant certains neurotransmetteurs. D'après SHARMA et al. (1982), il toucherait tout particulièrement les voies dopaminergiques.

Les eaux de déchet de l'industrie sulfate-cellulose apportent des modifications importantes dans le système neurosécréteur hypothalamohypophysaire des Téléostéens et en particulier sur les structures peptidergiques, lesquelles jouent un rôle important dans les phénomènes d'intégration (TISHCHENKO et al., 1980).

ESCOUBET (1974) a montré que le lindane diminue l'activité de la glucose 6 phosphatase de l'encéphale de la Rascasse. Or, un métabolisme glucidique normal, qui implique la pleine activité de cette enzyme, est essentiel pour que l'activité et l'intégrité structurale du cerveau soient maintenues.

Tels sont quelques uns des exemples qui montrent que les processus nerveux et neuro-endocriniens de conduction et d'intégration des messages transmis par les e. peuvent être perturbés par divers polluants.

Conclusions

Cet aperçu sur les divers types d'action des polluants sur les e. n'a pas seulement un objectif spéculatif mais pratique. Les polluants potentiels qui suscitent les plus pressantes recherches sont des substances nouvelles créées par l'industrie chimique. La constatation éventuelle qu'elles exercent un effet nocif sur les animaux ou les écosystèmes marins conduit à rechercher d'autres substances atteignant, pour l'usager, le même but, mais ne présentant pas le même inconvénient. Le fait de savoir à quel niveau se situe l'action des substances nouvelles serait très utile pour guider la recherche de produits de synthèse nouveaux ne présentant pas d'effets regrettables sur la vie marine.

Je voudrais enfin souligner combien ces travaux ont une importance économique. En effet, les communications chimiques jouent un rôle extrêmement important dans le comportement alimentaire, essentiel pour la fonction de nutrition et donc pour la croissance dans le comportement reproducteur dont l'accomplissement normal est évidemment essentiel pour le maintien des stocks, dans le comportement migratoire dont le déroulement normal est si important pour les pêcheries. L'action des nouveaux produits chimiques qui apparaissent continuellement sur le marché et qui sont trop souvent rejetés dans les eaux marines, ainsi que celles des produits résultant de leur dégradation, devrait donc être envisagée aux divers niveaux d'intervention qui viennent d'être évoqués.

Références

A. ALBERT, (1953). - Quantitative studies of avidity of naturally occuring substances for trace metals. - The Biochemical Journal, 3 : 646-654.

J.M. ANDERSON & M.R. PETERSON, (1969). - D.D.T. sublethal effects on brook trout nervous system. -Science N.Y., 164, 3878 : 440-441.

M. AUBERT, M. GAUTHIER, B. DONNIER, D. PESANDO, J.M. PINCEMIN, M. BARELLI, (1972). - Effets des pollutions chimiques vis-à-vis des télémédiateurs intervenant dans l'écologie microbiologique et planctonique en milieu marin. - Revue Internationale d'Océanographie Médicale, 28 : 129-166.

M. AUBERT, D. PESANDO & J.M. PINCEMIN, (1972). - Télémédiateurs chimiques et équilibre biologique océanique. Quatrième partie : seuil d'activité de l'inhibiteur de la synthèse d'un antibiotique produit par une Diatomée. - Revue Internationale d'Océanographie Médicale, 25 : 17-22.

M. AUBERT, M. GAUTHIER & D. PESANDO, (1975). - Effets des pollutions chimiques vis-à-vis de télémédiateurs intervenant dans l'écologie microbiologique et planctonique en milieu marin - 2e partie. - Revue Internationale d'Océanographie médicale, 37-38 : 69-88.

M. AUBERT, M. GAUTHIER, J. AUBERT & P. BERNARD, (1980-1981). - Les systèmes d'information des micro-organismes marins. Leur rôle dans l'équilibre biologique océanique. - Revue Internationale d'Océanographie Médicale, 60-61 : 231 p.

M. BARBIER, (1982). - Les phéromones, aspects biochimiques et biologiques. - Masson : 140 p.

J.E. BARDACH, M. FUJIYA & A. HOLL, (1965). - Detergents : effects on the chemical senses of the fish Ictalurus natalis Le Sueur. - Science N.Y., 148 (3677) :1605-7.

H.D. BLOOM, A. PERLMUTTER, R.J. SEELEY, (1978). - Effect of a sublethal concentration of zinc on an aggregating pheromone system in the zebrafish, Brachydanio rerio (Hamilton-Buchanan). - Environmental pollution, 17 (2) : 127-131.

J.E. BODAMMER, (1981). - The cytopathological effect of copper on the olfactory organs of larval fish (Pseudopleuronectes americanus and Melanogrammus aeglefinus). - Int. Counc. Explor. Sea., C.M. 1981/E : 46.

L.W. CHANG, (1977). - Neurotoxic of mercury. A review. - Environmental Resources, 14 : 329-373.

S. CHATTERJEE, N.B. JASH, S. BHATTACHARYA, (1982). - In vivo and in vitro activation of peroxidase in the head kidney of Channa punctatus (Bloch) by sublethal doses of carbofuran. - Comparative and Physiological Ecology, 7 (2) : 129-131.

V. D'AMELIO, G. RUSSO & D. FERRARO, (1974). - The effect of heavy metal on protein synthesis in Crustaceans and fish. - Revue Internationale d'Océanographie Médicale, t. XXIII : 111-117.

J.B. DERENBACH, M.V. GERECK, (1980). - Interference of Petroleum hydrocarbons with the sex pheromone reaction of Fucus vesiculosus (L.). - Journal of Experimental Marine Biology and Ecology, 44 (1): 61-65.

M.S. DUBALE, M. AWASTHI, (1982). - Biochemical changes in the liver and kidney of a catfish Heteropneustes fossilis exposed to dimethoate. - Comparative and Physiological Ecology, 7 (2) : 111-114.

P. ESCOUBET, (1974). - Effets d'une pollution alimentaire par un organochloré chez la Rascasse Scorpaena porcus Linne 1758. - Thèse Doctorat 3e cycle · Biologie appliquée : Université Aix-Marseille-3 (France), 1974 : 62 p. Publiée par Faculté des Sciences et Techniques de St-Jérome, Marseille.

M. FONTAINE, S. PILLAPORT, A.M. SASPE, (1963). - Présence de ptérines dans la peau dorsale d'Anguilla anguilla L., et leur excrétion dans le milieu ambiant. Contrôle du métabolisme des ptérines par l'hypophyse. - Comptes rendus de la Société de Biologie, 157 (10) : 1721.

M. FONTAINE, (1970). - L'action des polluants sur la vie marine. Choix des critères expérimentaux. - Conférence F.A.O, Rome 1970. - In : Marine Pollution and Sea Life, F.A.O. Rome, 1972 : 194-203.

M. FONTAINE, J. MARCHELIDON, A. MOMZIKOFF, (1978). - Etude de la riboflavine de ptérines et des acides aminés libres dissous dans l'eau de mer d'une frayère de harengs. Oceanologica Acta, 1 (4) : 415-424.

M. FONTAINE, (1981). - Communications chimiques chez les poissons. - Journal de Psychologie (1) : 42-70.

M. FONTAINE, S. JEANJEAN & A. MOMZIKOFF, (1982). - Intervention possible de la riboflavine (vitamine B_2) comme télémédiateur chimique dans les écosystèmes aquatiques. - Comptes rendus de l'Académie des Sciences, 294, série III : 165-167.

M.T. FONTAINE, (1983). - Recherches sur la phéromone sexuelle et le comportement reproducteur de Carcinus maenas L. Crustacé decapode brachyoure). - Dissertation présentée pour l'obtention du grade de Docteur en Sciences. Facultés universitaires N.D. de la Paix, Namur (Belgique) (Département de biologie animale), 1983 : 201 p. + 54 planches.

H.C. FREEMAN & D.R. IDLER, (1975). - The effect of polychlorinated biphenyl and steroidogenesis on reproduction in the brook trout. - Canadian Journal of Biochemistry, 53 : 666-670.

H.C. FREEMAN & G.B. SANGALANG, (1977). - A study of the effects of Methyl Mercury, Cadmium, Arsenic, Selenium and a P.C.B. (Aroclor 1254) on adrenal and testicular steroidogenesis in vitro, by the gray seal Halichoerus grypus. - Archives of Environmental contamination and Toxicology U.S.A., 5 (3) : 369-383.

P.O. FROMM, (1980). - A review of some physiological and toxicological responses of freshwater fish to acid stress. - Environmental Biology of Fishes, 5 (1) : 79-93.

G.R. GARDNER & G. LA ROCHE, (1973). - Copper induced lesions in estuarine teleosts. - Journal Fisheries Research Board of Canada, 30 (3) : 363-368.

M. GAUTHIER, (1970). - Substances antibactériennes produites par les bactéries marines - 2e partie : Lipo-polysaccharides antibiotiques produits par certains germes marins appartenant aux genres Pseudomonas et Chromobacterium. - Revue Internationale d'Océanographie Médicale, t. XVII : 23-45.

D.J. GERHART, (1984). - Prostaglandin A_2 : an agent of chemical defense in the Caribbean gorgonian Plexaura homomalla. - Gordon Research Conference (Feb 12-17/84) on Marine Natural Products. Marine Ecology Progress series (sous presse).

E. GOULD & J.J. KAROLUS, (1974). - Physiological response of the cunner, Tautogolabrus adspersus, to cadmium. V. Observations on the biochemistry. - in : Physiological responses of the Cunner, Tautogolabrus adspersus, to Cadmium, pp.21-25. N.O.A.A. Technical Report, N.M.F.S., S.S.R.F.-681

E. GOULD, (1977). - Alteration of Enzymes in Winter Flounder, Pseudopleuronectes americanus, exposed to sublethal amounts of Cadmium Chloride. - In : Physiological responses of marine biota to pollutants. - F.J. VERNBERG ed. - New-York : Academic Press Inc : 209-224.

S. GUPTA, R.C. DALELA, P.K. SAXENA, (1983). - Effect of phenolic compounds on in vivo activity of transaminases in certain tissues of the fish, Notopterus notopterus, Environmental Research, 32 (1) : 8-13.

T. HANSSON, (1981). - Sex differences in the Hepatic in vitro metabolism of 4-Androstene-3, 17-dione in Rainbow Trout, Salmo gairdnerii. - General and Comparative Endocrinology,44 (2) : 181-188.

T.J. HARA, (1973). - Olfactory responses to amino acids in Rainbow trout Salmo gairdneri. - Comparative Biochemical Physiology, 44 : 407-416.

R. HOWELL, (1982). - The secretion of mucus by marine nematodes (Enoplus spp.) : A possible mechanism influencing the uptake and loss of heavy metal pollutants. - Nematologica, 28 (1) : 110-114.

E. JACKIM, J.M. HAMLIN & S. SONIS, (1970). - Effects of metal poisoning on five liver enzymes in the Killifish (Fundulus heteroclitus). - Journal Fisheries Research Board of Canada, 30 (4) : 383-390.

E. JACKIM, (1973). - Influence of lead and other metals on fish. - Aminolevulinate Dehydrase activity. - Journal of Fisheries Research Board of Canada, 30 (4) : 560-562.

S. JENSEN & A. JERNELOV, (1969). - Biological methylation of mercury in aquatic organisms. - Nature, 223 (5207) : 753-754.

C.B. JOERGENSEN, (1981). - Feeding and cleaning mechanisms in the suspension feeding bivalve Mytilus edulis. - Marine Biology, 65 (2) : 159-163.

J.S. KITTREDGE, M. TERRY & F.T. TAKAHASHI, (1971). - Sex pheromone activity of moulting hormone Crustecdysome, on male crabs, (Pachygrapsus crassipes, Cancer autonnarius, Cancer anthronyi). - Fishery Bulletin of United States, 69 : 337-343.

B.P. KREMER, J.W. MARKHAM, (1982). - Primary metabolic effects of cadmium in the brown alga, Laminaria saccharina. - Zeitschrift für Pflanzenphysiologie, 108 (2) : 125-130.

W.W. KUHNHOLD, (1970). - The influence of crude oils on fish fry. - In : Marine Pollution and Sea Life, Mario Ruivo ed. - London : Fishing News (Books) Ltd : 315-318.

A. LARSSON, K.J LEHTINEN, C. HAUX, (1980). - Biochemical and hematological effects of a titanium dioxide industrial effluent on fish. - Bulletin of environmental Contamination and Toxicology, 25 (3) : 427-435.

R.A. LOCK, A.P. VAN OVERBEEKE, (1981). - Effects of Mercuric Chloride and Methylmercuric Chloride on mucus secretion in Rainbow Trout, Salmo gairdneri Richardson. - Comparative Biochemistry and Physiology C., 69 (1) : 67-73.

E. LOPEZ, J. PEIGNOUX-DEVILLE, F. LALLIER, E. MARTELLY, Y.A. FONTAINE, (1981). - Anguilles contaminées par les hydrocarbures après l'échouage de l'Amoco-Cadiz. Modifications histopathologiques des ovaires, des branchies et des glandes endocrines. - Comptes rendus de l'Académie des Sciences, 292, serie III : 407-411.

P.G. Mc WILLIAMS, (1980). - Acclimation to an acid medium in the brown trout Salmo trutta. - Journal of Experimental biology, 88 : 269-280.

O.G. MIRONOV & T.L. SHCHEKATURINA, (1979). - Oil change in excretory products of mussels (Mutilus galloprovincialis). - Marines Pollution Bulletin, 10 (8) : 232-234.

A. MOMZIKOFF & F. GAILL, (1973). - Mise en évidence d'une émission d'isoxanthoptérine et de riboflavine par différentes espéces d'Ascidies. - Experientia, 29 1438-1439.

A. MONZIKOFF, (1977). - Substances fluorescentes (ptérines et flavines) dans les eaux de mer et des planctons marins. Essai d'interprétation écologique. - Université de Paris VI, 1977. - 240 p. Thèse d'Etat : Sciences naturelles.

S. OMATA, E. HIRAKAWA, Y. DAIMON, M. UCHIYAMA, H. NAKASHITA, T. HORIGOME, I. SUGANO & H. SUGANO, (1982). - Methylmercury-induced changes in the activities of neurotransmitter enzymes in nervous tissues of the rat. - Archives of toxicology, 51 : 285-294.

E. PAPATHANASSIOU & P.E. KING, (1983). - Ultrastructural studies on the gills of Palaemon serratus (Pennant) in relation to cadmium accumulation. - Aquatic toxicology, 3 (4) 273-284.

D. PESANDO & M. AUBERT, (1975). - Effets des pollutions chimiques vis-à-vis de télémédiateurs intervenant dans l'écologie microbiologique et planctonique en milieu marin. 3e partie. - Revue Internationale d'Océanographie Médicale, 39-40 : 109-116.

P. PREVOT, (1980). - (Intracellular action of cadmium : observations and absorption measurements). Etude de l'action intra cellulaire du cadmuim : Observations et mesures d'absorption. - Actualités biochimie marine, 2 : 117-134.

D.A. RAPPOPORT & H.F. DAGINAWALA, (1968). - Changes in nuclear RNA of brain induced by olfaction in catfish. - Journal of neurochemistry, 15 : 991-1006.

S. RASHATWAR & R. ILYAS , (1983). - Effect of chronic herbicide intoxication on in vivo activities of certain enzymes in the liver or freshwater fish Namachelius denisonii (Day). - Toxicology Letters, 16 (3-4) : 249-252.

I.S. RICHARDS, (1974). - Caudal neurosecretory system : possible role in pheromone production. - Journal of Experimental Zoology, 187 : 405-408.

G.B. SANGALANG & M.J. O'HALLORAN, (1972). - Cadmium-induced testicular injury and alteration of androgen synthesis in Brook Trout. -Nature, 240 : 470-471.

R.P. SCHNEIDER, (1975). - Mechanism of inhibition of rat brain (Na + K) adenosine triphosphatase by 2-2bis (p.chlorophenyl) 1, 1, 1, trichloroethane (D.D.T.). - Biochemical pharmacology, 24 : 939-946.

S.A. SHAFFI, (1982). - D.D.T. toxicity : Gluconeogenic enzymes and non-specific phosphomonoesterases in three teleosts. - Toxicology Letters, 13 (1-2) : 11-15.

R.P. SHARMA, C.N. ALDOUS, C.H. FARR, (1982). - Methylmercury induced alterations in brain amine syntheses in rats. - Toxicology Letters, 13 : 195-201.

J.F. SKIDMORE & P.W.A. TOVEL, (1972). - Toxic effects of zinc sulphate on the gills of rainbow Trout. Water Ressources, 6 (3) : 217-230.

A.W. SPERANZA, R.J. SEELEY, V.A. SEELEY & A. PERLMUTTER, (1977). - The effect of sublethal concentrations of zinc on reproduction in the zebrafish,Brachydanio rerio Hamilton-Buchanan. - Environmental Pollution, (12) : 218-222.

R.M. STAGG, (1981). - The effects of copper on branchial mechanisms of ionic regulation by the flounder (Platichthys flesus L.). - Thesis Ph.D. published by : Exeter University, Exeter, Devon (U.K.) : 271 p.

R.M. STAGG & T. J. SHUTTLEWORTH, (1982). - The accumulation of Copper in Platichthys flesus L. and its effects on plasma electrolyte concentrations. - Journal of Fish Biology, 20 (4) : 491-500.

T.L.M. SYVERSEN, (1977). - Effects of methylmercury on in vivo protein synthesis in isolated cerebral and cerebellar neurons. - Neuropathology and Applied Neurobiology, 3 : 225-236.

N.I. TISCHENKO, A.L. POLENOV & L.N. IVANOVA, (1980). - Réactions du système de neurosécrétion hypothalamohypophysaire du poisson Coregonus autumnalis aux eaux de rejets de l'industrie de la pâte à papier au sulfate. Zurnal evoljucionnoj Biochinii i fiziologii, 16 (Janv. 1980) : 53-59.

A. VIARENGO, M. PERTICA, G. MANCINELLI, R. CAPPELLI, M. ORUNESU, (1980 a). - The effects of Cu++ on the absorption of amino acids, on protein synthesis and on the ATP content in different tissues of Mytilus galloprovincialis. - Atti del 3. Congresso della Associazione italiana di Oceanologia e Limnologia, BERNARDI, R. de ed. - Pallanza (Italie) : A.I.O.L. : 441-449.

A. VIARENGO, M. PERTICA, G. MANCINELLI, G. ZANICCHI & M. ORUNESU, (1980 b). - The induction of proteins of binding Cu++ in the gills of mussels exposed to metal. - Atti del 3. Congresso della Associazone italiana di Oceanologia e limnologia, R. BERNARDI de ed. - Pallanza (Italie) : A.I.O.L. : 451-457.

F.S. VOGEL, (1959). - The deposition of exogenous copper under experimental conditions with observations on its neurotoxic and nephrotoxic properties in relation to Wilson's disease. - Journal of Experimental Medecine, 110 : 801-810.

J.L. WINKELHAKE, M.J. VODICNIK, J.L. TAYLOR, (1983). - Induction in rainbow Trout of an acute phase (C-reactive) protein by chemicals of environmental concern. - Comparative Biochemical Physiology, C. 74c (1) : 55-58.

J.E. WINTER, (1970). - Long-term laboratory experiments on the influence of ferric hydroxide flakes on the filter-feeding behaviour, growth, Iron content and mortality in Mytilus edulis L. - In : Marine Pollution and Sea Life, Mario RUIVO ed. - Londres : Fishing News (Books) Ltd : 392-396.

LES EFFETS DE LA POLLUTION SUR LES ECOSYSTEMES BENTHIQUES ET PELAGIQUES DANS LE GOLFE D'IZMIR (TURQUIE)*

par

A. KOCATAS, Z. ERGEN, S. MATER, I. OZEL,
T. KATAGAN, O. UCAL, T. KORAY et B. BUYUKISIK
Ege University, Faculty of Science, Biology Division,
Hydrobiyological Department, Bornova - Izmir (Turkey)

Abstract

This investigation is about the effects of pollution on the benthic and pelagic ecosystems of Izmir Bay. With this aim, 12 research stations have been selected, 6 from extremely polluted Inner Bay and 6 from the clear-water Gülbahçe Bay. Physico-chemical and biological results obtained during 1983 are as follows.

Both bay waters are quite similar in respect to temperature, though surface waters of Inner Bay are a little warmer. The pollution free waters of Gülbahçe Bay are much more transparent than those of Inner Bay. Gülbahçe Bay is slightly more saline than Inner Bay. Dissolved oxygen is around 7,5 mg/l in both surface and bottom waters of Gülbahçe Bay : whereas in surface and bottom waters of Inner Bay, this value is above 8 mg/l and below 6 mg/l, respectively. pH values are rather similar in both bays. Inner Bay waters are richer in nutrients (nitrite, nitrate, phosphate).

In both bays the plankton specimens belonging to Diatomophyceae Silicoflagellata, Dinoflagellata, Ciliata, Annelida (larvae) Mollusca (larvae) and Crustacae are encountered. Qualitatively, Gülbahçe Bay is especially rich in Diatomophyceae. Quantitatively Inner Bay is much richer in phytoplankton and zooplankton than Gülbahçe Bay. Gülbahçe Bay is richer, both qualitatively and quantitatively than Inner Bay in regards to benthic organisms. The number of species varies between 5-52 in Inner Bay stations and between 64-89 in those of Gülbahçe Bay. The number of individuals encountered in Gülbahçe and Inner Bays are 2341 and 1846 respectively. Qualitatively, Gülbahçe Bay, and quantitatively, Inner Bay are richer in regards to fish population.

*
* *

Introduction

Le golfe d'Izmir, au niveau économique et scientifique, est le golfe le plus important de la mer Egée. D'une part le port d'Izmir, dont l'activité commerciale est très importante et d'autre part agglomération d'Izmir, qui voit sa population et son industrie s'étendre chaque jour davantage, posent de graves problèmes pour l'équilibre écologique du golfe.

*Ce travail a été réalisé dans le cadre d'un projet UNEP/FAO.

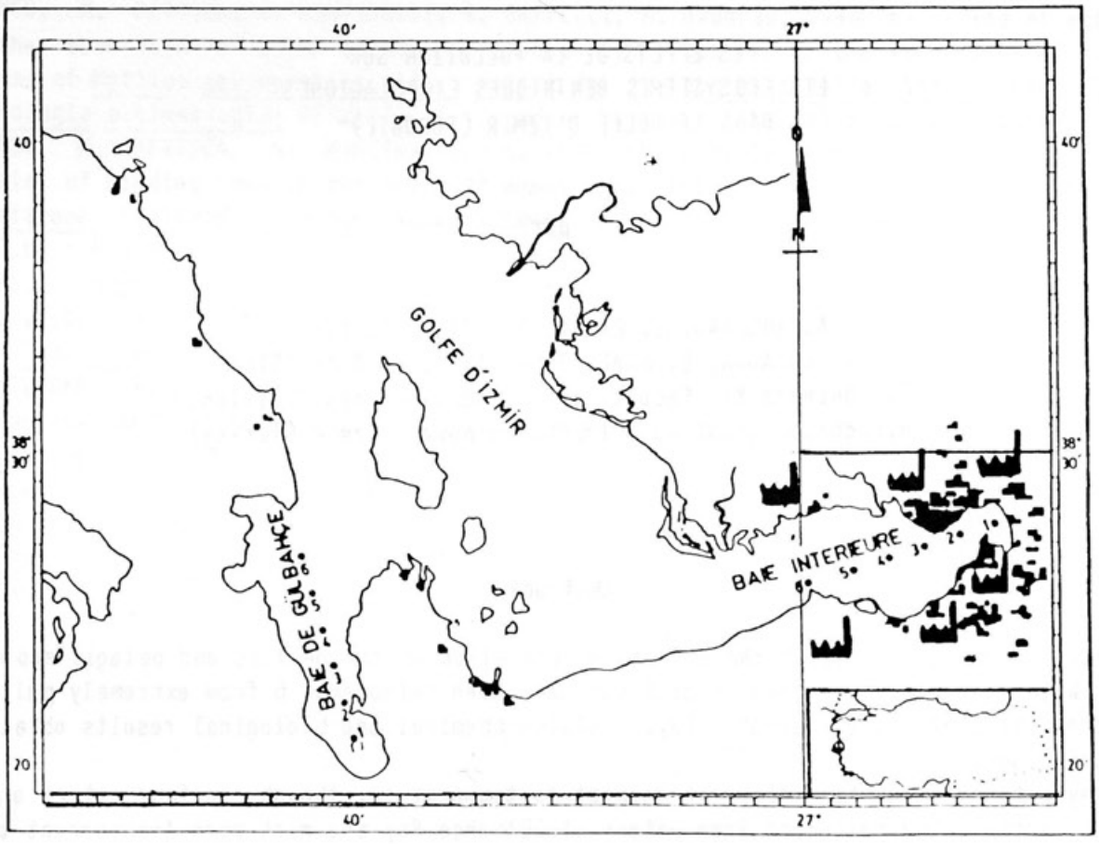


Fig. 1. - Carte du golfe d'Izmir : localisation des stations.

Du point de vue hydrographique et topographique, le golfe d'Izmir peut être divisé en deux zones : le golfe intérieur et le golfe extérieur : la partie intérieure s'étend à l'est du phare de Yenikale : la partie extérieure se situe entre le phare et la sortie du golfe (Fig. 1).

Le golfe intérieur peut être assimilé à une lagune, il est relié au golfe extérieur par un étroit chenal. Tout autour du golfe intérieur s'étend l'agglomération d'Izmir, sa banlieue (Bornova, Karsiyaka, etc.) et de nombreuses industries. La population d'Izmir qui ne comptait qu'un million d'habitants en 1975 atteignait 1.270.000 habitants en 1982 pour être actuellement estimée à 1,5 million. Dans le même temps l'industrie se développait et l'on compte aujourd'hui 1230 usines. A cela s'ajoute un important trafic maritime ainsi que de grands travaux d'aménagement : routes, etc.

En revanche autour du golfe extérieur, qui est beaucoup plus étendu, on ne trouve que quelques villages et peu d'usines.

Les effets néfastes de la pollution décelés dès 1970 augmentent chaque jour davantage. C'est en 1970 qu'a débuté une série d'études portant sur divers paramètres physico-chimiques (GELDIAY et al., 1975) : plancton (OZEL, 1979, MATER, 1979) : benthos (KOCATAS, 1978, 1981 : ERGEN, 1979: KOCATAS et GELDIAY, 1980) poissons (GELDIAY, 1969).

Sources de pollution

On peut diviser les sources de pollution du golfe d'Izmir en quatre catégories : rejets d'origine domestique, rejets industriels, trafic maritime (hydrocarbures), rejets solides (travaux publics).

Rejets domestiques : ces rejets qui augmentent parallèlement avec la population sont déversés directement dans le golfe par 128 émissaires et 10 petites rivières (GOKALP et al., 1983).

Dans ces eaux usées (168.910 m3/jour) on trouve une grande proportion de matière organique et inorganique. En 1980 on a estimé qu'il se déversait chaque jour 76.2 tonnes de matière organique, 1.575 tonnes de phosphates et 6.45.35 tonnes de nitrates et nitrites.

Rejets industriels : les 1230 usines qui bordent le golfe intérieur ont des activités très diverses : tanneries, alimentation et détergents, textiles, chimie et boissons : en 1980 ces industries rejetaient 9.969.950 m3/an d'eaux usées et 24.435 tonnes/an de matières déversées.

Trafic maritime : à l'activité commerciale du port d'Izmir qui est le port le plus important de Turquie s'ajoutent de nombreux bateaux de liaison ainsi que les bateaux de pêche. Le trafic maritime est donc intense dans cette partie du golfe.

Rejets solides : les rejets ont trois origines distinctes : la construction d'une nouvelle route autour du golfe intérieur : le remblai des zones peu profondes pour gagner du terrain sur la mer ; la construction de nombreux bâtiments en bord de mer.

Matériel et méthode

Six stations ont été choisies dans le golfe intérieur ainsi que dans la baie de Gülbahçe afin de suivre l'effet de la pollution sur les écosystèmes benthiques et pélagiques (Fig. 1).

Des prélèvements benthiques et pélagiques ont été effectués durant un an alors que les paramètres physico-chimiques tels que la température (YSI Modèl 33 SCT-mètre), la transparence, (Disque de Secchi), la salinité (Salinomètre-Beckman), pH (pH mètre), l'oxygène dissous (méthode de Winkler), les nitrites et les nitrates (méthode de WOOD) et les phosphates (méthode de STRICKLAND et PARSONS, 1972) ont été mesurés.

Le plancton était prélevé à l'aide de l'Hydrobios Universal serie water sample (5 litres). Les échantillons ont été précipités pendant une semaine et valarisés quantitativement : nous avons ensuite mesuré les individus et les cellules d'après SEMINA (1978).

Les prélèvements benthiques ont été effectués par la méthode de BELLAN (1967) à l'aide d'une benne "Orange-peel" qui permet de récolter chaque fois 4,5 litres de sédiment correspondant à environ un dixième de mètre carré. Dans chaque station, 9-10 litres de sédiments ont été tamisés avec un tamis à maille de 2 mm de côté.

Résultats

Paramètres physico-chimiques

Température : quand on compare les températures de l'ensemble du golfe, il semblerait que le golfe extérieur autour de Gülbahçe soit plus froid (Fig. 2). Les minima de température relevé (10°C) ont été mesurés dans la baie de Gülbahçe en janvier tandis que les maxima (27°C) étaient mesurés dans le golfe intérieur en juillet.

Transparence : il semble que la transparence varie de façon considérable entre le golfe intérieur et le golfe extérieur (baie de Gülbahçe) (Fig. 2). Les mesures effectuées fluctuent entre 183 et 340 cm dans le golfe intérieur et 960 et 1310 cm dans la baie de Gülbahçe. Si l'on considère l'ensemble du golfe, la valeur minimale a été relevée dans le golfe intérieur en septembre : 70 cm., tandis que la valeur maximale était relevée dans la baie de Gülbahçe en juin : 1830 cm.

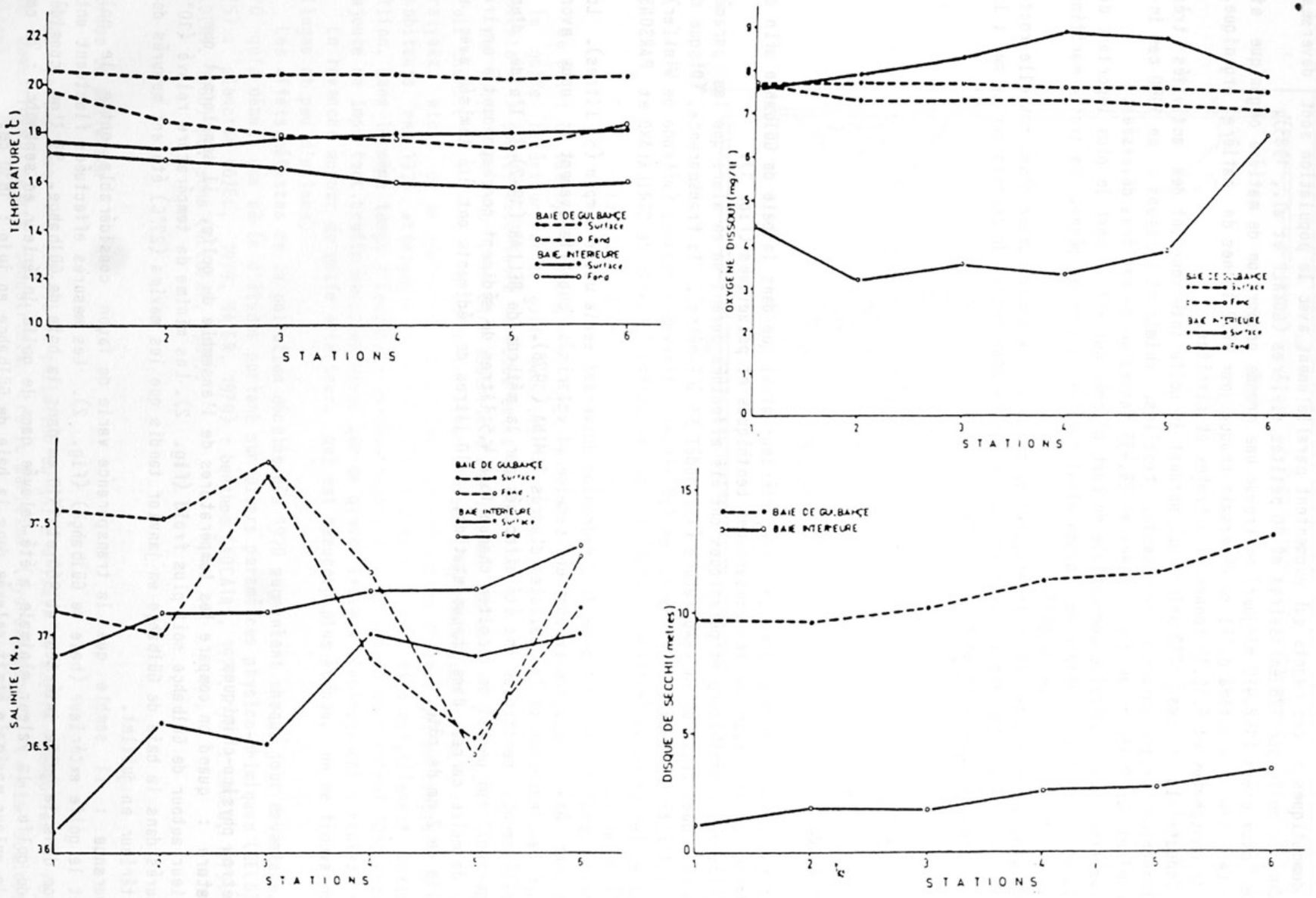


Fig. 2.
Variation moyenne de la température, de la transparence,
de la salinité et de l'oxygène pour chaque station.

Salinité : nous assistons à une variation régulère de la salinité du golfe intérieur vers la baie de Gülbahçe qui est sous l'influence directe des eaux de la mer Egée : ces fluctuations ont été enregistrées (Fig. 2). D'autre part, les mesures de salinité varient entre 36.1 ‰ à 37,4 ‰ dans le golfe intérieur et 36.52 ‰ à 37.78 ‰ autour de Gülbahçe. La salinité minimale (29.24 ‰) à été mesurée en février dans la baie de Gülbahçe et le maximum (38.60 ‰) dans le golfe intérieur en septembre.

Oxygène dissous : il a été observé que les mesures d'oxygène dissous varient entre 3.21 et 8.030 mg/l pour le golfe intérieur, tandis que pour la baie de Gülbahçe elles varient entre 7.1 et 7.7 mg/l (Fig. 2). Comme le montre la figure. la proportion d'oxygène dissous entre les eaux de surfaces et les eaux profondes, ne varie pas pour la baie de Gülbahçe tandis que pour le golfe intérieur, ces variations sont considérables. En juillet ainsi qu'en septembre on trouve dans les eaux profondes du golfe intérieur des valeurs très faibles d'oxygène, de l'ordre de 12.4 mg/lt.

pH : les valeurs de pH relevées dans les stations des deux régions du golfe sont sililaires (Fig. 3). C'est-à-dire qu'elles sont comprises entre 7.49 à 7.83 pour le golfe intérieur et 7.66 à 7.76 pour le golfe extérieur (baie de Gülbahçe). La valeur minimale (6.35) a été observée dans les eaux profondes du golfe intérieur en juillet tandis que la valeur maximale (8.40) était observée en septembre dans les eaux superficielles du golfe intérieur.

Nitrites : il a été observé que les concentrations en nitrites sont plus élevées dans le golfe intérieur (Fig. 3). Les valeurs relevées dans le golfe intérieur varient entre 1.20 et 11.30 ug/l tandis que dans la baie de Gülbahçe elles vareint entre 0.20 et 1.00 ug/l. Les concentrations en nitrites sont extrêmement faibles dans la baie de Gülbahçe en mai et juin ainsi que dans le golfe intérieur en juillet et septembre. La concentration maximale de nitrites (45.0 ug/l) a été mesurée en mars dans la baie intérieure.

Nitrates : les concentrations en nitrates révèlent des fluctuations entre les stations, le long des deux régions étudiées (Fig. 3). Les valeurs mesurées varient entre 14.9 ug/l dans le golfe intérieur et 26.5 ug/l dans les eaux de la baie de Gülbahçe . Les nitrates sont absents dans les eaux de la baie de Gülbahçe en mai et dans les eaux du golfe intérieur en septembre et octobre. La concentration maximale en nitrates (166.0 ug/l) a été observée en mars dans les eaux du golfe intérieur.

Phosphates : les concentrations en phosphates mesurées dans les eaux de la baie de Gülbahçe (1.3-5.6 ug/l) sont plus faibles que celles mesurées dans les eaux du golfe intérieur (14.5-124.2 ug/l) comme on peut le constater sur la Figure 3 Il a également été observé que les eaux de la baie de Gülbahçe, avaient des concentrations très faibles en phosphates en avril, mai et juin tandis que le maximum était trouvé en juillet (522.9 ug/l) dans le golfe intérieur.

Observations biologiques

Plancton : au cours de nos recherches dans l'ensemble du golfe d'Izmir nous avons observé que le nombre d'espèces dans le golfe intérieur varie entre 29 et 51 alors que dans la baie de Gülbahçe il varie entre 30 et 52 (Fig. 4). Au niveau qualitatif, le groupe des Dinoflagellés est largement dominant dans le golfe intérieur mais il est suivi de près par le groupe des Diatomées dans la baie de Gülbahçe.

Pour le zooplancton, les Ciliés sont plus abondants dans le golfe intérieur que dans la baie de Gülbahçe.

Au niveau quantitatif, le phytoplancton est plus important dans le golfe intérieur (valeur maximale 3x10 6 cellules/litre) que dans la baie de Gülbahçe (valeur maximale : 1500 cellules/litre).

Des observations similaires ont été effectuées pour le zooplancton : golfe intérieur, valeur maximale : 35.000 individus/l, baie de Gülbahçe, valeur maximale : 45 individus/l (Fig. 5).

Benthos : au cours de nos recherches benthiques, effectuées dans l'ensemble du golfe d'Izmir, nous avons trouvé 75 espèces et 1846 individus dans le golfe intérieur contre 176 espèces et 2341 individus dans la baie de Gülbahçe pour un même volume de sédiment (Tab. I).

Dans ces deux régions du golfe, les Polychètes sont dominants au niveau des espèces comme du nombre d'individus. Ils sont suivis par les Mollusques dans le golfe intérieur tandis que les Crustacés viennent en seconde position dans la baie de Gülbahçe.

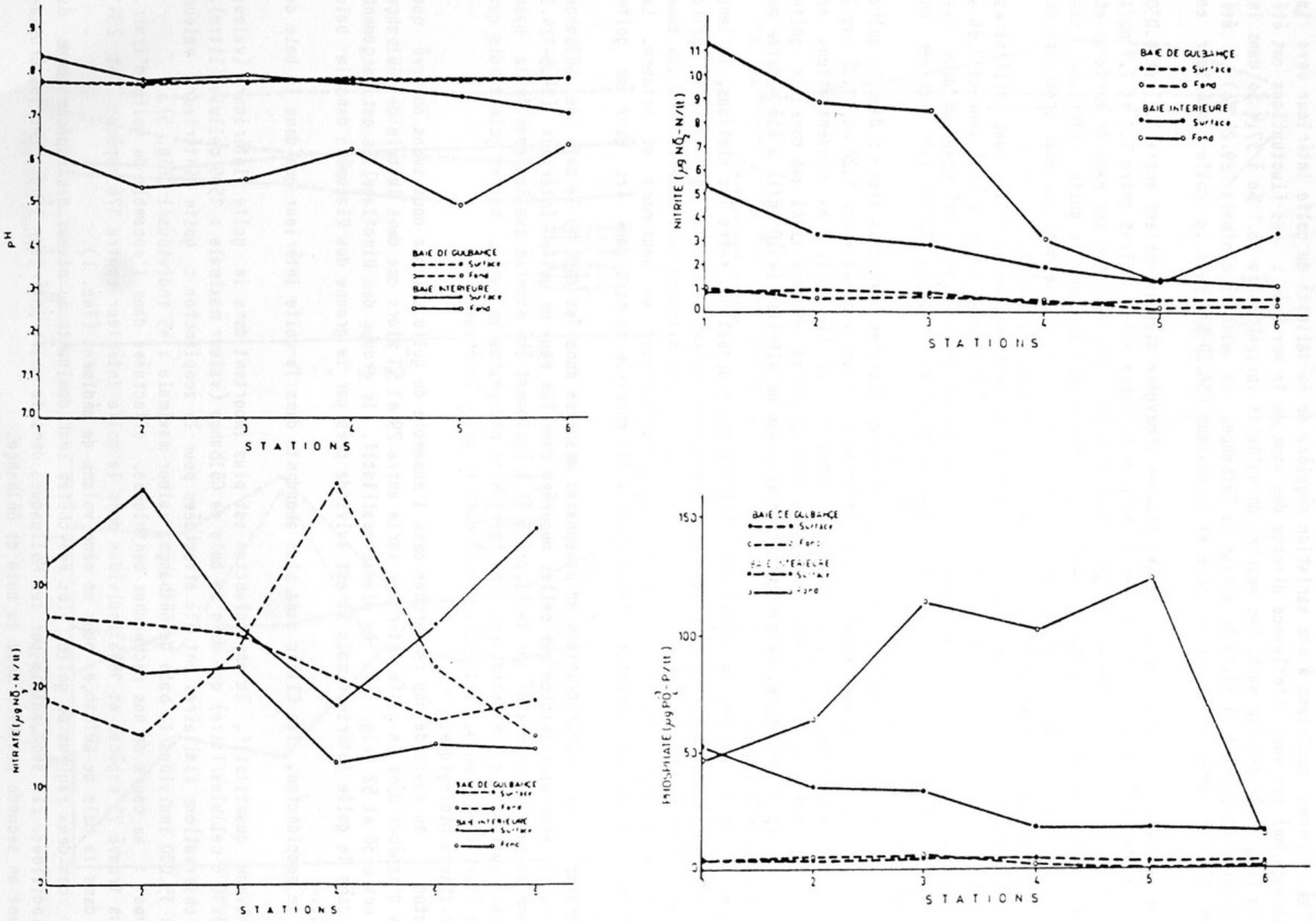


Fig. 3.
Variation moyenne du pH, des nitrites, des nitrates
et des phosphates pour chaque station.

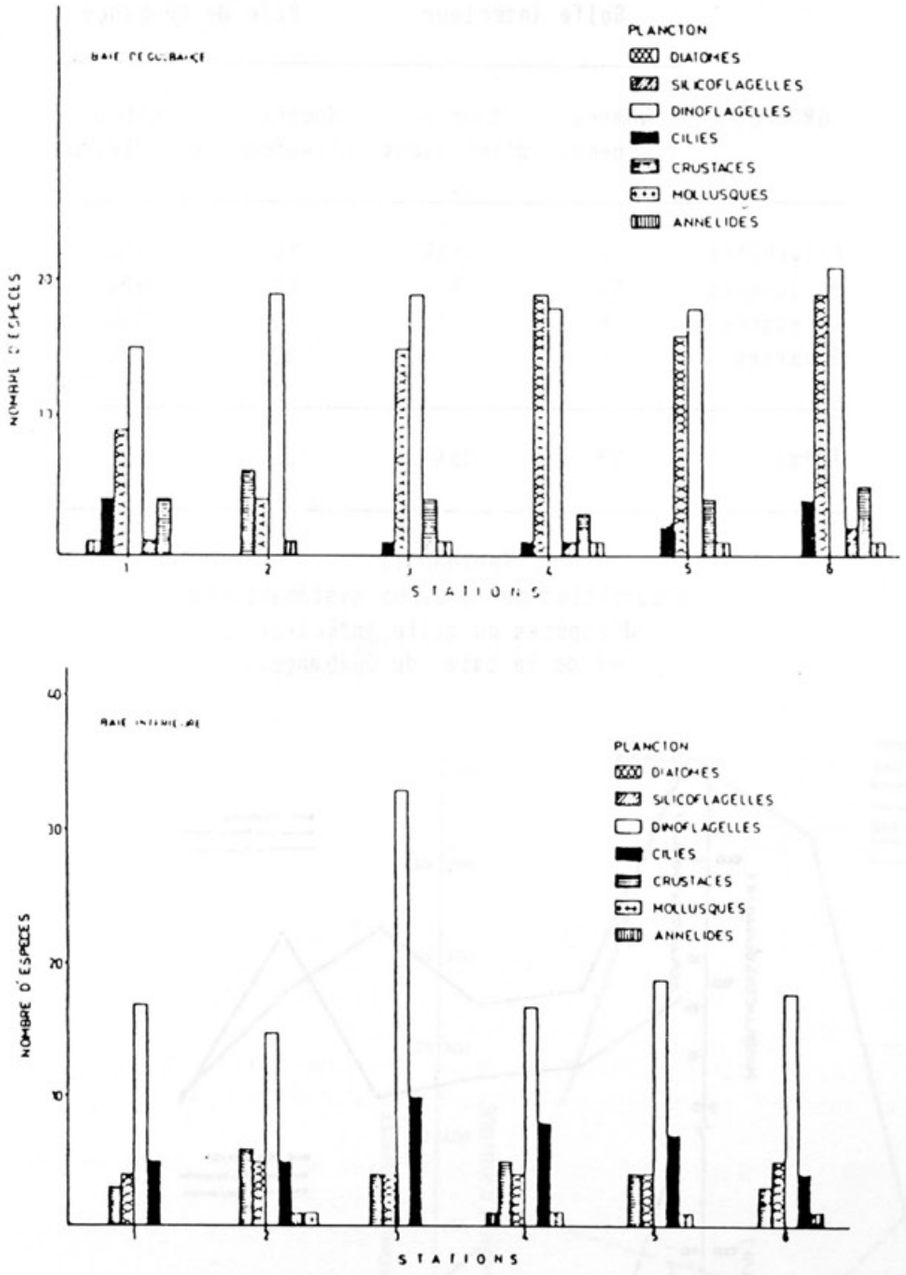


Fig. 4.
Variation du nombre d'espèces planctoniques
à chaque station.

Dans le golfe intérieur, le nombre d'espèces varie en fonction des stations entre 5 et 52 et le nombre d'individus varie entre 58 et 726. Pour la baie de Gülbahçe le nombre d'espèces varie entre 64 et 89, le nombre d'individus entre 334 et 436. A l'exception de la station 6 du golfe intérieur, quie se trouve dans une zone intermédiaire, le golfe intérieur est très pauvre en espèces benthiques. (Fig. 6).

GROUPES	Golfe intérieur		Baie de Gülbahçe	
	Nombre d'espèces	Nombre d'individus	Nombre d'espèces	Nombre d'individus
Polychètes	47	1324	76	1132
Mollusques	15	491	41	529
Crustacés	8	23	46	336
Diverses	5	8	13	114
Total	75	1846	176	2341

Tableau I.
Répartition par groupes systématiques
d'espèces du golfe intérieur
et de la baie de Gülbahçe.

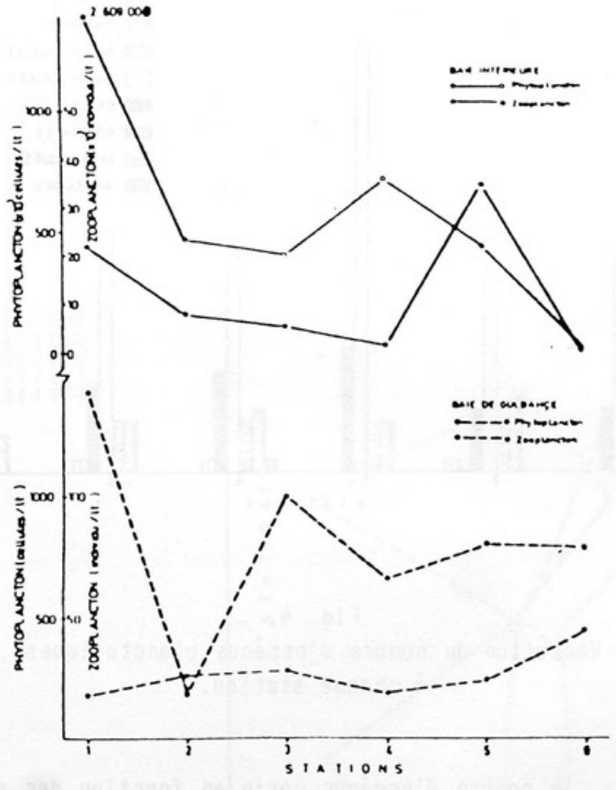


Fig. 5.
Variation du nombre d'organismes planctoniques
pour chaque station.

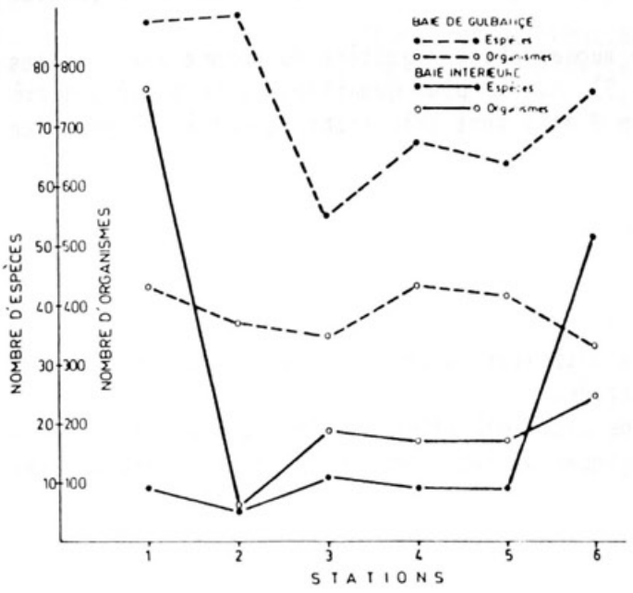


Fig. 6.
Distribution des différentes espèces benthiques et du nombre d'individus pour chaque station.

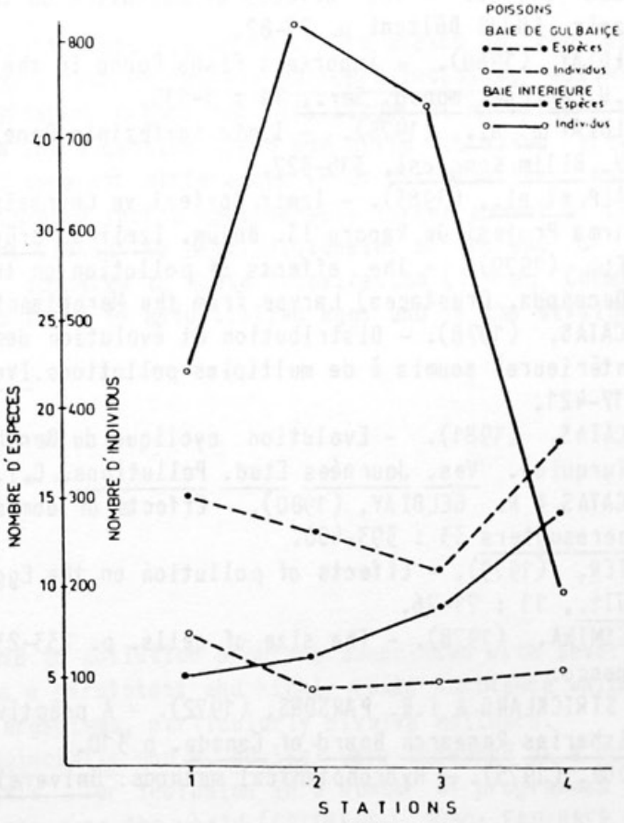


Fig. 7.
Distribution des différentes espèces de poissons et du nombre d'individus pour chaque station.

Poissons : il a été observé, au niveau qualitatif que le golfe extérieur (baie de Gülbahçe) est plus riche que le golfe intérieur.

Si nous considérons le golfe intérieur il y a une augmentation régulière du nombre des espèces de la partie intérieure vers la partie extérieure (Fig. 7). Sur le plan quantitatif, il a été observé que le golfe intérieur, et particulièrement les station 2 et 3 sont très riches grâce à l'abondance des anchois.

Conclusion

Ces recherches nous ont permis de comparer le golfe intérieur, soumis à de nombreuses sources de pollution, à la baie de Gülbahçe qui en est encore préservée.

Nous avons pu constater les effets très néfastes de cette pollution sur les écosystèmes benthiques du golfe intérieur alors que les écosystèmes pélagiques de cette partie du golfe d'Izmir semblent moins atteints et voient leur biomasse augmenter.

Références

G. BELLAN, (1967).- Pollution et peuplements benthiques sur substrat meuble dans la région de Marseille. Revue int. océanogr. méd., 6-7 : 53-87.

Z. ERGEN (1979). - The effects of pollution on the distribution of the Polychaeta in the Bay of Izmir. TUJJB Bülteni p. 77-82.

R. GELDIAY (1969). - Important Fishs found in the Bay of Izmir and their possible invasions. E.U.Fen Fak. monog. Ser., 11 : 1-135.

R. GELDIAY et al.. (1975). - Izmir Korfezinin Genel Hidrografisi Uzerine Ilk Gorüsler. T.B.T.A.K. V. Bilim Kongresi, 315-327.

N. GOKALP et al., (1983). - Izmir Korfezi ve Cevresinde Deniz Kirlenmesinin Tesbiti Uzerindeki Arastirma Projesi On Raporu II. Bolüm. Izmir Su Urünleri Bl. Müd., Arast. Proj. Rap. N°4-2, 1-21.

I. OZEL, (1979). - The effects of pollution on the distribution of Macrura Reptentia and Anomura (Decapoda, Crustacea) Larvae from the Meroplancton of Izmir Bay. T.U.J.J.B. Blt., 11 : 67-70.

A. KOCATAS, (1978). - Distribution et évolution des peuplements benthiques du golfe d'Izmir (Partie intérieure) soumis à de multiples pollutions.IVes Journées Etud. Pollution, C.I.E.S.M.,Antalya : 417-421.

A. KOCATAS, (1981). - Evolution cyclique du Benthos dans les zones de pollution du golfe d'Izmir (Turquie). Ves. Journées Etud. Pollutions. C.I.E.S.M. Cagliari.

A. KOCATAS & R. GELDIAY, (1980). - Effects of domestic pollution in Izmir Bay (Turkey). Helgolander Meeresunters 33 : 393-400.

S. MATER, (1979). - Effects of pollution on the Eggs of Teleost Fish in the Bay of Izmir.T.U.J.J.B. Bült., 11 : 71-76.

H.J. SEMINA, (1978). - The size of cells. p. 233-237. In : Phytoplankton manual. A. Sournia (Ed.) Unesco.

J.D.H. STRICKLAND & T.R. PARSONS, (1972). - A practival handbook of sea water analysis.Bull. N°. 167. Fisheries Research Board of Canada. p 310.

R.D. WOOD, (1975). - Hydrobotanical methods. University Park Press, Baltimore. p 173.

FORMATION OF INDUCIBLE Cd-BINDING PROTEINS SIMILAR TO METALLOTHIONEINS IN SELECTED ORGANS AND LIFE STAGES OF MYTILUS GALLOPROVINCIALIS

by

Jasenka PAVICIC*, Mirjana SKREBLIN*, Igor KREGAR**
Magda TUSEK-ZNIDARIC*** and Peter STEGNAR***
* Center for Marine Research Rovinj
"Ruder Boskovic" Institute, Rovinj, Yugoslavia
** Department of Biochemistry, *** Department of Nuclear Chemistry
"Jozef Stefan" Institute, "Edvard Kardelj" University,
Ljubljana, Yugoslavia

Abstract

Low molecular weight proteins similar to metallothioneins of mammalia which play an important role in the transport, storage and elimination of some toxic metals, were recently also isolated from several species of bivalve molluscs.

The aim of the present study was to determine inducible Cd-binding proteins in the digestive gland, gills and gonads, as well as in eggs and larvae of Mytilus galloprovincialis, assuming the importance of binding of the toxic metal in different organs and life stages of an edible species. The elution profiles of soluble fractions from the digestive gland and gills obtained after gel-filtration on Sephadex (G-75 and G-50) indicated apparent differences in Cd distribution, especially in chronically exposed mussels. It has been estimated that a considerably larger amount of Cd in the digestive gland was bound to inducible proteins similar to metallothioneins at the end of chronic exposure in sea-water containing 0.1 ug Cd/ml, compared to acute intoxication (1.3 ug Cd/ml). The formation of similar inducible proteins was also found in unfertilized eggs and in the straight-hinge veliger stage of the same species.

*
* *

Introduction

In recent years much attention has been paid to pollution problems associated with several trace metals. Cadmium has been generally considered as a persistent and highly toxic substance which may be accumulated to extremely high levels in marine organisms, particularly bivalve molluscs.

Mussels of the genus Mytilus (M. galloprovincialis and M. edulis) were accepted as a convenient indicator organism, particularly for trace metals, for inclusion in a number of programmes directed towards water quality control in different regions over the world (GOLDBERG, 1980; FAO/UNEP Mediterranean Action Plan).

Several studies, which appeared a few years ago, proved the formation of low-molecular weight inducible proteins similar to metallothioneins in different organs of the same organism (NOEL-LAMBOT, 1975; GEORGE et al., 1979; CARPENE et al. 1980, 1983). which besides a high affinity for cadmium and

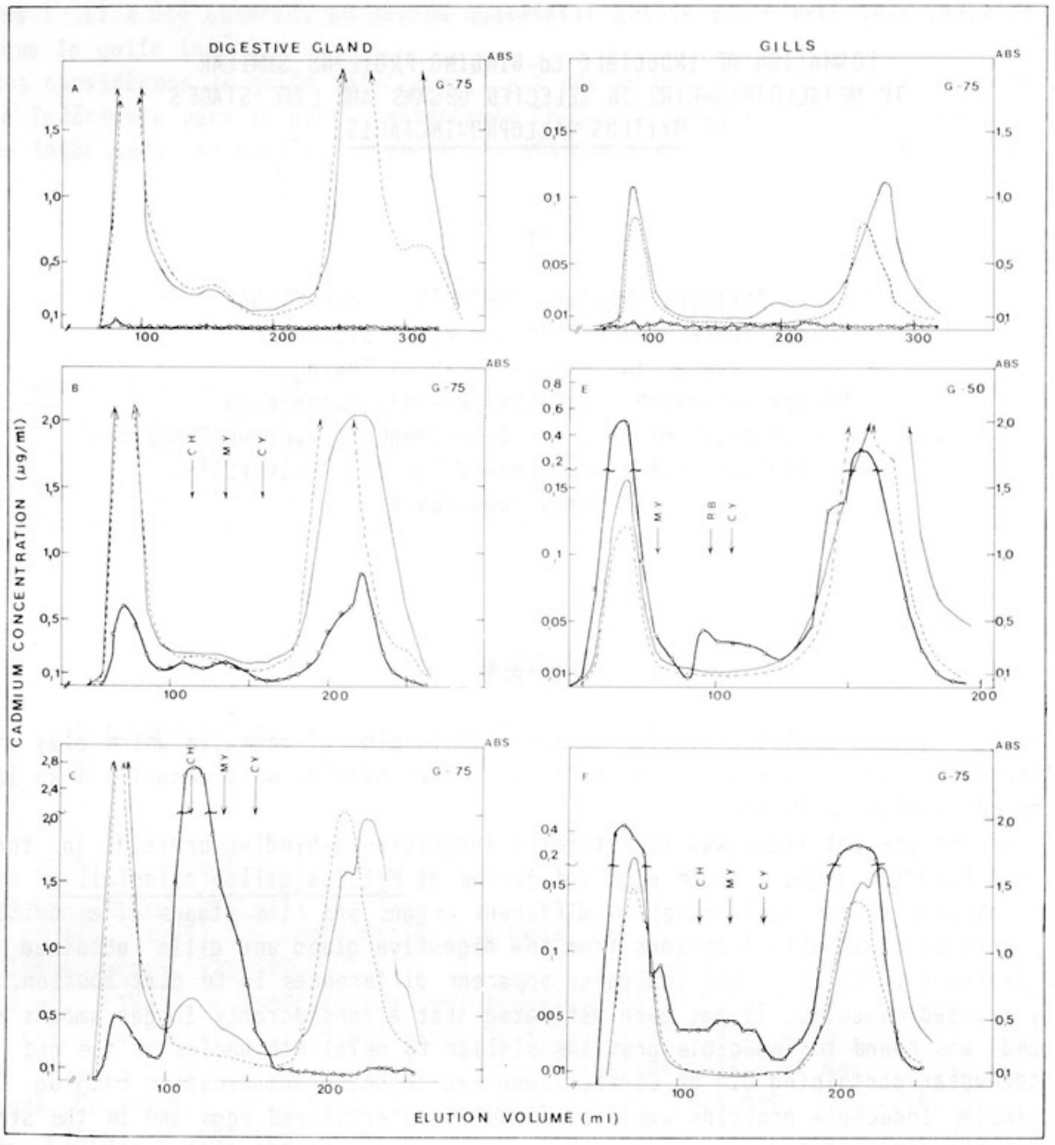


Figure 1. - Distribution of Cd (o-o) in the elution profiles obtained on gelfiltration of soluble fractions from the digestive gland and gills of M. galloprovincialis using different columns. Absorbances at 250 nm (——) and at 280 nm (---) are also presented.
A) Digestive gland, control, column size (77x2.5 cm)
B) Digestive gland, 7 days in 1.3 µg Cd/ml (65x2.4 cm)
C) Digestive gland, 120 days in 0.1 µg Cd/ml (65x2.4 cm)
D) Gills. control (77x2.5 cm)
E) Gills, 7 days in 1.3 µg Cd/ml (90x1.6 cm)
F) Gills, 120 days in 0.1 µg Cd/ml (65x2.4 cm)

some other metals, indicated, however, a certain disagreement in regard to amino-acid composition and molecular weight. Continuing investigations associated with better characterization and subcellular localization of metallothionein-like proteins (MLP) showed that the focal function has been postulated as the transport, storage and detoxification of Cd, Zn, Hg, Cu, etc. (GEORGE, 1980, ROESIJADI 1980).

The aim of the present study was to confirm the existence of MLP in M. galloprovincialis under different levels of exposure to Cd and its possible formation in eggs larvae of the same organism.

Material and methods

Adult mussels Mytilus galloprovincialis (length ranging between 4 and 8 cm) were collected from the rearing place in Limski Kanal on the north-western coast of the Istrian Peninsula, in the northern Adriatic. The water quality of the area in regard to the toxic metals level as reported by MARTINCIC et al. (1980) was considered as having "a low metal content" (total dissolved Cd at pH 2 was 0.015 ug/kg sea-water).

Adult organisms were exposed to cadmium as $CdCl_2 \times H_2O$ at two different concentration levels using an open, continous-flow sea-water system ($37\text{-}38 \times 10^{-3}$ salinity). Short-term intoxication was performed at 1.3 ug Cd/ml for 7 days at 20 °C (at the beginning of June). The experimental mussels were mostly at the beginning of their resting period. Chronic exposure took four months at a lower concentration level (0.1 ug Cd/ml) under decreasing temperature conditions (from 17 to 8 °C). The mussels were subjected to the toxicant for 120 days (from the end of October to the end of February) through a spawning season including the restoration of gonads.

Spawning of ripe adult mussels and rearing of their embryos were conducted following standard methods (LOOSANOFF and DAVIS, 1963). Fertilized eggs (density 100/ml) were exposed to Cd in four 10 litre glass containers to obtain sufficient quantity of larvae (collected on 50 um synthetic net) for further analysis and chromatography.

Composite samples of selected tissues, digestive gland, gills and gonads from the mantle were prepared using 15-20 specimens and stored deep-frozen until the beginning of tissue homogenization.

The tissues were homogenized in three volumes of 20 mM Tris-HCl buffer of pH 8.6 containing 0.1 mM PMSF by means of a motorized teflon pestle in an ice bath. The homogenate was centrifuged (Sorwal superspeed RC2-B centrifuge) at 4 °C and 27,000 g for one hour. Because of the lower inducibility of MLP in gills, both supernatants of gills prepared from Cd-intoxicated mussels were exceptionally freeze-dried under comparable conditions in order to obtain more concentrated samples (4x).

All fractionating operations were also performed at 4 °C using four different glass columns whose size is noted below the chromatograms.

Aliquots of previously filtered supernatants (2-6 ml) were fractionated by Sephadex gel-filtration chromatography using G-75 or G-50 gel types. The columns were calibrated with standard proteins of known molecular weights, chymotripsinogen A (25 000), myoglobin (17 800), ribonuclease (13 700) and cytochrome C (12 400). The position of the void volume was determined using blue dextran. The flow rate of the eluant buffer (also Tris-HCl, pH 8.6) in different chromatographic runs varied between 14-20 ml/H in order to obtain a fraction of 3-7 ml every 20 minutes by means of an automatic fraction collector.

The UV absorbance of each fraction was determined at 250 and 280 nm using a Beckman spectrophotometer. Cadmium concentrations in fractions were analysed by direct aspiration using a Varian atomic absorption spectrophotometer by the flame technique. The aliquots of tissue, homogenate and supernatant were previously digested in nitric-perchloric acid mixture (3/1) and analysed for Cd content on the same instrument.

Results and discussion

The elution profiles obtained after gel-filtration on Sephadex G-75 and G-50 of supernatants of homogenized mussel tissues (gills and digestive gland) from the control mussels and from those subjected to acute and chronic intoxication by Cd are presented in Figure 1. The supernatant patterns obtained correspond to the postlysosomal fraction being further resolved into three major peaks associated with different proportions of cadmium bound to the proteins or low-molecular weight compounds. The high molecular weight peak (HMW) is eluted just behind the void volume (V_0) of the column and contains most of the soluble enzymes (M.W > 80 000 on Sephadex G-75, and > 30 000 on Sephadex G-50). Metallothionein-like proteins (ML) were in 1-3 subfractions depending on the resolution of the column and on the tissue examined.

Cadmium is also associated with low-molecular weight compounds (LMW) too small to be resolved by the gels (M.W. < 3 000 on Sephadex G-75, and < 1 500 on Sephadex G-50).

The Cd-binding protein was partially characterized as a protein similar to metallothioneins because of several properties: being inducible by exposure to cadmium, heat-stable at 70 °C for 10 minutes (PAVICIC et al., unpublished results), showing a higher absorption at 250 nm because of Cd-mercaptide bonds, than at 280 nm because of a lack of aromatic aminoacids.

Apparently, the higher molecular weight ranging between 12 000 and 28 000 could be explained by the formation of dimers and trimers of the native protein (GEORGE, 1979, MINKEL et al., 1980).

Tissue	Exposure Cd (µg/ml)	Exposure Days	Cadmium content Tissue (wet weight) (µg/g)	Cadmium content Supernatant (µg/ml)	Extraction (%)	Cd distribution (%) HMW	MLP	LMW
Digestive gland	1.3	7	63.7	12.0	68	23.3	19.5	57.1
Digestive gland	0.1	120	121.0	28.5	84	10.5	86.8	2.7
Gills	1.3	7	35.2	2.7	30	43.4	9.9	46.7
Gills	0.1	120	32.1	2.8	37	44.9	8.7	46,4
Gonads (female)	0.1	120	16.5	1.2	29	9.2	90.8	0
Eggs (ripe)	0.1	55	9.2	0.8	41	78.9	11.8	9.3
Larvae	2.75	2	144.9	6.5	26	27.0	56.0	17.0

Table I. - Cadmium concentration in different fractions of selected tissues, eggs and larvae of Mytilus galloprovincialis. Cd determined is HMW, MLP and LMW fractions is expressed as proportion (%) of the total Cd in the supernatant.

The distribution of cadmium in different fractions obtained from selected tissues of adult mussels and those of their eggs and larvae at the end of defined exposure times is presented in table 1. By summation of the quantity of Cd in each of the chromatographic fractions, the proportions of Cd associated with each of the three major peaks were estimated in percentages.

Our results show apparent differences in the Cd distribution pattern of the digestive gland under low- and high-level exposures. During the short-term high-level exposure (7 days at 1.3 ug Cd/ml), Cd was accumulated to 64 ug/g on wet weight basis. It was predominantly bound to low molecular weight compounds (LMW) which were estimated to represent about 57 per cent of the total Cd in the soluble cytoplasmic fraction. The rest of the metal was distributed between HMW (23 per cent) and inducible MLP fractions (19.5 per cent). In the same tissue isolated from long-term low-level exposed mussels (120 days at 0.1 ug Cd/ml) the majority of Cd accumulated (121 ug Cd/g) was bound to the inducible MLP (87 per cent) only 10 per cent was associated with HMW, and less than 3 per cent with LMW compounds. Similar distribution patterns for cytosolic Cd in the digestive gland and the so called "soft body fraction" (midgut gland and kidney) have been reported in earlier studies (GEORGE et al., 1979; KOHLER and RIISGARD,1982). The formation of similar proteins was also evident in gills, although the inducibility was significantly lower and the distribution pattern of Cd markedly different from that in the digestive gland. Mussels subjected to both acute and chronic intoxication accumulated similar Cd concentrations in gills (35 and 32 ug Cd/g respectively). Also, similar elution profiles in regard to Cd distributions were obtained. Only 9-10 per cent Cd appeared to be bound to MLP and the major amount was almost equally distributed between HMW and LMW fractions (43-47 per cent).

Elution profiles of the soluble cytoplasmic fraction obtained from gonads, eggs and larvae of M. galloprovincialis are presented in figure 2. In female gonads sampled within the spawning phase of

the annual cycle, which were simultaneously subjected to low-level Cd intoxication, the metal was predominantly bound to MLP fraction (91 per cent), even though the total Cd concentration accumulated in gonads was several times lower than in the digestive gland. In contrast, ripe artificially spawned eggs indicated a significantly lower response (only 12 per cent of Cd was associated with the MLP fraction), although being exposed via adult mussels less than half the time (55 days) than the gonads.

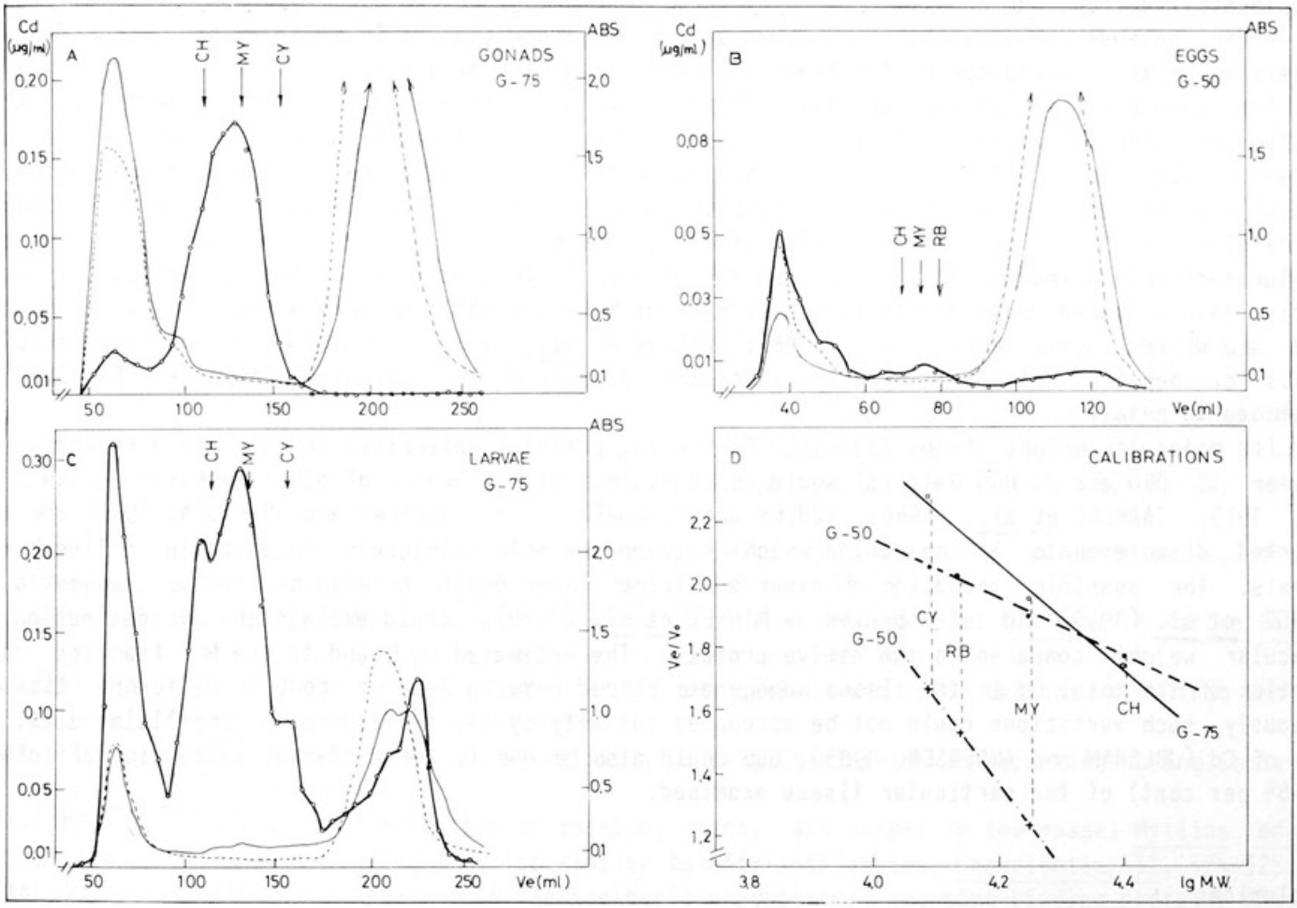


Figure 2. - Elution profiles of Cd, µg/ml (o-o) in soluble fractions of gonads, eggs and larvae of M. galloprovincialis. Absorbances at 250 nm (——) and at 280 nm (---) are also presented.
A) Gonads (female), 120 days in 0.1 µg Cd/ml (65x2.4 cm)
B) Eggs (ripe), 55 days in 0.1 µg Cd/ml (42x1.6 cm)
C) Larvae, 2 days in 2.75 µg Cd/ml (65x2.5 cm)
D) Calibrations of 3 different columns using standards :
Chymotripsinogen A (CH), M.W. 25 000
Myoglobin (MY), M.W. 17 800
Ribonuclease (RB), M.W. 13 700
Cytochrome C (CY), M.W. 12 400

Surprisingly, a strong inducibility in response to the high Cd concentration (2.75 ug Cd/ml) within two days of embryonic and early larval development was found in the straight-hinge veliger larvae. The high proportion of cytoplasmic Cd (56 per cent) associated with the MLP fraction could be partially explained by the estremely high tolerance towards Cd reported earlier of this particular life-history stage of the American oyster C. virginica (CALABRESE, 1973) and of M. galloprovincialis (PAVICIC, 1980).

A high proportion of Cd being bound to the MLP fraction in gonads and straigh-hinge veliger larvae would be consistent with the results from neonatal rats, where tissues undergoing rapid growth and development were able to synthesize high level of metallothioneins (BRADY, 1982). Very recently the first evidence appeared concerning the existence of metal-binding proteins in eggs of sea-urchins (OHTAKE et al., 1983) and of crab larvae (SANDERS et al:, 1983).

In isolating MLP using gel-filtration chromatography we were able to estimate roughly the proportion of Cd bound to this particular fraction as a proportion of the total Cd in the soluble cytoplasmic fraction. Our results provide evidence that the digestive gland could serve as an indicator organ because the inducibility, expressed as the proportion of Cd bound to the MLP fraction, appears to be the consequence of the level and duration of the exposure.

The possible application of metallothioneins as an additional monitoring parameter is still questionable with regard to specificity. It is well known that these kinds of proteins are normally present in organisms, taking part in the metabolism of some essential metals (Zn, Cu). Recently, the possibility has been recognized that a stimulus other than metals can act as an independent inducer of metallothioneines although the ability of metals would be much greater (20-50 fold increase) than of glucocorticoid hormones (2-5 fold increase) compared to the basal level (BRADY, 1982). Only a few studies have appeared dealing with the occurrence of MLP in aquatic organisms under field conditions (RAY and WHITE, 1981; ROCH et al., 1982; WIEDOW et al., 1982) supporting the statement that the levels of hepatic metallothionein are a specific measure of the biological response of fish and crustacea to metals.

The molecular weight of the inducible Cd-binding proteins determined in this study (fraction 1-3 between 12 000 and 28 000 Daltons) would be consistent with a number of other authors (GEORGE, et al., 1979, CARPENE et al., 1980; KOHLER and RIISGARD, 1982: JULSHAM and ANDERSEN, 1983) who also reported disagreements in molecular weight between the metallothionein fraction in molluscs and mammals. The possible formation of dimer and trimer forms due to disulphide bridges suggested by GEORGE et al. (1979) and later proved by MINKEL et al. (1980), could explain the overestimation of molecular weight compared to the native protein. The estimated Cd bound to the MLP fraction as a fraction of the total Cd in the tissue homogenate ranged between 2-72 per cent in different tissues. Obviously, such variations could not be accounted for only by the difference in subcellular localization of Cd (JULSHAM and ANDERSEN, 1983), but could also be due to the different extraction efficiency (26-84 per cent) of the particular tissue examined.

Conclusions

The induction of metallothioneins-like proteins has been demonstrated in selected tissues of M. galloprovincialis and also the formation of similar proteins in eggs and larvae of the same organism, by application of gel-filtration chromatography.

A considerable difference in the degree of induction of proteins similar to metallothioneins was found in the digestive gland between acutely and chronically exposed mussels, supporting the view that this particular fraction represents a measurable biological response of organisms to metals.

Further developments in the method to improve the selectivity, would give an opportunity to intensify studies on the physiological function of these proteins dealing with accumulation and toxicity of metals in marine organisms.

Aknowledgements

The authors are indebted to the Self-management Communisties of Interest for Scientific Research of S.R. and Croatia ans S.R. Slovenia, Yugoslavia,as well as the UNEP (MED POL, Research Programme) for financial support. Thanks are also due to Dr. A.R. Byrne for language corrections, and to Miss

A. Hrelja for typing of the manuscript. The valuable tachnical assistance of Mrs. M. Pregalj and Mrs. M. Spenko, as well as the help of Mr N. Ukmar in graphical presentations, is gratefully acknowledged.

References

BRADY F.O. (1982). - The physiological function of metallothionein. TIBS, 7(4), 143-145.

CALABRESE A. et al. (1973). - The toxicity of heavy metals to embryos of the American oyster Crassostrea virginica. Mar. Biol 18, 162-166.

CARPENE E. et al. (1980). - Cadmium-binding proteins from the mantle of Mytilus edulis (L.) after exposure to cadmium. Thalassia Jugosl. 16(2-4), 317-323.

GEORGE G. et al. (1979). - Characterization of cadmium-binding proteins from ussels, Mytillus edulis (L.) exposed to cadmium. Biochim. Biophys. Acta, 580, 225-233.

GEORGE S.G. (1980). - Correlation of metal accumulation in mussels with the mechanisms of uptake, metabolim and detoxification: A rview. Thalassia Jugosl., 16(2-4), 347-365.

GOLDBERG E.D. (1975). - The mussel watch: A first step in global marine monitoring. Mar. Poll. Bull. 6, 111.

JULSHAM K. and K.J. ANDERSON (1983). - Subcellular distribution of major and minor elements in unexposed molluscs in Western Norway: II The distribution and binding of Cadmium, zinc. copper, magnesium, manganese and iron in the kidney and digestive system of the common mussel Mytilus edulis. Comp. Biochem. Physiol., 75 A (1), 13-16.

KOHLER K. and H.U. RIISGARD (1982). - Formation of metallothioneins in relation to accumulation of cadmium, in the common mussel Mytilus edulis. Mar. Biol. 66, 53-58.

LOOSANOFF V.L. and H.C.? DAVIS (1963). - Rearing of bivalve mollusks. In Advances in Marine Biology 1, (F.S. Russel, Ed.), 1-130, Academic Press; London and New-York.

MARTINCIC D. et al. (1980). - Toxic metal levels in bivalves and their ambient water from the Lim Channel. Thalassia Jugosl. 16 (2-4), 297-315.

MINKEL D.T. et al. (1980). - On the sensitivity of metallothioneins to oxidation during isolation. Biochem. J. 191, 475-485.

NOEL-LAMBOT F. (1975). - Distribution of cadmium, zinc, and copper in the mussel Mytilus edulis. Existence of cadmium-binding proteins similar to metallothioneins. Experientia, 32, 324-325.

OHTAKE H. et al. (1983). - sea urchin (Anthocidaris crassispina) egg zinc-binding protein. Biochem. J., 211, 109-118.

RAY S. and WHITE M. (1981). - Metallothionein-like protein in lobsters (Homarus americanus). Chemosphere, 10 (11/12), 1,205-1,213.

ROESIJADI G. (1980). - The significance of low molecular weight metallothionein-like proteins in marine invertebrates: Current status. Mar. Environ. Res., 4, 167-179.

ROCH M. et al. (1982). - Hepatic metallothionein in rainboxw trout (Salmo gairdneri) as an indicator of metal pollution in the Campbell River system. Can. J. Fish. Aquat. Sci., 39 (12), 1,596-1,601.

SANDERS B.M. et al. (1983). - Free cupric ion activity in sea-water: Effects of metallothionein and growth in crab larvae. Science, 222, 53-55.

WIEDOW M.A. et al. (1982). - Cadmium-binding proteins from blue crabs (Callinectes sapidus) environmentally exposed to cadmium. Environ. Res., 328, 164-170.

QUANTIFICATION OF CRITERIA FOR GENOTOXIC RISK ASSESSMENT IN THE MARINE ENVIRONMENT: ATTEMPT TO DEFINE THRESHOLD QUALITY OF WATER

by

B. KURELEC, K. AL-SABTI
and K. ZAHN
Center for Marine Research, Ruder Boskovic Institute
YU-41001 Zagreb and Akademie der Wissenschaften und der Literatur
6500 Mainz (F.R. Germany)

Abstract

Screening the frequency of chromosomal aberrations (CA) in mussel populations inhabiting increasingly polluted sites gave a maximum at 7.7 per cent aberrant mitoses. Control mussels exposed for two days to such a maximally polluted site in a "mussel watch" experiment induce up to 6.8 per cent aberrant mitoses. Sites with a potential to induce higher percentages of aberrations within two days of exposure (i.e., 8.0 and 10.1 per cent) were not inhabited by mussels Mytilus edulis. Thus pollution with the potential to induce 6.8 per cent of aberrations after two days of exposure seems to represent the tolerable threshold level for mussel populations. Based on this criterion it is possible to re-interpret data on other parameters that have been gathered on other occasions in the same area. Using the CA as a standard, the threshold quality of water with respect to mussel populations was defined using as criteria the following eight parameters (maximal rate of CA in natural population of mussels, CA induced in mussels exposed for two days, activity of benzo(a)pyrene monooxygenase in the livers of the resident fish, "Mixed function oxydase induct test", damage of the DNA in the gills of mussels, Ames-microsomal test response to the extract of two litres of water, Ames-test response to the extract of 1 g of mussel tissue and the CA response of Allium roots exposed to the extract of 1 g mussel tissue).

*
* *

Introduction

The mussel Mytilus edulis accumulates genotoxins which than may be extracted and quantified by chemical or biological methods (PARRY et al., 1976: PARRY and AL MOSSAWI, 1979). Mussels can activate premptagens as was demonstrated by chemical analyses (STEGEMAN, 1981) as well as by mutagenicity testing of the metabolic products of their metabolism (ANDERSON and DOOS, 1983). This potential has been exploited in a mussel-host mediated assay for detection of premutagens in sea-water (PREZZA et al., 1982). Bioactivation of precarcinogens to the ultimate forms is responsible for at least a part of neoplasms found in mussels (MIX et al., 1979) as well as for the induction of chromosomal

structure aberrations and sister chromatid exchanges (SCE; DIXON and CLARKE, 1982). It is thus obvious that Mytilus edulis offers the potential for monitoring the genotoxic compounds among pollutants in the marine environment.

In this work we have exploited CA as indicator of the presence of genotoxins in actual field conditions as well as compared its correlativity to other biochemical indices relevant to the assessment of the genotoxic risk in the marine environment.

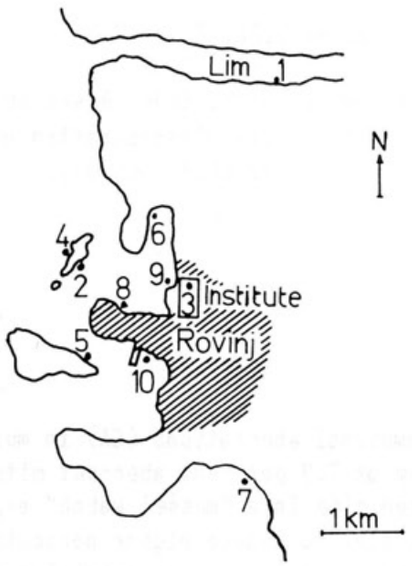


Figure 1. - Mussel collection sites in the Rovinj area.
Arrows : Main points of pollutional loadings.

Material and methods

Mussels. Specimens of the adult common mussel (Mytilus edulis), 5 to 7 cm in shell length, were collected from the low water neap tide level in the area of Rovinj, North Adriatic, at several sites that are under the influence of the local sources of mixed organic pollution (Figure 1). They were used within an hour after collection for chromosomal preparations.

Exposure experiments. The specimens collected in the Lim Channel served as controls and as material for exposition at other sites ("mussel watch"-type experiment, GOLDBERG et al., 1978). Ten specimens, placed in one nylon net, were anchored at the low neap tide level at selected sites for exposure for two days. Groups of 10 specimens (600 ml sea-water in glass bakers) were used in a laboratory two day exposition experiment with sea-water polluted by benzo(a)pyrene (BaP). Different concentrations of BaP (Roth, Karlsruhe, FRG) were prepared by dissolving BaP in acetone and consecutive dilution in sea-water. The additions of BaP in acetone were repeated after 24 h of exposure. The experiment was done in a dark room.

Chromosomal preparation. For the preparation of chromosomes the method of DIXON and CLARKE (1982) with slight modifications as described by AL-SABTI et al: (1983) was used. The mussel were first exposed to 0.04 per cent colchicine for 6 hours. Then the excised gills were treated with 50 per cent and then with 25 per cent sea-water for 30 min each. Subsequently the tissues were fixed in

Carnoy (3 ethanol: 1 glacial acetic acid), placed on clean slides at -5 °C, homogenized by forceps, dried in a flame, left at room temperature for three hours and finally stained with 20 per cent Giemsa solution for seven min.

Microscopy. One slides were examined by light microscopy (Opton, FRG) and photographs taken at a magnification of 10x and 100x on an EFKA 14 film.

Slides were scored "blind", with seven to nine mussels per group. On the average, in 3,200 microscopical fields per slide, 20 metaphases could be evaluated taking 2 h or, 2 days per group. The results presented in figures 2. and 3. require 44 full-time days of continuous scoring, or, more than two working months.

Definition of aberrations. In this work only breaks and fragments were evaluated. Aberrant mitoses were counted as single events irrespective of whether there were single or multiple aberrations per mitosis.

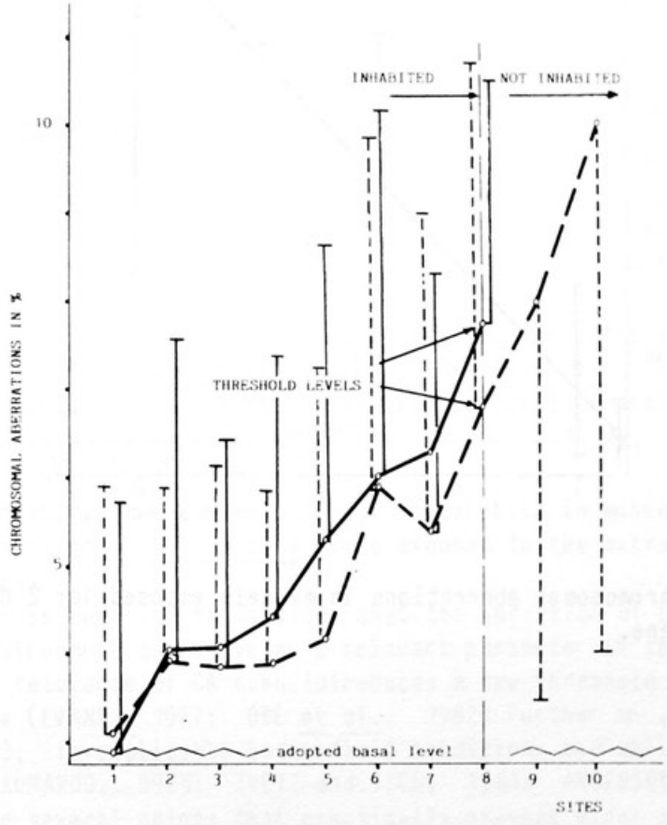


Figure 2. - Induction of chromosomal aberrations in mussels exposed to increasingly polluted waters. Full line : natural population. Dotted line: control specimens exposed for 2 days. Means ± SD are given.

Results

The frequency of aberrations in the gills of mussels from different sites of Rovinj area (Fig. 1.) are shown in figure 2. The maximal frequency of CA in this population was found to be 7.7 per cent (site 8). Since the Lim Channel is a highly protected area and since the number of aberrations found there (site 1) was the lowest, its frequency of 2.9 per cent was adopted as a basis.

In the "mussel watch" experiment, in the mussels transferred for two days to polluted sites, an increase in CA number was induced, the highest inductions of 3.5 and 2.9 fold being found in mussels

transferred to the harbour of Rovinj (site 10) and to the Institute pier (site 9), respectively. Both sites are not inhabited by mussel populations. The induction rates in mussels transferred to other sites approach the one found in the respective resident populations (Figure 2.). The highest CA induction frequency found for any site inhabited by mussels was 6.8 per cent (site 8).

In the groups of mussels exposed for two days to 1, 5 and 10 ppb of benzo(a)pyrene a dose response of CA induction could be observed (Figure 3).

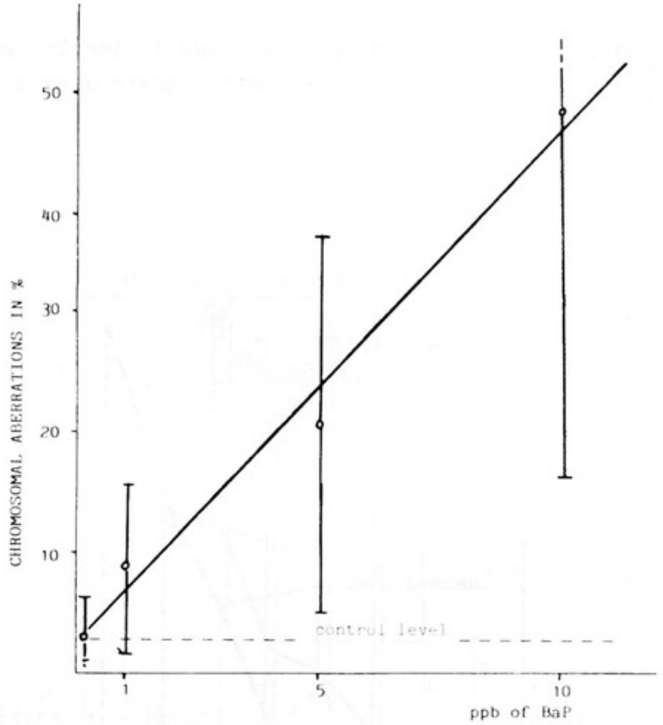


Figure 3. - Induction of chromosomal aberrations in mussels exposed for 2 days to sea water polluted by benzo(a)pyrene.

Discussion

The area under investigation in this work is defined with regard to the pollutional loads. The harbour of Rovinj (site 10, fig. 1) is a recipient of untreated domestic wastes as well as typical harbour wastes. At site 9., a constant source of pollution exists in the form of untreated wastes from a fish cannery. The waste products are intermittently visible at sites 2, 4 and 6. In addition, during the short summer tourist season wastes from touristic activities locally affect site 7.

The number of CA in the gills of mussels collected in August 1983 at different points mirror the quality of their environment (Figure 2). The number of CA is at sites 2, 4, 6 and 9 correlated to both the level of the environmentally induced benzo(a)pyrene monooxygenase activity found in the liver of the local fish Blennius pavo (KURELEC et al., 1977) and to the level of hexane extractable xenobiotica and mutagenic substances, as demonstrated in the BPMO-induct test as well as the Ames-microsomal test (KURELEC et al., 1979). According to these criteria the quality of water in this area decreases in the order of 1 to 10, very much in the same way as the induction of CA increases, more than doubling its rate from the basal one to the rate found in the mussels exposed to the most polluted sites.

Another purpose of this work was to find out whether the principle of the "mussel watch" could be applied to the genotoxicity assessment even of such areas as are not inhabited by mussels. Since exposure of naive, control mussels to benzo(a)pyrene increase the frequency of CA induction in a dose-response-like manner after only two days (Figure 3), this period was chosen as a standard time of exposure in the "Mussel watch" experiment. The CA frequency of Lim mussels exposed to the pollution of a given site has been found to approach the level of the one in the mussel population inhabiting that site. Even the clastogenic effect of waters at sites not inhabited by mussels (harbour, Institutes pier) can be assessed by the exposure of control mussels, for two days (Figure 2). Actually, at these sites the increase in CA frequency is highest. It is correlated to the pollutional loads and their toxic effects in such areas.

Based on this criterion it is possible to re-interpret data on other parameters that had been gathered on other occasions in the same area. Using the method for determination of CA frequency, the threshold quality of water with respect to mussel populations could be defined as:

1. - the water with the potential to induce more than 7.7 per cent aberrations in the gills of Mytilus edulis;
2. - the water that induces more than 6.8 per cent CA in the gills of the control mussels exposed for two days:
3. - the water that induces half-maximal activity of benzo(a)pyrene monooxygenase in the livers of the resident fish (KURELEC et al., 1977):
4. - the water that causes half-maximal induction of benzo(a)pyrene monooxygenase activity in the livers of experimental fish treated i.p. with the extract of 2 l of water in the "Mixed function oxydase - induct test" (KURELEC et al:, 1982):
5. - the water that causes the damage of DNA in the gills of resident mussels with more than one single strand scission in every second DNA domaine;
6. - the water that doubles the number of revertants per two litre extract in the Ames-microsomal test using TA 100 strain of Salmonella typhimurium (KURELEC et al:, 1979);
7. - the water that allows the accumulation of xenobiotica in mussels that double or triple the number of revertants of Salmonella typhimurium strains TA 100 or TA 98, respectively, per gram of tissue extracted (PARRY and AL-MOSSAWI, 1979): and,
8. - the water that allows the accumulation of xenobiotica in mussels that cause more than 10 per cent chromosomal aberrations in Allium cepa roots exposed to the extract of 1 g of mussel tissue (AL-SABTI and KURELEC, 1985).

At first glance it is tempting to conclude that the induction of CA in the gills of mussels transferred to a new environment can serve as a relevant parameter in the assessment of genotoxic chemicals present. This relevance of CA even introduces a new threshold quality: it measures the consequence of DNA damage (EVANS, 1977; OBE et al., 1982) Further on ,CA , contrary to the sister chromatid exchange (SCE), is applicable under field conditions and while SCE fails to detect some chemicals (THILAGAR and KUMAROO, 1983; IVETT and TICE, 1981: ANDERSON et al., 1981) CA is more reliable. Still, there are several points that practically prevent wider environmental application of this method 1. - the score of CA is obscured by its reliance on the skill and objectivity of the scorere, 2. - results are not easily accessible to statistical evaluation, 3. - the method is not as sensitive as the SCE or micronuclei test, and finaly, 4. - the method is very time consuming. All these points do not satisfy the principles and requirements that are expected from a reliable environmental method. This impedes the use of CA in mussel as monitors of marine pollution.

Acknowledgement

The present work was done within the framework of the activities of the UNEP CO-ordinated MED POL Programme. Financial support was received from the Authority for Scientific Research of the SR of Croatia and the Academy of Science and Letters, Mainz.

References

AL-SABTI K., B. KURELEC and N. FIJAN (1983). - A simple and fast technique for the chromosome preparation in fish. Veterinarski Arhiv 53,283-290.

AL-SABTI K. and B. KURELEC (1985). - Chromosomal aberrations in onion (Allium cepa) induced by water chlorination by-products. Bulletin of Environmental Contamination and Toxicology, 34, 80-88.

ANDERSON R.S., C.S. GIAM, L.E. RAY and M.R. TRIPP (1981). - Effect of environmental pollution on immunological competancy of the clam Mercenaria mercenaria: impared bacterial clearance. Aquatic Toxicology 1, 187-195.

ANDERSON R.S. and J.E. DOOS (1983). - Activation of mammalian carcinogens to bacterial mutagens by microsomal enzymes from a pelecypod mollusk. Mutation Research 116, 247-2.

DIXON D.R. and K.R. CLARKE (1982). - Sister Chromatid Exchange: A sensitive method for detecting damage caused by exposure to environmental mutagens in the chromosomes of adult Mytilus edulis. Marine Biology Letters 3, 163-172.

EVANS H.J. (1977). - Molecular mechanisms in the induction of chromosomal aberrations. In: Scott D., B.A. Bridges and F.H SOBELS (eds.): Progress in Genetic Toxicology, Elsevier North Holland, Amsterdam, pp 57-74.

FREZZA D., B. PEGORARO and S. PRESCIUTTINI (1982). - A marine host-mediated assay for the detection of mutagenic compounds in polluted sea-waters. Mutation Research 104, 215-223.

GOLDBERG E.D., V.T. BOWEN, J.W. FARRINGTON, G. HARVEY, J.H. MARTIN, P.L. PARKER, R.W. RISEBROUGH, W. ROBERTSON, E. SCHNEIDER and E. GAMBLE (1978). - The mussel watch. Environmental Conservation 5, 1-25.

IVETT J.L. and R.R. TICE (1981). - Diethylstibestroldiphosphate induces chromosomes aberrations but not sister chromatid exchanges in marine bone marrow cells in vivo. Environmental Mutagenesis 3, 445-452.

KURELEC B., S. BRITVIC, M. RIJAVEC, W.E.G. MULLER and R.K. ZAHN (1977). - Benzo(a)pyrene Monooxygenase induction in marine fish molecular response to oil pollution. Marine Biology 44, 211-216.

KURELEC B., Z. MATIJASEVIC, M. RIJAVEC, M. ALACEVIC, S. BRITVIC, W.E.G. MULLER and R.K. ZAHN (1979). - Induction of benzo(a)pyrene monooxygenase in fish and the salmonella test as a tool for detecting mutagenic/carcinogenic xenobiotics in the aquatic environment. Bulletin of Environmental Contamination and toxicology 21, 799-807.

KURELEC B., M. PROTIC, M. RIJAVEC, S. BRITVIC, W.E.G. MULLER and R.K. ZAHN (1982). Induction of benzo(a)pyrene monooxygenase in fish after i.p. application of water hexane extract: a prescreen tool for detection of xenobiotics. In: Richards N.L. and B.L. Jackson (eds.), EPA-600/9-82-013, 124-136.

MIX M.C., S.R. TRENHOLM and K.I. KING (1979). - Benzo(a)pyrene body Murdens and the prevalence of proliferative disorders in mussels (Mytilus edulis) in Oregon: In: Animals as Monitors of Environmental Pollutants, National Academy of Sciences, Washington, P. 52-62.

OBE G., A.T. NATARAJAN and P. PALITTI (1982). - Role of DNA double-strand breaks in the formation of radiation-induced chromosomal aberrations. In: Natarajan A.T., G. Obe and H. Altmann (eds.) Progress in mutation research. Vol. 4. Elsevier Biomedical Prep. Amsterdam. pp, 1-9.

PARRY J.M., D.J. TWEATS and M.A.J. AL-MOSSAWI (1976). - Monitoring the marine environment for mutagens. Nature (Lond.) 264, 538-540.

PARRY J.M. and M.A.J. AL-MOSSAWI (1979). - The detection of mutagenic chemicals in the tissue of the mussel Mytilus edulis, Environmental Pollution 13, 175-179.

STEGEMAN J.J. (1981). - Polynuclear aromatic hydrocarbons and their metabolism in the marine environment. In: Gelboin H.U and POP Ts'o (eds.) Polycyclic Hydrocarbons and Cancer. Academic Press, New York, P. 1-60.

THILAGAR A.K. and V. KUMAROO (1983). - Induction of chromosomal damage by methylene chloride in CHO cell. Mutation Research 116, 361-367.

LES COMPOSES ORGANOSILICIES ET L'ENVIRONNEMENT MARIN

par

R. FIRMIN et A.L.J. RAUM
Dow Corning Europe, Bruxelles (Belgique)

Législations

Les composés organosiliciés sont interdits d'immersion par le protocole de Barcelone, de 1976, étant considérés comme "dangereux" un pour l'environnement marin méditerranéen par des dispositions plus sévères que celles de la convention de Londres du 29 décembre 1972 sur la prévention de la pollution résultant de l'immersion de déchets pour l'ensemble des mers du globe.

Néanmoins, à ce jour, aucune trace ni d'effet n'a été décelé en Méditerranée.

Les seuls indicateurs existants de la présence de ces composés sont, d'une part, les publications de Pellenbarg relatives aux sédiments des eaux intérieures de la côte est des Etats-Unis (moins de 50 ppm aux sites de déposition des déchets de New-York et de Baltimore : baies de New-York et de Chesapeake, ainsi que dans la baie de Delaware) et d'autre part, les quelques traces en composés silicones volatiles (D4 et hexaméthyldisiloxane) décelées en surface des eaux du Rhin aux Pays-Bas en 1978 et 1979 (ppt) et dans les eaux et sédiments de la rivière Nagara au Japon en 1983.

Aucune trace ni effet n'a été décelé dans les eaux et sédiments tombant sous la juridiction des conventions relatives à une dizaine de mers régionales dotées de plans d'action P.N.U.E. ayant entre autres objectifs, de définir des protocoles similaires au protocole d'Athènes du 11 mai 1980, visant à lutter contre les pollutions telluriques dues aux déversements par les fleuves, établissements côtiers émissaires ou "toute autre source".

Le protocole d'Athènes concernant entre autres les eaux intérieures de la Méditerranée, il n'est pas exclu d'y trouver un jour des traces de composés organosiliciés.

Toxicité humaine et sort dans l'environnement

Une documentation scientifique abondante est disponible pour démontrer la remarquable innocuité généralisée des silicones utilisés, en raison de leur inertie physicochimique et de la dimension exceptionnelle de leurs molécules, dans diverses applications intéressant au premier chef la santé de l'homme, que ce soit comme additif ou en contact alimentaire, en cosmétologie, en pharmacie ou en médecine.

Les silanes, et en particulier, les chlorosilanes, précurseurs des silicones, sont corrosifs. Ils sont généralement utilisés au lieu même de leur fabrication en tant qu'intermédiaires dans la chimie des silicones et sont décomposés instantanément au contact de l'humidité, en silanols et siloxanes dont l'innocuité relative pour l'être humain est démontrée de longue date.

Le Dr BOITEAU a établi d'excellents résumés de la documentation toxicologique disponible pour la C.E.E. Ces évaluations ont été complétées pour les composés de dégradation environnementale, et des rapports sont actuellement en cours de finalisation pour le Gesamp par le professeur JERNELOV et le Dr MAGOS. Une équipe du T.N.O. d'Utrecht a remis son rapport final sur la question le 1er octobre 1984 à la C.E.E. Peu de publications publiques existaient en 1976 quant à la faible écotoxicité

relative des composés organosiliciés, y compris les silicones, vis-à-vis des organismes aquatiques, en particulier sur l'absence de bioaccumulation et de bioamplification.

Aucun mécanisme de dégradation n'était connu à l'époque de l'élaboration du protocole de Barcelone, alors qu'actuellement on connaît des mécanismes de dégradation physicochimique dans le sol, dans l'air et dans l'eau. Ces processus de dégradation peuvent expliquer pourquoi on ne décèle que des traces infimes de composés organosiliciés susceptibles de contaminer l'environnement aquatique.

Il est actuellement prouvé que si l'on déposait intentionnellement ces composés dans les sédiments marins, par immersion de boues contaminées, il n'y aurait aucune migration de composés o.s. dans la colonne d'eau, la mobilité des substances fixées étant limitée à celle des sédiments proprement dits.

Il est également prouvé que les organismes benthiques ne semblent pas significativement perturbés par la présence de quantités de composés o.s. largement supérieures aux concentrations maximales que l'on pourrait rencontrer dans la pratique, dans les stations d'épuration situées dans les zônes d'utilisation les plus intensives.

Nous nous proposons de résumer succinctement les principales données publiées sur l'écotoxicité vis-à-vis d'organismes aquatiques des composés organosiliciés.

Ecotoxicité vis-à-vis d'organismes aquatiques

Nous étudierons d'abord les effets causés par le diméthylpolysiloxane (D.M.P.S.) composé principal (plus de 50 %) et colonne vertébrale de tous les silicones, pour étendre ensuite l'investigation aux compositions soit dérivées du D.M.P.S., soit aux composés précurseurs ou intermédiaires y compris les monomères et oligomères ou les substances de dégradation environnementale.

Les polysiloxanes sont très peu écotoxiques en raison de leur faible solubilité aqueuse, de leur inertie et des volumes moléculaires empêchant en général l'absorption au travers de membranes biologiques.

La grande perméabilité du D.M.P.S. à l'oxygène doit être soulignée, sa limite de saturation pour cet élément étant supérieure à celle d'une solution saline isotonique. CABRIDENC a revu récemment l'évaluation des résidus du D.M.P.S., 50 cS, dans l'environnement aquatique en raison des possibilités de substitution des P.C.B.'s par des huiles silicones dans les transformateurs électriques.

1. Effets du D.M.P.S. sur les micro-organismes, les algues et le plancton.

Les principaux résultats sont résumés aux tableaux I et II. Aucune écotoxicité significative n'a été relevée en particulier pour les organismes impliqués dans la fermentation des boues activées les résultats obtenus confirmant les nombreuses analyses de BOD indiquant une inertie complète.

Les effets sur la croissance des algues ont été observés surtout sur Selenastrum capricornutum et sur Anabaena flos-aquae tant pour ce qui concerne les monomères et oligomères cycliques ou linéaires que pour les polymères. Des études réalisées sur un plancton marin démontrent que des flagellates, des diatomées et divers autres organismes (citons Dunaliella tertiolecta, Tetraselmis suesica, Phaeodactulum tricornutum, Gyrosigma spenceri...) se comportent normalement à des concentrations nominales de 10 ml/l de D.M.P.S. de viscosités variant de 100 à 12.500 cS.

L'équipe du CERBOM de Nice sous la direction du professeur M. AUBERT, ne détecte une TL50 de 75 h. sur Tetraselmis qu'à partir d'une concentration nominale atteignant 10.000 ppm, sans déceler d'effets significatifs sur Artemia salina pour une exposition à des concentrations de l'ordre de 20.000 ppm d'une émulsion acqueuse de D.M.P.S.

MEURICE n'a exposé cette dernière espèce qu'à des concentrations de 500 ppm sans obtenir d'effets sur le comportement après 24 heures d'exposition et sans déceler la moindre mortalité.

Daphnia magna Str. est sensible à l'engluement physique causé par les liquides D.M.P.S. en milieu clos d'aquarium, mais il ne s'agit pas d'une écotoxicité au sens biochimique du mot. En microcosme, à des concentrations environnementales réalistes, aucun effet significatif n'est obtenu

sur la mortalité, la croissance ou la reproduction de daphnies en présence de D.M.P.S. insoluble ou de diméthylsilanediol soluble : a fortiori en milieu marin soumis à une dynamique normale.

Les émulsions aqueuses de D.M.P.S., utilisées comme agent antimousse dans l'industrie alimentaire et dans les stations d'épuration ont un effet d'immobilisation moins marqué sur la daphnie par rapport aux essais en laboratoire. Les CI50 varient en laboratoire de 73 à plusieurs dizaines de milliers de ppm selon la concentration et l'écotoxicité des émulsifiants.

2. Toxicité du D.M.P.S. vis-à-vis des Annélides

Des études de toxicité aiguë et subchronique ont été exécutées sur le polychète Nereis diversicolor avec du D.M.P.S. de 50 cS.

Aucune mortalité, ni bioaccumulation significative dans les tissus n'a été mise en évidence avec ce silicone pur à la concentration de 10.000 ppm de D.M.P.S. pur.

Néanmoins, AUBERT et coll. ont déterminé une TL100 de 168 heures à cette dernière concentration en utilisant une émulsion aqueuse de D.M.P.S. Dans ce cas, la solution témoin ne contenant que les émulsifiants s'est avérée aussi écotoxique, de sorte que l'on ne peut conclure que la faible toxicité relevée soit due au D.M.P.S. plutôt qu'aux émulsifiants. La température, excessive au moment des essais du CERBOM, peut expliquer partiellement les différences obtenues.

3. Effets sur Mollusques et Crustacés

Des essais sur coques (Prothaca spaninca), crabes (Pachygraphus crassipes) et sur crevettes brunes (Penaeus oxtecus) témoignent d'une TL50. 96 heures supérieure à 1.000 ppm.

MAGGI et ALZIEU n'ont observé aucun effet sur Ostrea edulis, Littorina littorea, Mytilus edulis, Artemia salina, Palaemonetes varians et Clinabarius misanthropus après exposition de 96 heures à des D.M.P.S. de 100 à 12.500 cS.

Aucun effet n'a pu être observé sur le crabe vert méditerranéen, carcinus maenas, après exposition à une émulsion de 10.000 ppm de D.M.P.S. pendant 216 heures. Cette concentration calculée et observée sur une émulsion aqueuse de D.M.P.S., correspond à la TL50. 96 heures pour la moule. La concentration similaire de la solution d'émulsifiants sans D.M.P.S. agit encore plus rapidement sur la moule, une TL50 n'atteignant que 80 heures.

La moule (Mytilus edulis) a été particulièrement bien observée dans deux études séparées d'écotoxicité aiguë sur des émulsions similaires à celle rapportée ci-dessus. La CL50 de 96 heures atteint 9900mg/l de concentration vraie d'une émulsion de D.M.P.S., sans mortalité, ni effets sublétaux ou de comportement à 1020 ppm de D.M.P.S. pur. Cette concentration de 1020 ppm n'a pu être dépassée dans des essais d'émulsification de D.M.P.S. par ultrasons dans l'eau sans intervention d'émulsifiants chimiques.

4. Effets sur les reflexes d'organismes benthiques

Des études sur les réflexes d'organismes benthiques ont été exécutées avec du D.M.P.S. pur de diverses viscosités ainsi qu'à l'aide d'émulsions aqueuses de ces mêmes composés sans effets significatifs notables sur Hydratinia echinata, Cordylophora caspia et Clava squamata. L'hydractinie réagit à la présence de D.M.P.S., en l'absence de toute proie naturelle, en ouvrant les bouches de polypes dans une tentative d'ingestion qui , si elle a lieu, se limite à un rejet sans effets notables sur la digestion de proies normales.

Aucune influence n'a été observée sur la filtration ou l'osmorégulation de la moule et du crabe vert ainsi que sur la respiration de ce crabe.

5. Effets sur les poissons

a) Espèces d'eau douce et d'eaux saumâtres

Quelques résultats sont résumés au Tableau III.

L'écotoxicité est aussi faible pour les oligomères cycliques et linéaires que pour les polymères D.M.P.S. comme en témoignent les CL50. 96 heures > 20.000 ou à 10.000 ppm selon la concentration maximale testée sur Lepomis macrochirus, Salmo gairdnerii, Fundulus heteroclitus, Pimephales promelas et Phoxinus phoxinus.

MEURICE a exposé le poisson-zèbre (Brachidanio rerio) pendant 105 jours à une concentration de 10 ppm de liquides siliconés polymères méthylés et phénylés, ainsi qu'à des copolymères silicone polyéther solubles, sans obtenir aucun effet significatif sur le comportement. En particulier, les études exécutées avec des oligomères cycliques (D4 et D() tant sur Lepomis que sur Salmo à des concentrations de 1.000 ppm et de 500 ppm max sur Brachidanio ne témoignent d'aucun effet nocif sur le comportement de ces poissons.

b) Espèces marines

Quelques résultats sont résumés au tableau IV. Des essais relatifs à la toxicité aiguë ont été exécutés sur plie juvénile (Pleuronectes platessa) sans obtenir de mortalité ou d'effets sur les réflexes, par exposition de 96 heures à des concentrations de 3110 mg/l de D.M.P.S. en émulsion aqueuse à 20 % .

Dans un essai de simulation d'un épanchement marin de D.M.P.S., 50 cS aucun symptôme écotoxique (y compris de perturbations de réflexes) n'a pu être observé sur la plie juvénile à des concentrations nominales de 10.000 ppm, correspondant à une concentration mesurée maximale de 5mg/l de solution vraie de D.M.P.S.

MAGGI et ALZIEU ont également démontré la faible écotoxicité de D.M.P.S. de diverses viscosités sur Pomatoschistus minutus et sur Gasterosteus aculeatus.

L'équipe du CERBOM a établi, par une série d'expériences préliminaires que l'écotoxicité d'une émulsion de D.M.P.S. 50 cS est plus faible sur Scorpaena porcus et sur Carassius auratus que la même émulsion aqueuse sans D.M.P.S. comme en témoignent des TL50 de 42,5 heures et de 22 heures à 2.000 ppm pour l'émulsion de D.M.P.S. et la solution d'émulsifiants de même concentration sur Carassius. A 10.000 ppm les TL50 sont respectivement de 235 heures et de 18 heures avec et sans D.M.P.S. Les résultats sur Scorpaena sont encore plus nets, soit pour l'émulsion de D.M.P.S. des TL50 de 120 heures à 1.000 ppm, 50 heures à 2.000 ppm et 23,4 heures à 10.000 ppm en comparaison à des TL50 de 50 heures à 10.000 ppm et de 15 heures à 1.000 ppm pour des concentrations similaires de la solution d'émulsifiants sans D.M.P.S. Des constatations similaires sur d'autres espèces semblent indiquer un certain effet protecteur de tampon du D.M.P.S. par rapport à l'écotoxicité d'autres composés chimiques. Ces résultats sont confirmés dans un mémoire récent présenté par GUILLEMAUT C. à l'Université d'Aix-Marseille en octobre 1984.

c) Etude embryo-larvaire

Une étude dynamique par exposition d'oeufs, d'embryons et de larves de Cyprinodon variegatus pendant 33 jours, à la même émulsion de D.M.P.S., 50 cS que celle qui fut utilisée sur Scorpaena et Carassius conduit à des constatations similaires témoignant d'un certain effet protecteur à des concentrations intermédiaires allant jusqu'à 606 mg/l par rapport aux mêmes concentrations d'émulsifiants sans D.M.P.S. Sur l'éclosion, un effet significatif ne se manifeste pour l'émulsion de D.M.P.S. qu'à partir de très fortes concentrations de l'ordre de 635 mg/l. En particulier, l'effet réducteur sur le poids et la longueur de la larve est manifeste pour la solution d'émulsifiants alors que la formulation contenant du D.M.P.S. témoigne au contraire d'une augmentation significative en poids et en longueur.

6. Bioaccumulation

L'hydrophobicité et le potentiel de bioaccumulation d'oligomères cycliques et linéaires ont été estimés suite à des expériences d'ingestion pendant six semaines par Poecilia reticulata en comparaison avec des PCB's.

Le contraste est frappant et témoigne de l'absence de bioaccumulation de composés organosiliciés, les molécules de ces composés étant particulièrement volumineuses. Ces résultats ont été confirmés récemment par OPPERHUIZEN et coll. avec excrétions très rapides. Quant aux polymères possédant des volumes moléculaires encore plus conséquents, les ingestions de D.M.P.S. C14 de 50 et de 300 cS, respectivement par Ictalurus melas et par Lepomis macrochirus ne peuvent que confirmer cette absence de bioaccumulation.

Le CERBOM démontra au surplus l'absence de bioamplification d'émulsion de D.M.P.S. 50 cS, dans quatre chaînes alimentaires marines dont deux benthiques. Les facteurs de concentration dans les poissons, crustacés et mollusques étudiés sont insignifiants et varient de 0.04 à 0.25.

Ecotoxicité d'autres composés organosiliciés

1. Polyméthylphénylsiloxanes

La toxicité et l'écotoxicité de ces composés sont similaires à celles du D.M.P.S., si l'on tient compte de leur volume moléculaire et de leur insolubilité aqueuse, ce qui explique que leur utilisation est autorisée en contact alimentaire dans de nombreux pays étant entendu que le 2,6-Cis diphénylhexaméthylcyclotétrasiloxane, oligomère à propriétés oestrogènes, n'est pas fabriqué industriellement et n'existe pas à l'état d'impureté dans les composés commerciaux considérés.

Divers oligomères utilisés comme matériaux de départ pour la fabrication de polymères, tel que le tris (triméthylsiloxy) phénylsilane et composés similaires, font preuve d'une très faible toxicité mammélienne ainsi que d'une écotoxicité des plus réduites sur l'artémie et le poisson zèbre. Aucun effet sur la croissance d'algues, telles que Anabaena et Selenastrum n'a été observé à des concentrations de 10.000 ppm.

2. Copolymères silicone-polyéther

Ces composés témoignent également d'une faible toxicité mammélienne expliquant leur usage dans l'industrie des cosmétiques ou de l'emballage alimentaire, dans divers pays européens.

Quelques effets mineurs ont été observés à hautes concentrations sur les organismes d'épuration des eaux quoique aucun BOD.20 jours ne soit enregistré à 1.000 ppm.

L'écotoxicité sur Anabaena est des plus limitées, la CE50 étant de 791.5 ppm pour un composé commercial des plus communs. Pour l'artémie, la CL1 est supérieure à 500 ppm, concentration maximale expérimentée.

Ces composés étant solubles, l'écotoxicité est également faible pour la daphnie, la CL50. 48 heures étant supérieure à 1.000 ppm (25). De même les essais chroniques exposant cette espèce pendant 21 jours à 10 ppm ne donnent lieu à aucune mortalité.

Des études de CL50. 96 heures sur poissons témoignent de résultats similaires : B. rerio > 500 ppm (conc. max. expérimentée) L. macrochirus > 1.000 ppm S. gairdneri > 245 ppm. L'exposition de B. rerio à 10 ppm pendant 105 jours n'entraîne aucun effet.

3. Chlorosilanes

Ces composés sont rapidement hydrolysés au contact de l'eau avec obtention de polysiloxanols ou de silanols atoxiques (voir 4) ainsi que d'acide chlorhydrique. Ce dernier composé est écotoxique et

classé avec raison comme produit dangereux pour l'environnement aquatique d'eaux douces. Par contre comme produit de réaction dans l'eau de mer, il est rapidement neutralisé. Les résultats d'essais de CL50. 96 heures en aquarium sur Crangon crangon et Agonus cataphractus varient de 140 à 320 ppm selon la quantité et la durée d'exposition à HCl.

4. Méthylsilanols et Siloxanols

Ces composés solubles peuvent résulter d'une dégradation environnementale de silanes (eau) ou de siloxanes (sol.). Le diméthylsilanediol (D.M.S.D.), représentant principal de ce groupe, peut subir une déméthylation photolytique oxidative en solution aqueuse, en présence de traces de chromophores tels que NOx pour donner de l'acide silicique et du CO2. Cet acide est assimilable par Navicula pelliculosa (41) comme certains silatranes le sont par Cyclotella.

Le D.M.S.D. n'est pas biodégradable et, à ce jour, aucun BOD n'a pu être décelé.

En microcosme, aucune influence délétère n'a pu être constatée après de nombreux mois d'exposition, non seulement à 100 ppm de D.M.S.D. mais aussi par action de triméthylsilanol résultant de la dégradation de D.M.S.D., tant sur la daphnie, que sur des ostracodes, des bactéries ou des algues telles que Anabaena ou Selenastrum. De même des essais sur Salmo, Lepomis et Fundulus confirment la faible écotoxicité même à de fortes concentrations.

Pimephales promelas a été exposé à 10 ppm de D.M.S.D. pendant 90 jours à partir du stade oeuf sans effets sur le comportement, l'éclosion, la croissance, la mortalité et sans bioconcentration significative dans les tissus.

5. Autres composés

Divers autres composés polymériques ou oligomèriques organosiliciés ne témoignent pas davantage d'une écotoxicité importante sur les organismes aquatiques considérés.

Le vinyltriméthoxysilane (V.T.M.O.) utilisé par exemple comme agent couplant dans la fabrication de tuyaux en polyéthylène en contact avec l'eau potable, ne laisse aucun résidu dans le produit fini. Son écotoxicité propre reste relativement faible, comme celle du vinyltriacetoxysilane produisant par hydrolyse de l'acide acétique et du vinylsilanetriol :

	V.T.M.O. (ppm)	Vinyltriacetoxysilane
S. capricornutum	CE50 = 210	110 ppm
A. flos aquae	CL50 > 1000	> 100
D. magna	CL50.48 h. > 100	> 100
S. gairdnerii	CL50.96 h. = 191	= 51
L. macrochirus	CL50.96 h. > 1000	= 68 (16,48)

La plupart des silatranes sont peu toxiques et les quelques composés arylés toxiques se dégradent en composés peu écotoxiques dans l'environnement aquatique. Leur importance quantitative est quasi nulle du point de vue commercial et a fortiori les possibilités pratiques de rejet en mer.

Conclusions générales et signification écologique

Les composés organosiliciés ne semblent pas représenter un danger significatif pour l'environnement de la Méditerranée si l'on se base sur les données obtenues à ce jour en laboratoire. Etant donné l'absence quasi générale à ce jour de résidus importants détectés dans l'environnement marin

soumis à des juridictions internationales, il n'est pas possible d'établir des comparaisons avec des dommages observés en milieu aquatique réel. Les seuils de sensibilité détectés en laboratoire sont néanmoins suffisamment élevés en comparaison des productions des usines et des probabilités de dissémination des déchets pour estimer l'absence probable de dangers potentiels.

Les expériences signalées dans cet inventaire des données existantes ont suivi les protocoles expérimentaux recommandés par l'O.C.D.E. et/ou par des organisations internationales ou par les pays dans lequels ces expériences ont eu lieu (E.P.A., I.S.O., F.A.O. etc.).

L'écotoxicité réduite est prouvée sur une centaine d'espèces aquatiques. L'absence de bioaccumulation significative et surtout de bioamplification est vérifiée non seulement sur les polymères mais sur les oligomères et produits de dégradation solubles. L'absence de traces généralisées de ces composés dans l'environnement semble s'expliquer partiellement par l'existence de trois voies de dégradation physicochimiques dans le sol et l'air, et partiellement par l'absence d'immersion ou de contamination tellurique intentionnelle, les quantités étant limitées, les produits chers et généralement recyclables.

Garder ces composés malgré tout sous un statut de composés potentiellement dangereux parce qu'ils peuvent être persistants dans les sédiments, sans tenir compte de leur faible écotoxicité directe ou induite, ni des processus de dégradation existants pourrait, conduire à une ségrégation de ces composés pouvant être utiles à l'environnement en tant que substituants potentiels de composés réellement dangereux. (ex : P.C.B.'s dans les transformateurs). Admettre que la liaison SiO_2 est non biodégradable mais refuser d'admettre qu'il peut en être occasionnellement de même pour la liaison $Si-CH_3$ est un choix indépendant d'évidences scientifiques.

On ne peut néanmoins jamais affirmer, qu'un jour un composé organosilicié encore inconnu ne "pourrait" avoir des effets écotoxiques. On constatera peut-être un jour qu'un composé connu a un effet perturbateur sur un des milliers d'organismes marins. Cette insécurité relative peut s'appliquer à divers groupes de composés chimiques tant naturels que xénobiotiques et justifie la continuation systématique de recherches en conséquence. Elle justifie aussi la révision périodique des annexes des conventions internationales.

Références

R. FIRMIN, C.L. FRYE and A.L.J. RAUM (1984). - The Environmental Impact of Organosilicon Compounds on the Aquatic Biosphere. Martox Symposium Ghent. Ecotoxicological Testing for the Marine Environment, Vol. I, 591-623.

R. FIRMIN (1984). - Mémoire sur les Composés Organosiliciés et l'Environnement Marin. Université libre de Bruxelles (ULB) 700 pp. 27 mars.

INITIAL ASSESSMENT OF MARINE BIOLOGICAL FOULING AND FOULING CONTROL, TAKING INTO CONSIDERATION ENVIRONMENTAL PROTECTION ASPECTS, FOR A NUCLEAR POWER PLANT TO BE SITED IN THE EAST MEDITERRANEAN

by

C.F. GOKCAY, S. SOYUPAK and M.E. HATIPOGLU
Department of environmental engineering
middle east technical University (Turkey)

The growth of biological film on the inner surfaces of heat exchangers is a serious problem in nuclear power plants depending on sea-water for coolant. Fouling organisms which grow attached to surfaces prevent heat transfer in the heat exchangers thus lowering plant efficiency.

This study has been carried out for the Turkish Electricity Board in order to suggest the appropriate anti-fouling measures to be employed in the future nuclear power plant to be sited on the east Mediterranean coast of Turkey, namely at Akkuyu Bay.

There are various anti-fouling measures available to the industry. Of these, biocides such as chlorine or heat shocks are the most used (1, 4). For example, McLean reports that 90 per cent of all nuclear power plants in the USA use chlorine as the biocide, usually administering it in shock doses three times a day, each application lasting ten minutes (1).

In this study bio-fouling potential in the area was investigated using a fouling raft manufactured according to the OECD specifications (2). The raft had 12 movable arms dipped 80 cm into the sea-water. Stainless steel (30 cm x 20 cm) plates were bolted on each arm and the raft was anchored at Akkuyu Bay.

The steel plates were shaded from sunlight to prevent growth of algae. At pre-determined time intervals plates were removed and the bio-fouling film was investigated under the microscope. The check-list of bio-fouling organisms recovered on these plates is presented in table 1 with the most abundant organisms indicated with an asterisk. In addition to that the bio-fouling potential of the area was investigated by examining rock surfaces. Further organisms were identified as significant foulers during these investigations and were appended to table 1.

In experiments with the steel plates it was observed that biomass accumulation rate on naked steel surfaces is much lower than that on already-fouled surfaces. Biomass accumulation rate on a steel surface was recorded as 0.71 g.m.-2.d-1 (dry-weight), at the highest (during summer), whereas biomass accumulation rate on plates covered by a primary fouling film was recorded almost ten times higher, i.e. 6.45 g.m-2 d-1.

In order to decide on the type of anti-fouling measure to be employed bio-assays with fouling organisms were undertaken. Bio-assays were carried out in a large fish tank with dimensions 100 x 25 x 40 cm which was filled with fresh sea-water brought from Akkuyu Bay. Test animals collected daily were first put into a similar storage tank and than transferred into the test tank to initiate the experiments. The sea-water inside the tanks was changed every hour and its temperature was maintained at 21° C as in the Akkuyu Bay.

Table 1
Check-list of fouling organisms found on stainless steel plates between 7.1.1983 and 25.6.1983, in the dark

* Balanus amphitrite	Ciona intestinalis
Balanus eburneus	Venerupis rhomboides
* Balanus perforatus	* Bowerbankia sp.
Pomatoceros triqueter	Scrupocellaria bertholettii
Hydroides norvegica	Scrupocellaria reptans
Filograna sp.	Botryllus schlosseri
Spirorbis pagenstecheri	Schizoporella unicornis
Spiorbis mediterraneus	Ascidia sp.
* Aetea truncata	Porifera sp.
Sycon raphanus	Leucosolenia sp.
* Gonothyraea gracilis	Campanularia flexuosa
Anomia ephippium	Sabella sp.
Anomia sp.	Podocoryne sp.

Chek-list of significant fouling species found on rock surfaces:

Paracentrotus lividus	Patella lusitanica
Sabella sp.	Chthamalus stellatus
Actinia equina	Monodonta turbinata

Table 2
Results of bio-assay experiments with chlorine on various groups of fouling organisms

Species tested	n° of individuals	Residual pean	Chlorine (ppm) range	Time for complete kill (min)	Remarks
Patella lusitanica	5	0.8	0.9 - 0.7	-	No sign of death could
	5	2.5	2.5 - 2.4	-	be detected within 20
	5	9.1	9.2 - 9.0	-	minutes.
Sabella sp.	3	0.8	0.9 - 0.7	-	"
	3	2.5	2.5 - 2.4	-	"
	3	9.1	9.2 - 9.0	-	"
Paracentrotus lividus	3	0.8	0.9 - 0.7	-	"
	3	2.5	2.5 - 2.4	-	Reduced activity
	3	9.1	9.2 - 9.0	-	Inactive at the end of 20 minutes but resumed activity when transferred to storage tank
Actinia equina	3	0.8	0.9 - 0.7	-	No sign of stress
	3	2.5	2.5 - 2.5	-	Signs of stress.
	3	0.1	9.2 - 9.0	(4)	2/3 of specimens released substrate surface, one specimen unaffected.
Monodonta turbinata	2	0.8	0.9 - 0.7	-	No sign of stress
	2	2.5	2.5 - 2.4	-	Nos sign of stress
	2	9.1	9.2 - 9.0	5	All (2/2) specimens released substrate surface.

For the chlorine bio-assay, at zero hour hypochlorous acid was added to the test tank. Immediately after mixing and after every minute, samples were drawn off from the test tank and the free residual chlorine concentration in the tank was checked colourimetrically (3). The maximum duration of these experiments was limited to only 20 minutes considering the average 10 minutes retention time of cooling water in the system and the common 10 minutes' practice (4). At the end of each experiment supposedly dead animals were transferred to the storage tank and signs of activity were observed there for four hours. Criteria of death and the results of chlorine bio-assay experiments are presented in table 2.

From Table 2 it can be seen that most bio-fouling organisms tested are resistant to high doses of chlorine, such as 9 ppm free residual.

In a later experiment the effect of heat-shock on the bio-fouling organisms was investigated in a similar experimental set-up. However chlorine was replaced this time by a thermostatically controlled heater maintaining the temperature of the test tank at the level required. The results of bio-assay experiments with raised temperature are summarized in table 3.

Table 3

Results of bio-assay experiments on fouling organisms with raised temperature. The temperature increase was 20° C and temperature shock was obtained by transferring test organisms at 20 ° C into water at 40° C.

Species tested	n° of individuals tested	Criteria of death	Time of complete death of test organisms (min)	Remarks
Paracentrotus lividus	3	Movement of tubular feet and jaws on the mouth plate .	4	-
Sabella sp.	5	Body and gill movements	4	Body movements were stimulated physically
Actinia equina	3	Tentacular movements and adherence to substrate surface	7	One individual resisted death for 7 min, others died in 4 min.
Monodonta turbinata	2	Adherence to substrate surface and opercular movements.	4.5	-
Patella lusitanica	7	Adherence to substrate surface	7	5/6 of tested individuals were killed within 5 minutes of exposure
Chthamalus stellatus var communis	22	Opercular movements upon physical stimulus, extrusion of gills trough opercular openings upon death.	10	Upon death opercula remained open and did not respond to physical stimuli
Balanus perforatus	10	Same as for Chthamalus.	3	Same as above

The results indicate that heat shock with a ΔT of 20° C kills all the test animals within 10 minutes' contact time. Hence heat shock application stands out as an anti-fouling measure much superior to chlorine.

A final attempt was made to determine the combined effects of both heat and chlorine on fouling organisms. An experiment was designed to seek the combined effects statistically by devising a linear equation . Design matrices were constructed by increasing temperature in 2.5° C increments within the range of 0-10° C ΔT over ambient temperature while also increasing chlorine concentration by 2.5 ppm increments within the 0-10 ppm residual chlorine concentration range. Thus all the possible combinations were tried. The results of these experiments, which are presented in table 4, indicate that at even the extreme case, when ΔT was 10° C and residual chlorine concentration was 10 mg/l, none of the test organisms were killed within 10 minutes' contact time.

Table 4
Results of combined effect experiments

T (°C)	OCl^- (mg/l)	n°. of test organisms killed over n°. tested		
		Patella lusitanica	Chthamalus stellatus	Monodonta turbinata
2.5	0	0/6	0/25	0/6
2.5	2.5	0/6	0/25	0/6
5	2.5	0/8	0/25	0/7
5	5	0/6	0/25	0/6
5	7.5	0/13	0/25	0/10
7.5	7.5	0/11	0/25	0/10
2.5	10	0/6	0/25	0/7
10	10	0/11	0/25	0/9

Chlorine application for fouling control inevitably affects biota on the receiving end. Fish being the most susceptible organism to chlorine suffer most. On the other hand chlorination is normally carried out as shock doses of high concentrations which gradually dilute to harmless levels in the receiving basin. Therefore the effect of extreme shock doses on local fish is of importance for the assessment of impact caused by this method i.e. doses which kill fish in a matter of minutes before fish can have a chance to escape.

Table 5
Results of chlorine bio-assay studies with fish

Species of fish	No. of individuals	Residual chlorine ppm		Time for complete kill (min)	Remarks
		mean	range		
Oblada melanura (8 - 24 cm)	3	0.97	0.98-0.95	4	Signs of agitation and vigorous swimming from zero minute onwards
Oblada melanura (20 - 25 cm)	4	0.3	0.34-0.25	5	Signs of agitation and vigorous swimming after two minutes onwards

In an experimental set-up very similar to that described previously for bio-assay with chlorine, experiments with the commonest local fish, namely Oblada melanura, were undertaken. Fish were caught in Akkuyu Bay with gill nets and within 10 minutes of netting were quickly transferred to the laboratory in sea-water bins filled with sea-water with a minimum of handling. Fish were never exposed directly to the air during this time and were kept in a well aerated storage tank in the lab. for ca. 5 hours for acclimatizion. Sea-water in the storage tank was changed every half an hour with fresh sea-water from Akkuyu Bay. Experiments were started by carefully transfering fish to the test tank and a population of four fish were kept in the storage tank during the experiments and then after wards for 20 hours as control.

The results of these experiments presented in table 5 indicate that the local fish are extremely susceptible to chlorine even at sub-ppm levels, i.e. 0.3 ppm.

The present study suggests that short-time chlorine application even at high concentrations such as 10 ppm chlorine residual will not produce antifouling effects while causing detrimental effects to local fish at the receiving end. However, using heat shocks which may be obtained simply by slowing cooling-water flow or by re-circulating it may yield the desired effect.

References

1. McLEAN R.C. (1973). - Chlorine and Temperature Stress on Estuarine Invertebrates. Journal of Water Pollution Control Federation, 45 (5), 837-841.
2. Unifying of Tests on Rafts (1961). - Organisation for European Economic Community, Directorate of Scientific Affairs, Committee for Scientific Research. Das./61 592, Paris, Sept.
3. Anonymus (1971). - Standard Methods for the Examination of Water and Waste Water, APHA, AWWA, 13th edition, American Public Health Association.
4. JENSEN P.: J. KLITGAARD and K.M. PEDERSEN (1977). - Chlorine decay in cooling water and discharge into sea-water. Journal of Water Pollution Control Federation, August. 1832-1841.

SUBTANCES LIANT LE CADMIUM, LE PLOMB ET LE CUIVRE DANS CERTAINS ORGANES DE POISSONS MEDITERRANEENS

par

G.N. FLATAU, R.L. CLEMENT et M.J. GAUTHIER
I.N.S.E.R.M., U. 40 - 1, av. Jean Lorrain, 06300 Nice (France)

Résumé

Le but de cette étude était de voir sous quelles formes pouvaient être fixés le Cadmium, le Plomb et le Cuivre après leur absorption par des poissons marins. L'étude a porté sur des échantillons de peau et de muscle sains ou nécrosés et de foie d'une daurade pêchée en Méditerranée, et sur le foie d'un loup élevé dans un centre aquicole méditerranéen. Les deux sujets présentaient de nombreuses lésions à caractère nécrotique.

La peau et le muscle de la daurade ne contenaient pas de métaux. Par contre, le foie en contenait et souvent en concentration importante. Le Cuivre et le Plomb étaient associés à des molécules de poids moléculaires d'environ 25.000, 14.000, et entre 6.000 et 8.000. Le Cadmium n'était associé qu'à des subtances de PM situés entre 6.000 et 8.000. Ces produits pourraient être entre autres, des métallothionéines, et leurs di-, tri- ou tétramères, des produits semblables aux métallothionéines, et des polysaccharides capables de lier certains métaux.

*
* *

Introduction

Les métaux déversés en mer sont, à plus ou moins long terme, absorbés par les organismes marins. De nombreux travaux expérimentaux ont été réalisés sur l'accumulation des métaux par les bactéries (BARKAY et COLWELL, 1983 ; MACASKIE et DEAN, 1982), le plancton (HARDSTEDT-ROMEO et LAUMOND, 1980), les mollusques (PHILLIPS, 1976, 1976a) et les poissons (AMIARD et coll., 1980 ; HOLDWAY et SPRAGUE, 1983). La nature des organes cibles a été également précisée (KOHLER et RIISGARD, 1982 ; FLATAU et GAUTHIER, 1983 ; BREITTMAYER et ZSURGER, 1983). Depuis quelques années des travaux ont permis la mise en évidence de molécules biologiques fixant "in vivo", certains métaux dangereux dans divers organismes marins comme la moule Mytilus edulis (KOHLER et RIISGARD, 1982) ou certains poissons (OVERNELL et coll., 1979).

Le but de ce travail était de détecter des subtances liant le Plomb, le Cadmium et le Cuivre dans le foie et les muscles de poissons méditerranéens atteints de nécroses. Un des échantillons étudié était une Daurade (Sparus auratus) de 38 cm pesant 1,8 kg. Trouvée agonisante au large de Menton (Alpes-Maritimes, France) elle montrait de nombreuses nécroses dispersées sur l'ensemble du corps. Le deuxième échantillon analysé était un jeune Loup (Dicentrarchus labrax) de 8 g possédant des foyers nécrotiques, et récolté mort dans un centre aquicole méditerranéen.

Matériel et méthodes

Les subtances liant les métaux ont été purifiées partiellement par la technique proposée par NAKAMURA et coll. (1981). La colonne était remplie de Sephadex G-100 préalablement équilibrée par du tampon TRIS 0,01M-HCl (pH 8,6) et tarée par un mélange de protéines de poids moléculaires (PM) connus. Les PM des subtances liées aux métaux ont été estimés d'après leur temps de rétention. Le Cadmium, le Plomb et le Cuivre ont été dosés par redissolution anodique (ESA 2011).

Résultats

Le Cadmium, le Plomb et le Cuivre n'ont pu être détectés dans les échantillons de muscle. Par contre, certaines fractions chromatographiées des échantillons de foie (Daurade et Loup) en conte-

Métaux	PM 6.000	7.000	8.000	15.000	25.000
Cd		13,7			
Pb			72	16	14,4
Cu	52,6			34,7	22,1

Tableau 1. - Répartition des métaux (ug/l) dans les fractions chromatographiées, en fonction des PM dans le foie de la Daurade.

naient et parfois à des concentrations importantes. Les tableaux 1 et 2 montrent les concentrations les plus élevées des métaux, respectivement dans le foie de la Daurade et du Loup et les PM des subtances auxquelles ils étaient associés. Ces PM étaient voisins, voire indentiques, dans le foie des deux poissons.

Métaux	PM 8.000	14.000	16.000
Cd			
Pb			32
Cu	18	=35	

Tableau 2 - Répartition des métaux (ug/l) dans les fractions chromatographiées, en fonction des PM dans le foie du Loup.

Discussion

Les métaux comme le Cadmium, le Plomb et le Cuivre, absorbés par les poissons, sont généralement accumulés par le foie et les reins. Dans ces tissus, ils peuvent être liés à des subtances de haut PM comme les polysaccharides, à des substances de PM moyens (entre 10.000 et 30.000), ou à des subtances plus légères (PM inférieurs à 5.000) comme certains polypeptides. On connaît en particulier le rôle fixateur joué par les métallothionéines (MT) dont le PM est situé entre 6.000 et 10.000, et riches en résidus SH (CHERIAN et GOYER, 1978).

Dans le foie de la Daurade, les trois métaux étaient associés à des subtances qui pouvaient être des MT. En effet, ces PM étaient voisins de ceux trouvés chez la Plie Pleuronectes platessa, par comparaison des distances de migration en électrophorèse sur gel (OVERNELL et COOMBS, 1979). La détermination des PM des MT de la Plie par filtration sur gel fournissait des valeurs situées entre 13.000 et 15.000. Ces valeurs deux fois plus élevées que les valeurs réelles seraient dues à la forme de ces molécules plutôt ellipsoïdales et non globulaires comme celles des protéines servant à tarer le gel de filtration (WEBB, 1979). D'autres auteurs estiment que les MT possèdent des PM approximativement voisins mais que durant leur purification et leur identification elles copolymérisent pour former des produits à PM plus élevés (WEBB, 1979). Les subtances à faibles PM (6.000-8.000) seraient des MT monomères et les substances à PM situés aux alentours de 15.000 pourraient correspondre à leurs dimères. Le Cuivre et le Plomb étaient également associés à des molécules plus massives (PM = 25.000), qui pourraient être des trimères de Pb-MT et les tétramères du Cu-MT, ou bien des MT liées à d'autre protéines ne contenant pas de métal. Ce phénomène a été vérifié dans le cas du Cuivre (WEBB, 1979). Les métaux peuvent être piégés par certains éléments constitutifs de la cellule, comme des sites (chargés ou non), des polysaccharides comme chez certains micro-organismes (RENDELMAN, 1978). La présence de métaux (Zinc, Cadmium, Plomb, Cuivre,...) dans le foie d'organismes plus évolués déclenche la synthèse de métallothionéine (WEBB, 1979). Ainsi complexés, ils deviennent moins diffusibles et donc moins disponibles. Le rôle prépondérant des métallothionéines serait donc un rôle de détoxification de certains métaux.

Références

J.C. AMIARD, C. AMIARD-TRIQUET, C. METAYER, J. MARCHAND, (1980). - Etude du transfert de Cd, Pb,Cu et Zn dans les chaînes trophiques nérétiques estuariennes. II.Accumulation biologique chez les poissons planctonophages. Water Res., 14 : 1327-1332.

T. BARKAY, R.R. COLWELL, (1983). - Altered mercury transfert in a mutant of Pseudomonas fluorescens B 69 J. Gen. Microbiol., 129 : 2945-2950.

J.P. BREITTMAYER, N.V. ZSURGER (1983). - Accumulation du Mercure dans les organes de la Moule : effets de la dose contaminante et de la taille des organismes. Rev. Int. Océanogr. Méd., 70-71 : 87-97.

G.M. CHERIAN, R.A. GOYER, (1978). - Metallothioneins and their role in the metabolism and toxicity of metals. Life Sci., 23 : 1-10.

G.N. FLATAU, M.J. GAUTHIER, (1983). - Accumulation du Cadmium par Mytilus edulis en présence de souches bactériennes sensibles ou résistantes à ce métal. Can. J. Microbiol., 29 : 210-217.

M. HARDSTEDT-ROMEO, F. LAUMOND, (1980). - Zinc, copper and cadmium in zooplankton from the N.W. Mediterranean. Mar. Pollut. Bull., 11 : 133-138.

D.A. HOLDWAY, J.B. SPRAGUE, J.G. DICK, (1983. - Bioconcentration of vanadium in american flagfish over on reproductive cycle. Water. Res., 17 : 937-941.

K. KOHLER, H.U. RIISGARD, (1982). - Formation of metallothioneins in relation to accumulation of cadmium in the common mussel Mytilus edulis. Mar. Biol., 66 : 53-58.

L.E. MACASKIE, A.C.R. DEAN, (1982). - Cadmium accumulation by microorganisms. Environ. Tech. Lett., 3 : 49-56.

H.Y. NAKAMURA, S. KATAYAMA, Y. OKADA, F. SUZUKI, Y. NAGATA, (1981). - The isolation and characterization of a cadmium and zinc binding protein from Tetrahymena pyriformis. Agric. Biol. Chem., 45 : 1167-1172.

J. OVERNELL, T.L. COOMBS, (1979). - Purification and properties of plaice metallothionein, a cadmium binding protein from the liver of the plaice Pleuronectes platessa. Biochem. J., 183 : 277-283.

D.J.H. PHILLIPS (1976). - The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals. Mar. Biol., 38 : 59-69.

D.J.H. PHILLIPS (1976a). - The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead and copper. III. Relationships of metals in the mussel to those discharged by industry. Mar. Biol., 38 : 71-80.

J.A. RENDELMAN, (1978). - Metal-polysaccharides complexes. I. Food Chemistry, 3 : 47-49.

M. WEBB, (1979). - The metallothioneins. In : The chemistry, biochemistry and biology of cadmium. M. WEBB, Editeur Elsevier North-Holland, 195-266.

THE IMPORTANCE OF TEMPERATURE AND LIGHT CONDITIONS ON THE TOXICITY OF OIL, OIL DISPERSANT AND OIL/DISPERSANT MIXTURE TO ARTEMIA SALINA

by

M. MORAITOU-APOSTOLOPOULOU, G. VERRIOPOULOS
and N. CHRISTOU
Zoological Laboratory
University of Athens
Athens 15771 (Greece)

Abstract

In the framework of the research programme MED-POL phase II of UNEP, we have studied the influence of temperature (14°, 22 °C) and light conditions (continuous light, continuous dark, photoperiod), on the acute toxicity (LC50 48h) of three toxic substances (oil, oil dispersant and oil/dispersant mixture) to the brine shrimp Artemia salina.

Oil (Tunisian crude oil zarzaitine type) proved more toxic under continuous light conditions (LC50 48h = 9,982.8 ppm) than under dark conditions (LC50 48h = 11,865 ppm), while under photoperiod (12h light, 12h dark) oil presented the highest of the three light conditions toxicity (LC50 48h = 7,309.8 ppm), at 14 °C.

Light also seems to influence, but to a lesser degree, the toxicity of oil dispersant (Finasol OSR-2). Under continuous dark conditions Finasol proved very toxic to Artemia (LC50 48h = 1.83 ppm), while under continuous light conditions Finasol was less toxic (LC50 48h = 2.42 ppm). A further decrease of Finasol toxicity was noticed when Artemia was exposed under photoperiod (LC50 48h = 8.001 ppm). The same influence of light conditions was found for the oil/dispersant mixture (LC50 48h = 34.44 pp under dark conditions, 63.293 at light and 137.21 at photoperiod).

A pronounced increase in the toxicity of the three toxicants to Artemia was noticed with the elevation of temperature from 14 ° to 22 °C.

*
* *

Introduction

Crude oil contains thousands of different hydrocarbons and other compounds. Oils from different fields may differ widely in physical. chemical and thus toxicological properties. Apart from the type of oil other factors markedly influence the toxicity of crude oils to marine organisms: state of oil (thin oil films, thick oil films, water-in-oil emulsions, oil-in-water emulsions), climatic and weather conditions (wind and waves, temperature and sunlight). Finally the toxicity of oil may be altered through interactions with other compounds such as dispersants used in cleaning-up processes and other chemicals such as phenol, sulphides, ammonium compounds, etc.

Solvent-based dispersants are among chief ways of removing oil from shores. Although recently new dispersants have been developed (usually by replacement of the highly toxic aromatic hydrocarbons

of their solvent part by aliphatic hydrocarbons) which are much less toxic than the earlier ones, dispersants are still toxic substances. As for oils, the harmful effects of dispersants on aquatic life may be altered by the influence of environmental factors and the presence of other compounds acting in the environment, especially oil.

In this paper, we have studied the toxicity of an oil (Tunisian crude oil zarzaitine type), an oil dispersant (Finasol OSR-2) when acting alone and when acting together with the oil, to the brine shrimp Artemia salina. In order to evaluate the influence of two environmental conditions (temperature and light) on the toxicity of the three toxicants (oil, dispersant, oil/dispersant mixture) all toxicity tests were run under two different temperature and three different light conditions.

With the aim of investigating if larval stages of Artemia are more sensitive than adults to oil and dispersant, and also if the responses of young individuals are the same as those of adults when exposed to toxicants under different light conditions, we repeared the toxicity experiments using larvae of Artemia.

Material and methods

Artemia salina hatched from commercially available cysts was used as test animal: adults in all types of experiments, but also larvae (36 ± 12 hours old) for some experiments. The acute toxicity was estimated by determination of LC50 48h (concentration of a toxicant which kills 50 per cent of the test animals after 48 hours of exposure) according to the BLISS (1938) method. All experiments (static bioassays) were run in constant temperature rooms at 14 ± 0.5 °C and 22 ± 0.5 °C. Adults of Artemia were put individually into 30 ml glass jars sealed with Teflon lined caps. Larvae were put in groups of about 30 in 200 ml Erlenmeyer flasks. Oil-water mixtures for use in bioassays were prepared from oil-in-water dispersions (OWDs). OWDs were prepared by adding measured volumes of oil (Tunisian crude oil zarzaitine type) to artificial sea-water (prepared my mixing distilled water with Instant Ocean synthetic sea salts) and shaking the mixture vigorously for 15 minutes at approximately 2,000 cycles/min on a shaker. Detailed characteristics of the OWDs tested and the resulting concentrations of hydrocarbons at various times could not be obtained. The dispersant solutions were prepared by diluting a stock solution of 80 ppm Finasol. The oil/dispersant mixture contained equal parts of oil and Finasol. In no one case were the test mixtures aerated during the experiments.

The range of experimental concentrations were fixed by preliminary tests.

Generally six to seven concentrations of the test mixture plus a control were used in each bioassay. A minimum of 30 animals were exposed at each concentration in all cases. For the adults of Artemia the acute toxicity (LC50 48h) was estimated at two temperatures: 14° and 22 °C and for the larval stages (36 ± 12 hours old) at 22 °C. For both adults and larvae the bioassays were performed under three light conditions (continuous light, continuous dark and photoperiod (12h light, 12h dark). The light intensity ranged from 220 to 300 lux. At 24 and 48 hours the containers were examined, mortalities were recorded and at 24h dead individuals removed. Test animals were put into containers immediately after the preparation of the mixture, because as had previously been found (VERRIOPOULOS and MORAITOU-APOSTOLOPOULOU, 1983) for all three mixtures tested a significant decrease in their toxicity is noticed with time.

The differences between the linear regressions by which the LC50 under various experimental conditions were determined, were tested statistically by the t-test (statistically significant between 95 and 99,99 per cent level).

Results

Table 1 gives the LC50 48h of the three toxicants calculated according to the Bliss method.

Table 1

LC50 48h (ppm) of the three toxic solutions for Artemia at various temperature and light conditons.

Light conditions	Continuous dark			Photoperiod			Continuous light		
Temperature	14 °C	22 °C		14 °C	22 °C		14 °C	22 °C	
Stage	adults	adults	larvae	adults	adults	larvae	adults	adults	larvae
Finasol	1.83	0.83	2.72	8.001	1.19	3.427	2.42	1	2.95
Oil/Finas	34.446	4.24	5.85	137.21	8.2	7.99	62.5	6.04	7.66
oil	11.865	677.27	260.84	7,309.8	464	201.4	9,982.8	616.74	250.84

Oil presented a low toxicity to Artemia. On the contrary Finasol proved very toxic. The mixture oil/Finasol exhibited an intermediate toxicity, its harmful effects being obviously mainly the result of its dispersant constituent.

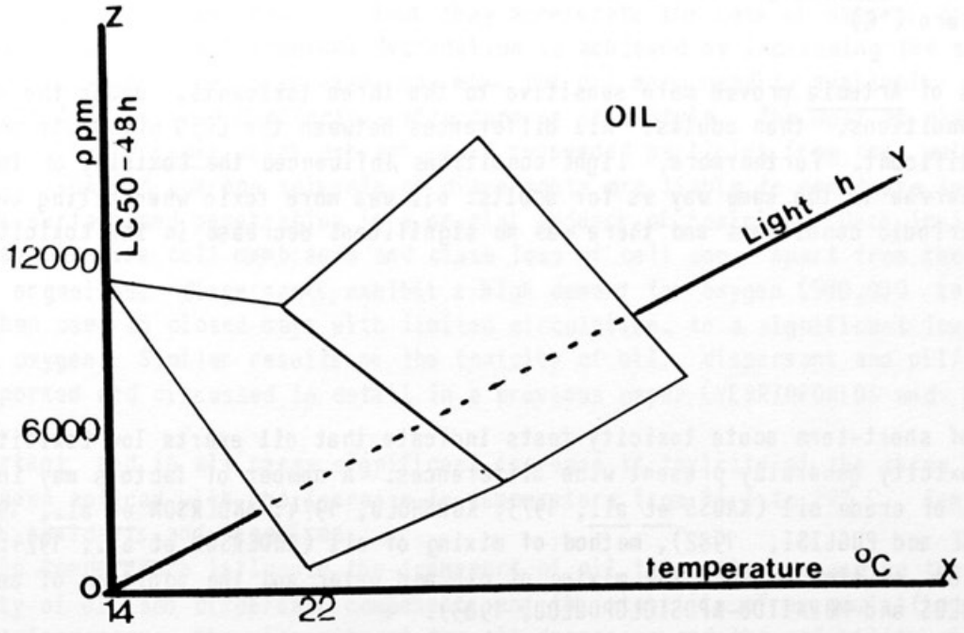


Figure 1. - Impact of temperature and light conditions to the LC 50 48h of oil (Tunesian Crude Oil Zarzaitine Type) to Artemia.

Temperature seems an important factor in the level of toxicity to Artemia of the three tested solutions. Acting under the same light condition all solutions were much more toxic at the higher tested temperature (22° C). All differences between the calculated LC50 48h values were statistically significant with one exception: when Finasol is acting under continuous dark conditions the resulting LC50 at 14 ° and 22 °C are not statistically different.

Light conditions also seem to influence the toxicity of the three toxicants to Artemia. However only in the case of oil the differences between the calculated LC50 when oil is acting at the same temperature but under different light conditions, were in all cases statistically significant. Oil exerted the lowest toxicity on Artemia when acting under continuous dark conditions. A statistically significant increase of its toxicity was noticed under continuous light and especially under photoperiod conditions. The influence of light conditions on the toxicity of Finasol and Finasol/oil mixture was the same. Contrary to what was found for oil, both solutions showed themselves to be more toxic when they were acting in the dark. Under light conditions the two solutions were less toxic and a further decrease in their toxicity was noticed under photoperiod. However the differences observed between the LC50 values when the two solutions were acting in the light and in the dark were significant only in one case (out of four): for the mixture of Finasol/oil at 22 °C. More pronounced was the decrease in toxicity from light to photoperiod conditions: only in one case (out of four) was the difference between the calculated LC50 values not significant: in the case of Finasol/oil acting at 22 °C.

Figure 1 illustrates the impact of temperature and light conditions on the LC50 of oil to Artemia, Figure 2 that of Finasol and Figure 3 that of Finasol/oil mixture. The estimation of multiple linear regression analysis is:

for oil:	$z = 26{,}187.66 - 1{,}141.63x - 40.48y$	Coefficient of Determination $R^2 = 0.92950$
Finasol:	$z = 9{,}2833 - 0.385x + 0.0158y$	" " " $R^2 = 0.38355$
Finasol/oil:	$z = 196.406 - 8.987x + 0.621y$	" " " $R^2 = 0.59503$

where z = concentration of the toxic solution (in ppm)
y = light (duration of light in hours)
x = temperature (°C)

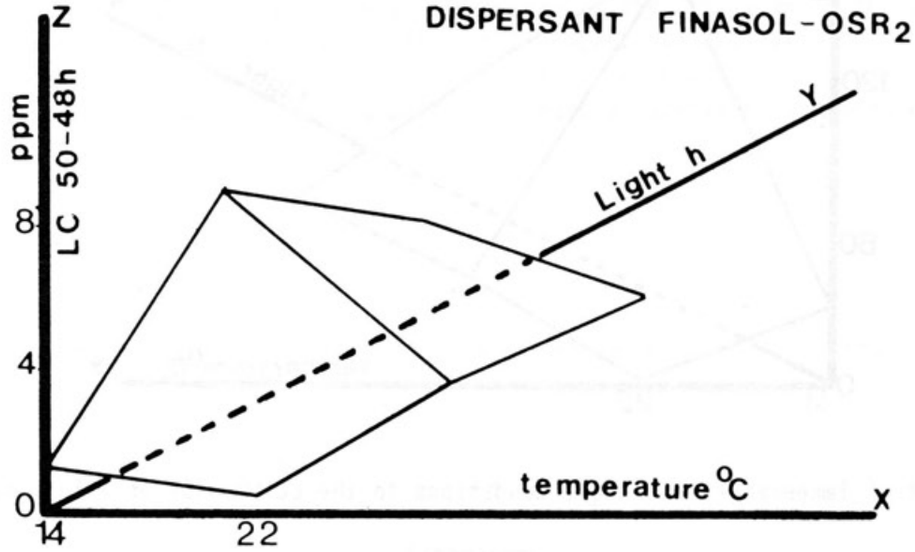
Larval stages of Artemia proved more sensitive to the three toxicants, under the same temperature and photic conditions, than adults. All differences between the LC50 of adults and larvae were statistically significant. Furthermore, light conditions influenced the toxicity of the three toxic solutions to the larvae in the same way as for adults: oil was more toxic when acting under light and especially photoperiodic conditions and there was no significant decrease in its toxicity under dark.

Discussion

The results of short-term acute toxicity tests indicate that oil exerts low toxicity to Artemia. Results of oil toxicity generally present wide differences. A number of factors may influence this variability: type of crude oil (KAUSS et al., 1973; KUHNHOLD, 1974: ANDERSON et al., 1974; PULICH et al., 1974: HEDTKE and PUGLISI, 1982), method of mixing of oil (ANDERSON et al., 1974: TEMPLETON et al., 1975), period of time between the mixing of oil and water and the addition of animals to the mixture (VERRIOPOULOS and MORAITOU-APOSTOLOPOULOU, 1983).

Although an analysis of the hydrocarbon content of the test solutions was not possible, data from the literature show that a small fraction of the oil added as dispersed fine droplets is measured as oil-in-water phase. According to ANDERSON et al. (1974), for the Kuwait crude oil, when 100 ppm were added to the test solution, the total hydrocarbons (as determined by IR) in the aqueous

Figure 2. - Impact of temperature and light conditions to the LC 50 48h of oil dispersant (Finasol OSR-2) to Artemia.



phase of the oil-in-water dispersion was 20 ppm (for 1,000, it was 27 and for 10,000.36). Unlike the water soluble fractions (WSF) of oils which are richer in the more soluble aromatic hydrocarbons. the OWDs are expected to resemble those of the parent oil since most of the hydrocarbons are present in dispersed droplets. The dispersant Finasol OSR-2 proved very toxic to Artemia. The theory behind the use of dispersants in oil incidents is that they accelerate the rate of natural degradation of the oil. The acceleration of the biological degradation is achieved by increasing the surface area of oil by dispersion. However the dispersant may make the oil more readily available to organisms and particularly to those with a distinctive structure or life style. The most at risk are probably the filter feeders like Artemia which extract small suspended particles from the water. According to COWELL (1976), the hydrocarbon solvents of dispersants are liable to penetrate into plants through the lipophilic surface and penetration is a crucial adjunct of toxicity. Once inside the plants, the detergents may dissolve cell membranes and cause loss of cell sap. Apart from their evident direct toxicity to organisms, dispersants exhibit a high demand for oxygen (500,000 to 1,000,000 mg/l) resulting, when used in closed bays with limited circulation, to a significant lowering or depletion of dissolved oxygen. Similar results on the toxicity of oil, dispersant and oil/dispersant mixture have been reported and discussed in detail in a previous paper (VERRIOPOULOS and MORAITOU-APOSTOLOPOULOU, 1983).

An important and in all cases significant increase in toxicity of the three tested toxic solutions has been noticed with the increase in temperature from 14 ° to 22° C. Temperature changes influence both toxicants and organisms.

Changes in temperature influence the transport of oil into water, changing the viscosity of oil, the solubility of oil and dispersant components and the stability of an emulsified or suspended oil. As temperature increases, the viscosity of the oil decreases and the solubility of the non-volatile components, which remain longer than the volatiles in the test solution, increases.

The elevation of temperature causes, within the tolerance range of an organism, a metabolic rate increase and also numerous biochemical and physiological changes. Temperature also changes the permeability of cellular membranes.

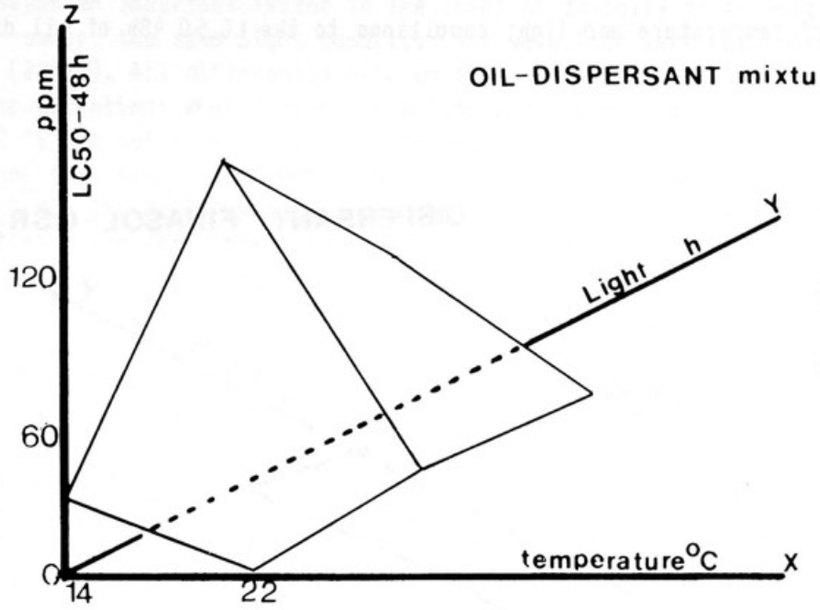


Figure 3. - Impact of Temperature and light conditions to the LC 50 48h of oil/dispersant mixture to Artemia.

Similar results, i.e. an increase in the toxicity of pollutants with the elevation of temperature have been referred to by other authors (EISLER, 1971: MORAITOU-APOSTOLOPOULOU et al., 1979). The pronounced increase observed in the sensitivity of Artemia to oil and Finasol with the elevation of temperature from 14 ° to 22 °C is of particular importance in temperate regions where marine animals encounter similar temperatures during summer.

The impact of light conditions on the toxicity of oil mainly but also, to a lesser degree, of Finasol and Finasol/oil mixture, seems very important. The increase in the toxicity of oil under continuous light and photoperiod conditions must be attributed to the direct effect of light both on oil and Artemia. There is evidence that polar hydrocarbon derivatives are generated from oil by photo-oxydation (LYSYJ and RUSSELL, 1974). These polar hydrocarbons tend to dissolve into solution from an oil slick which raises the total concentration of oil-derived hydrocarbons with time. Exposure of petroleum constituents to oxygen and light results in their oxidation. This oxidation usually produces more soluble compounds and/or toxic acids. For example the oxidation of n-octanol (solubility 1 ppm) yields n-octanic acid (solubility 600 ppm). The susceptibility of animals to oil dispersants has been found to vary over dark and light periods in the case of animals such as the limpet Patella vulgata which demonstrate circadian rhythms (DICKS, 1975).

Light also directly influences the physiology of marine organisms, e.g. by increasing their respiratory rates, thus rendering oil more available to animals. In the planktonic copepod Calanus finmarchicus exposure to light results in a sharp increase in respiratory rate (MARSHALL et al., 1935). The decapod crustacean Hemigrapsus oregonensis showed a higher oxygen consumption under light than crabs kept in constant darkness (DEHNEL, 1955).

Finally, there is evidence that light may activate the harmful effects of oil. CUSACHS and STEELE (1969) have suggested that carcinogenesis by polycyclic aromatics may result from sublethal photodynamic effects. These authors have proposed the involvement of singlet oxygen in polycyclic aromatics carcinogenesis. KHAN and KASNA (1976) have proposed an optical residue singlet oxygen theory of photocarcinogenicity in which the polycyclic aromatics were first bound to a cellular constituent. The resulting residual molecule could continue to absorb light from the environment and sensitize the formation of singlet oxygen. The observed tendency of decrease in dispersant toxicity to Artemia when acting under light must probably be attributed to the photo-oxidation of some part of its solvent and/or surfactant constituent which becomes less active, less soluble or more volatile.

The oil/dispersant mixture demonstrated the same reaction to photic conditions as the dispersant because its toxicity is mainly due to the dispersant part.

A further decrease in toxicity of dispersant and oil/dispersant mixture compared with their toxicity under light was observed when the two solutions were acting under photoperiod conditions. As photoperiod in our experiments began with 12 hours light, it might be supposed that light during this period exerted its "beneficial" effect on dispersant and oil/dispersant toxicity. During the following dark period Artemia individuals probably demonstrated the reaction in the dark refered to in the literature, i.e. lower respiratory and filtration rates. In this way the experimental animals absorbed smaller amounts of pollutants and thus proved more resistant.

The increase in oil toxicity observed under photoperiod conditions compared with their toxicity under light seems difficult to explain.

Achnowledgements

This work is part of the MED POL phase 2 research programme of UNEP. We thank UNEP for financial support.

References

ANDERSON J.W, J.M. NEFF, B.A. COX, A.E. TATEM and G.M. HIGHTOWER (1974). - Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish, Marine Biology 27: 75-88.

BLISS C.I. (1938). - The determination of dosage mortality curve from small numbers. Quarterly Journal of Pharmacology 2: 192-216.

COWELL E.B. (1976). - Oil pollution of the sea, In: Marine Pollution. R. Johnston Editor, Academic Press, London, New York, San Francisco: 353-403.

CUSACHS L.C. and R.H. STEELE (1969). - Physicochemical Mechanisms in Carcinogenesis. E. Bergman Editor: 59-63.

DEHNEL P.A. (1955). - Rates of growth of gastropods as a function of latitude. Physiological Zoology 28: 115-144.

DICKS B. (1975). - The importance of behaviour in toxicity testing ecological prediction. In: Marine Biology and Oil Pollution: J.M. Baker Editor, Applied Sciences, London.

EISLER R. (1971). - Cadmium poisoning in Eundulus heteroclitus and other marine organisms. Journal of Fisheries Research Board of Canada. 28: 1,225-1,234.

HEDTKE S. and F. PUGLISI, (1982). - Short-term toxicity of five oils to four freshwater species. Environmental Contamination and Toxicology. 11: 425-230.

KUHNHOLD W.W. (1974). - Investigations on the toxicity of water soluble extracts of three crude oils egg of cod (Gadus morhua). Ber. Konn. Meeresforsuchnung 23: 165-180.

KAUSS P.T.C., C. HUTCHINSON, C. SOTO, J. HELLEBUST and M. GRIFFITHS (1973). - The toxicity of crude oil and its ccomponents to freshwater algae. In: Proceedings of sixth Conference on Prevention and control of oil spills: 703-719. American Petroleum Institute, Washington.

KHAN A.N and M. KASNA. Trans. annals New York Academy of Sciences, 171: 24-32.

LYSYJ J. and E.C. RUSSELL (1974). - Dissolution of petroleum-derived products in water. Water Research 8: 863-868.

MARSHALL S.M., A.G. NICHOLIS and A.P. ORR (1935). - On the biology of Calanus finmarchicus. 6. Oxygen consumption in relation to environmental conditions. Journal of Marine Biological Association of the United Kinkdom 20: 1-28.

MORAITOU-APOSTOLOPOULOU M., G. VERROPOULOS and P. PALLA (1979). - Temperature and adaptation to pollution as factors influencing the acute toxicity of Cd to the planktonic copepod Acartia clausi. Iethys 9(2): 97-101.

PULICH W.M.Jr., K. WINTERS and C. VAN BAALEN (1974). - The effects of a n° 2 fuel oil and two crude oils on the growth and photosynthesis of microalgae. Marine Biology 28: 87-92.

TEMPLETON W.L., F.A. SUTTON, R.M. BEAN, R.C. ARNETT, J.W. BAYLOCK, R.E. WILDING and H.J. MOORE (1973). - Oil pollution studies on Lake Maracaibo, Venezuela. In: Proceedings of 1975 Conference on prevention and control of oil pollution: 419-468. American Petroleum Institute, Washington. DC.

VERRIOPOULOS G. and M. MORAITOU-APOSTOLOPOULOU (1983). - Comparative toxicity of oil (Tunisian crude oil zarzaitine type), oil dispersant (Finasol OSR-2) and oil/dispersant mixture on Artemia salina. VIes Journées d'Etudes sur les Pollutions marines, C.I.E.S.M., Cannes.

LE PHYTOPLANCTON DU LITTORAL ROUMAIN DE LA MER NOIRE SOUS L'INFLUENCE DE L'EUTROPHISATION

par

Nicolae BODEANU
Institut Roumain de Recherches Marines
Constantza (Roumanie)

Abstract

In the past 10-15 years a series of new important characteristics regarding the quantitative development and the structure of the phytoplankton of Romanian Black Sea coast have been registred : high global biomasses and densities ; frequent processes of ample blooming phenomena : the presence of a great number of mass species ; the participation of numerous fresh water origin species in the development processes - beside the aboriginal marine and brackish water ones ; a decrease in the role of the Diatoms and an increase in the importance of the Dinophlagellates and other algae groups.

The high ratios between the phytoplankton and zooplankton biomasses registered at present point to the existence in excess of planktonic algae stocks over long periods during the year, which represent an important nutritive supply for an increase on the bioproductivity of the Romanian coastal waters.

*
* *

Le renforcement de l'action anthrophique dans le bassin pontique et dans les fleuves tributaires a entraîné des modifications évidentes dans le régime chimique des eaux du littoral roumain de la mer Noire (5,6), la plus importante de celles-ci consistant dans l'augmentation des quantités de sels minéraux et substances organiques. C'est ainsi que durant l'intervalle 1975-1980, le stock des phosphates dans le secteur de Constantza a été en moyenne 19 fois plus grand que celui de la décennie 7, et le stock des nitrates 8 fois plus élevé · durant la même période, la teneur en substances organiques a augmenté avec 40 % (Tableau 1). De cette manière, les algues planctoniques se sont trouvées dans une situation nouvelle, complètement différente de celle du passé, leur milieu de vie devenant une véritable solution nutritive complexe, et cela ne pouvait pas demeurer sans implications sur le phytoplancton (1, 2, 4, 10).

Dans le développement quantitatif et dans la structure du phytoplancton du secteur roumain de la mer nous avons enregistré, pendant les dernières années, une série de caractéristiques essentielles nouvelles que nous allons présenter ci-dessous, dues au processus d'eutrophisation et qui interviennent dans l'augmentation de la productivité biologique de la zone.

Tableau n° 1

Valeurs moyennes multiannuelles de la teneur en phosphates, nitrates et substances organiques dans les eaux voisines du rivage de Constantza (d'après les données obtenues par COCIASU, POPA et CHIRILA - Institut Roumain de Recherches Marines).

Période	$P-PO_4$ (ug/l)	$N-NO_3$ (ug/l)	Substances organiques (mg O_2/l)
1960-1970	10,5	22,5*	1,96**
1971-1975	177,5	-	2,32
1976-1980	197,9	188,8	2,25
1981-1983	114,5	103,4	2,70

* Conformément aux données existantes seulement pour les années 1959-1960.

** Conformément aux données existantes seulement pour 1970.

Augmentatiom des quantités globales de phytoplancton

Comparativement à la valeur moyenne de 495 mg/m^3 citée pour la période 1959-1963 dans la zone comprise entre les isobathes de 20 à 50 m du secteur roumain de la mer (12), la biomasse a été 1,5 fois plus élevée dans la première moitié de la décennie 8, et d'environ 5 fois dans la seconde moitié de la même décennie (quand la moyenne multiannuelle a été de 2.244 mg/m^3).

Les quantités de phytoplancton ont augmenté surtout dans la zone du proche voisinage de la côte, grâce à l'influence directe de l'eutrophisation due aux déversoirs d'eaux résiduaires. Les valeurs moyennes que nous avons mentionnées pour cette zone (jusqu'à l'isobathe de 30 m), loin d'être insignifiantes, pendant la période 1960-1970 - de 1627 mg/m^3 et 1256 mille cell/l (1) - semblent très réduites par rapport aux chiffres correspondants de la dernière décennie. Par exemple, la biomasse moyenne enregistrée dans la même zone était, en 1976, 14 fois plus grande (23.080 mg/m^3) que dans la période 1960-1970, et la densité 6 fois (7.248 cell/l).

Apparition fréquente des phénomènes amples de floraison

Antérieurement considérés comme phénomènes exceptionnels, ils se produisent à présent annuellement (1, 3, 4, 9) pendant toutes les saisons sauf en hiver. Les densités des formes responsables de la floraison (Sceletonema costatum, Goniaulax polygramma, Exuviaella cordata, Cerataulina bergonii et d'autres dépassent beaucoup les valeurs enregistrées antérieurement pour n'importe quelle espèce du phytoplancton du littoral roumain (Tableau 2).

Au cours des intenses processus de floraison se produisent des biomasses phytoplanctoniques extrêmement grandes. Si jusqu'au début de la décennie 8 la biomasse maximale signalée pendant les floraisons de la partie nord-ouest de la mer était de 52 g/m^3 (8) nous avons maintenant enregistré, dans la zone de Constantza, 360 g/m^3 (en juillet 1982), et dans la zone de Portita 310g/m^3 (en septembre 1980) ; près de la zone nord du secteur roumain, dans le golfe d'Odessa, NESTEROVA a trouvé, en 1977, une valeur encore plus élevée, de 420 g/m^3 (11).

N'étant pas produites par des espèces nocives, les floraisons de la mer Noire ne constituent pas de phénomènes de toxicité. On peut constater que les phénomènes de floraison qui y ont lieu présentent un côté positif par leur conséquence directe (augmentation de la biomasse du phytoplancton) et surtout par l'effet de cette conséquence sur la croissance de la bioproductivité du pélagial. Ce fait se reflète dans l'augmentation, durant les dernières années, des quantités de poissons planctonophages - sprat et anchois -, ce qui a déterminé l'extension de leur capture de la zone voisine à la côte, où l'on agissait surtout par l'intermédiaire des installations de pêche fixées, vers la haute mer, par l'intermédiaire des navires.

Tableau n° 2

Densités maximales (mille cell/l) de certaines espèces de masse du phytoplancton du littoral roumain pendant les périodes 1960-1970 et 1971-1982*.

Espèces	1960-1970	1971-1983
Exuviaella cordata Ostf.	50.814	462.700
Goniaulax polygramma Stein.	-	97.600
Sceletonema costatum (Grev.) Cl,	18.080	97.360
Cyclotella caspia Grun.	28.072	300.000
Cerataulina bergonii Perag.	922	14.000
Eutreptia lanowii Steuer	-	34.000
Sceletonema subsalsum (A.Cl.) Bethge	-	3.310
Thalassiosira parva Pr.-Lavr.	360	2.060
Thalassiosira subsalina Pr.-Lavr.	216	636
Rhizosolenia fragillissima Berg.	404	6.100
Chaetoceros socialis Laud.	1.342	2.610
Chaetoceros similis Cl.	470	6.800
Chaetoceros curvisetus Cl.	144	878
Thalassionema nitzschioides Grun.	739	1.200
Microcystis pulverea (Wood.) Forti	-	19.403
Rhizosolenia calcar-avis Schultze	3.200	-
Leptocylindrus minimus Gran.	6.834	425
Leptocylindrus danicus Cl.	7.075	864
Detonula confervacea (Cl.) Gran.	935	310
Chaetoceros lorenzianus Grun.	632	206
Diatoma elongatum (Lyngb.) Ag.	437	-
Nitzschia seriata Cl.	3.072	3.000
Nitzschia delicatissima Cl.	21.000	4.500

* Le signe (-) signifie que les densités maxima n'ont pas atteint le seuil de 100.000 cell/l (et non pas leur absence complète) pendant la période de référence.

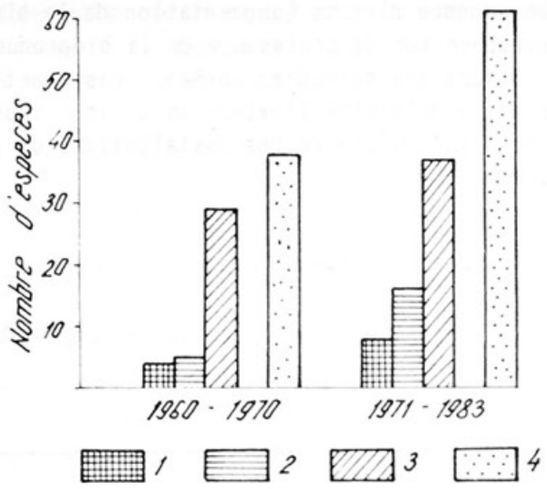


Fig. 1

Nombre d'espèces de masse dans le phytoplancton du littoral roumain pendant les périodes 1960-1983 (1 - avec des densités maximum plus de 10 millions cell/l ; 2 - avec des densités maximum de 1-10 millions cell/l ; 3 - avec des densités maximum de 100 mille-1 million cell/l ; 4 - total espèces dont les densités dépassaient 100 mille cell/l).

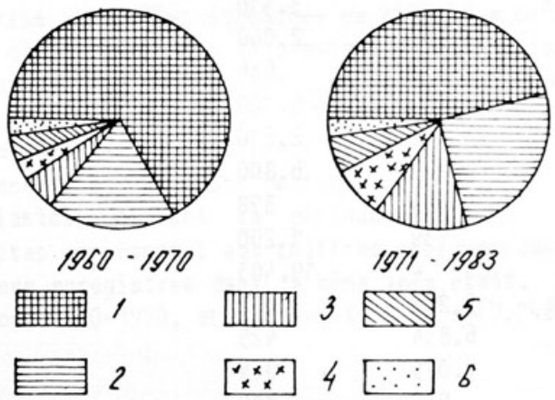


Fig. 2

Structure par groupes systématiques du phytoplancton du littoral roumain pendant les périodes 1960-1970 et 1971-1983 (1 - Bacillariophyta ; 2 - Pyrrophyta ; 3 - Chlorophyta ; 4 - Cyanophyta ; 5 - Chrysophyta ; 6 - Euglenophyta et Xantophyta).

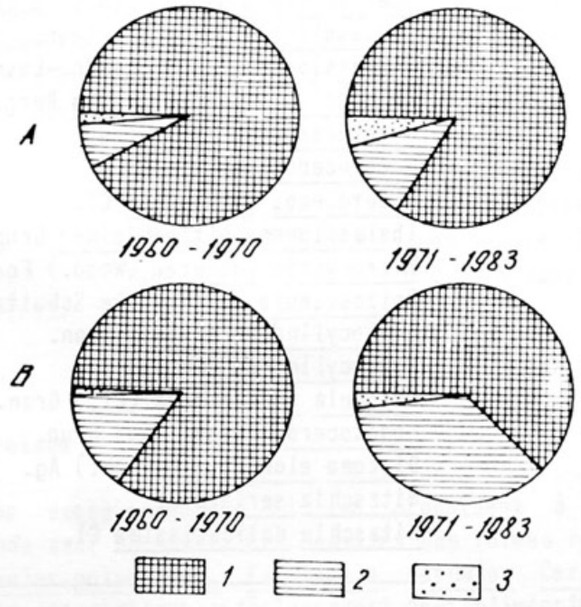


Fig. 3

Structure par groupes d'algues en densités (A) et biomasse (B) du phytoplancton du littoral roumain pendant les périodes 1960-1970 et 1971-1983 (1 - Diatomées ; 2 - Péridiniens ; 3 - Chlorophytes, Cyanophytes, Chrysophytes, Euglenophytes et Xantophytes).

Mais, si les floraisons du printemps restent sans effets directs sur la dégradation du milieu aquatique, les températures basses et l'hydrodynamisme intense, spécifique à la saison, empêchant de telles conséquences, les choses changent dans le cas des floraisons qui se produisent en été. Les processus cataboliques qui ont lieu dans les agglomérations algales, en conditions d'accalmie et températures élevées, déterminent l'installation de l'hypoxie, le taux d'oxygène de l'eau de mer diminuant au-dessous de 2-3 mg O_2/l. En même temps, la grande quantité d'algues planctoniques vieillies et mortes dans l'eau, ainsi que leurs produits d'excrétion, déterminent des croissances importantes de la teneur des eaux en substances organiques (3). L'installation de l'hypoxie et l'asphyxie par la colmatation des branchies des animaux filtrateurs avec les grandes quantités de suspensions algales existantes déterminent la mortalité en masse, sur de larges étendues du fond de la mer, de certains animaux benthiques tels que les poissons, les crustacés et surtout les mollusques (3, 7). Certes, le déclenchement de la mortalité massive des animaux benthiques constitue un nouveau facteur de pollution secondaire, d'origine biologique, qui détruit la qualité de l'eau de mer et les conditions de vie des organismes.

Présence d'um grand nombre d'espèces de masse

L'excès de sels nutritifs, rendant nulle la compétition pour la nourriture entre les populations phytoplanctoniques, détermine la végétation quasi-permanente - y compris durant les phénomènes de floraison - d'une grande diversité d'espèces (1, 3, 4) ; beaucoup d'entre elles connaissent d'importants développements quantitatifs aux côtés des formes responsables de la floraison.

La plupart des principales espèces du phytoplancton ont des densités supérieures à celles d'autrefois (Tableau 2). Ce fait implique la présence d'un plus grand nombre d'espèces de masse. Si de 1960 à 1970 nous avons trouvé dans le secteur roumain de la mer seulement 38 espèces dont les densités dépassaient 100 mille cell/l, le nombre des espèces de la même catégorie de 1971 à 1983 a été de 61 : le nombre des espèces ayant des densités de l'ordre de millions de cell/l a augmenté de 9 dans la décennie 7, à 24 pendant l'intervalle 1971-1983 (Fig. 1).

Réduction de la densité numérique de certaines espèces de masse

Parallèlement à l'augmentation de densité en majorité des formes de masse, on constate l'existence d'espèces dont les densités ont diminué au-dessous des valeurs enregistrées pendant les années 60, ce qui supposerait l'aggravation de certaines conditions de végétation, qu'elles exigent. C'est surtout le cas de quelques Diatomées d'importance quantitative antérieurement primordiale (Tableau 2).

Participation aux processus de développement du phytoplancton, à côté des espèces autochtones marines et saumâtricoles, de nombreuses espèces d'origine dulçaquicole

Parmi les 61 espèces dont les densités dépassaient 100 mille cell/l dans la période allant de 1971 à 1983, 21 sont dulçaquicoles : quelques-unes (telles que Sceletonema subsalsum, Microcystis pulverea, Gloeocapsa crepidinium, etc.), se développent jusqu'à des millions de cellules par litre. L'ample développement des espèces dulçaquicoles met en évidence non seulement leur capacité d'adaptation, fondée sur leur halophilie, dans les conditions de salinité du milieu pontique, mais aussi la capacité actuelle du milieu respectif de nourrir toujours plus de populations d'algues planctoniques.

Réduction du taux de Diatomées et augmentatiom du taux de Péridiniens et d'autres groupes d'algues

Les changements d'ordre quantitatif du phytoplancton se reflètent aussi dans les proportions résidant entre les groupes taxonomiques, qui différent aujourd'hui de celles connues autrefois. Ces changements concernent la structure qualitative du phytoplancton ainsi que celle quantitative (Fig. 2-3) et consistent essentiellement dans la diminution de la contribution des Diatomées en faveur des Péridiniens, des Chlorophytes et des Cyanophytes, parmi lesquelles on retrouve beaucoup d'espèces à affinités mixotrophes. Il faut souligner que l'augmentation du taux des Péridiniens (Fig. 3) représente, surtout dans la structure quantitative du phytoplancton, l'expression de la croissance de la fréquence et de l'ampleur des phénomènes de floraison qu'ils produisent.

Le problème de la mesure dans laquelle les consommateurs directs du phytoplancton utilisent ces stocks, augmentés pendant les dernières années, présente un intérêt particulier.

En l'absence d'études concernant le budget énergétique de l'écosystème du secteur roumain de la mer Noire, on peut effectuer une appréciation globale sur le problème posé en discutant les rapports entre les biomasses du phytoplancton et celles de son consommateur direct, le zooplancton. Dans une acception élargie, ces rapports peuvent être considérés comme indices indirects de la consommation du

Tableau n° 3

Rapports entre les biomasses du phytoplancton (Fpk) et du zooplancton (Zpk) dans le secteur Constantza (depuis la côte jusqu'à 30 milles marins au large) dans les années 1977 et 1980.

1977				1980			
	Biomasse (mg/m3)				Biomasse (mg/m3)		
Mois	Fpk	Zpk	Fpk:Zpk	Mois	Fpk	Zpk	Fpk:Zpk
I	153,43	4,10	37,4:1	I	233,20	5,21	44,8:1
II	5.890,57	4,87	1.209,6:1	II	1.292,78	28,15	45,9:1
III	1.121,25	7,52	149,1:1	III	1.950,98	23,05	84,6:1
IV	9.610,14	68,10	141,1:1	IV	1.590,16	17,55	904,3:1
V	2.763,65	97,42	28,1:1	V	12.096,45	532,12	22,7:1
VI	1.933,21	782,17	2,5:1	VI	2.181,73	275,27	7,9:1
VII	560,65	954,22	0,6:1	VII	1.334,85	338,92	3,9:1
VIII	446,62	245,02	1,8:1	VIII	7.589,07	266,22	28,5:1
IX	2.386,73	63,38	37,7:1	IX	2.009,49	137,99	14,6:1
X	2.114,99	435,42	4,9:1	X	187,13	263,85	0,7:1
XI	547,21	427,43	1,3:1	XI	515,19	909,19	0,6:1
XII	122,88	79,02	1,6:1	XII	915,69	1.401,79	0,7:1

* Les données sur biomasse du zooplancton ont été obtenues par A. Petran (IRCM-Constantza).

Tableau n° 4

Rapports entre les biomasses du phytoplancton (Fpk) et du zooplancton (Zpk) dans les eaux voisines à la côte (jusqu'à l'isobathe de 10 m) dans la zone sud du littoral roumain en 1983.

Secteur Navodari				Secteur Eforie Sud			
	Biomasse (mg/m3)				Biomasse (mg/m3)		
Mois	Fpk	Zpk	Fpk:Zpk	Mois	Fpk	Zpk	Fpk:Zpk
II	199,07	4,82	41,3:1	II	146,13	0,74	197,5:1
IV	251,95	9,87	24,5:1	IV	18,00	1,53	11,8:1
V	420,47	6,61	63,6:1	V	173,81	0;96	181,1:1
VI	9.412,54	7.613,83	1,2:1	VI	745,08	182,83	4,1:1
VII	5.008,45	721,81	41,1:1	VII	2.525,50	1.502,50	1,7:1
VIII	877,83	15,30	57,1:1	VIII	1.165,68	9,04	128,9:1

* Les données sur les biomasses du zooplancton ont été obtenues par A. Petran (IRCM-Constantza).

Tableau n° 5

Rapports entre les biomasses du phytoplancton (Fpk) et du zooplancton (Zpk) dans les eaux de la zone nord du littoral roumain au printemps et en été de l'année 1983.

Mars				Août			
	Biomasse (mg/m3)				Biomasse (mg/m3)		
Secteur	Fpk	Zpk	Fpk:Zpk	Secteur	Fpk	Zpk	Fpk:Zpk
Sulina	2.150,22	2,48	867,0:1	Sulina	3.734,14	98,62	37:9:1
Mila 9	1.014,52	4,18	242,7:1	Mila 9	21.351,05	348,49	61,3:1
Sf.Gheorghe	1.731,52	2,49	695,4:1	Sf.Gheorghe	9.427,71	153,55	61,4:1
Portita	5.152,34	1,24	4.155,1:1	Portita	19.532,09	150,18	130,1:1
Gura Buhaz	12.377,42	5,16	2.398,7:1	Gura Buhaz	3.704,02	159,27	23,3:1

* Les données concernant la biomasse du zooplancton ont été obtenues par A. Petran (IRCM-Constantza).

phytoplancton, la mesure de son utilisation étant d'autant plus grande que les indices respectifs sont plus petits.

Partant du point de vue bien connu que le rendement écologique minimum d'un chaînon trophique inférieur à celui immédiatement supérieur est d'environ 10 %, il résulte que pour une consommation

normale, les herbivores aquatiques ont besoin d'une quantité de substance organique végétale au maximum 10 fois supérieure à leur poids corporel.

En analysant la dynamique des rapports mensuels entre les biomasses du phytoplancton et zooplancton sur une radiale Est Constantza - avec 5 stations entre 1 et 30 milles marins de distance de la côte - on constate que durant de longs intervalles de l'année, comprenant la période de janvier à mai, la fin de l'été et le début de l'automne, les rapports sont nettement supérieurs à la valeur 10:1, ce qui met en évidence l'excès des stocks de phytoplancton (Tableau 3).

Dans une autre zone caractéristique du secteur roumain de la mer - eaux du proche voisinage de la côte dans la moitié sud de notre littoral (jusqu'à l'isobathe de 10 m)- les rapports respectifs sont presque toujours nettement supérieurs à la valeur 10:1, en enregistrant des diminutions au-dessous de cette valeur seulement dans la première partie de l'été (Tableau 4). En ce qui concerne la partie nord du littoral roumain, soumise à l'influence directe du Danube, les rapports sont extrêmement élevés au printemps (jusqu'à 4155:1 dans le secteur Portitza), demeurant en été supérieurs à la valeur 10:1 (Tableau 5).

On peut conclure que les données concernant les rapports entre les biomasses du phyto- et zooplancton pendant les dernières années, suggèrent l'existence de stocks excessifs d'algues planctoniques durant de longs intervalles de l'année, dans toutes les zones caractéristiques de notre littoral. Nous considérons que les grands stocks de phytoplancton représentant une importante réserve nutritive pour l'augmentation de la bioproductivité du secteur roumain de la mer Noire, en agissant en ce sens par la croissance dirigée des animaux filtrateurs et phytophages intéressants du point de vue économique, et par l'acclimatation de nouvelles espèces de la même catégorie, qui puissent utiliser plus complètement la base trophique primaire.

Références

(1) N. BODEANU (1978). - Compozitia si dinamica fitoplanctonului si microfitobentosului din apele costiere românesti ale Marii Negre. Ministerul Educatiei si Invatamîntului, Institutul de stiinte Biologice : 1-25, Bucuresti.

(2) N. BODEANU (1983). - Modifications concernant le développement quantitatif et la structure du phytoplancton du littoral roumain de la mer Noire. Rapp. Comm. int. Mer Médit., 28, 9 : 91-92.

(3) N. BODEANU, A. ROBAN (1975). - Données concernant la floraison des eaux du littoral roumain de la mer Noire avec le péridinien Exuviaella cordata Ostf. Cercetari marine, I.R.C.M., 8 : 43-62.

(4) N. BODEANU, A. ROBAN, M. USURELU (1978). - Eléments concernant la structure et la dynamique du phytoplancton du littoral roumain de la mer Noire pendant la période 1972-1977. Cercetari marine, I.R.C.M., 11 : 61-76.

(5) A. COCIASU, L. POPA (1980). - Observations sur l'évolution des principaux paramètres physico-chimiques de l'eau marine de la zone Constantza. Cercetari marine, I.R.C.M., 13 : 51-61.

(6) A. COCIASU, L. POPA, L. DOROGAN (1981). - Dynamique de la matière organique dans les eaux marines devant le littoral roumain de la mer Noire (d'après les données sur l'oxydabilité). Cercetari marine, I.R.C.M., 14 : 19-29.

(7) M.-I. GOMOIU (1976). - Modificari în structura biocenozelor bentale de la litoralul românesc al Marii Negre. Cercetari marine, I.R.C.M., 9 (supl.) : 119-142.

(8) A. IVANOV (1967). - Fitoplankton. Biologhiia severo-zapadnoi ciasti Cernogo moria. Kiev. 57-75.

(9) P.-E. MIHNEA, E. CUINGIOGLU (1982). - Particularités des phénomènes de "floraison". Cercetari marine, I.R.C.M., 15 : 27-59.

(10) P.-E. MIHNEA, E. CUINGIOGLU, I. BILAL (1981). - Modifications des communautés phytoplanctoniques littorales sous l'influence du phénomène de pollution. Ves Journées Etud. Pollutions : 869-876.

(11) D.A. NESTEROVA (1977). - Razvitie fitoplanktona severo-zapadnoi ciasti Cernogo moria v vesenii, letnii i osenii periodî. Biologhiia moria, 43 : 17-23.

(12) H.V. SKOLKA (1967). - Consideratii asupra variatiilor calitative si cantitative ale fitoplanctonului litoralului românesc al Marii Negre. Ecologie marina, 2 : 193-293, Bucuresti.

STUDY ON THE TOXICITY OF THE FINAL WASTE WATER OF SOME FACTORIES IN ALEXANDRIA (EGYPT)

by

Hamed HAMED SALEH
Alexandria Institute
of Oceanography and Fisheries, Alexandria (Egypt)

Abstract

Laboratory experiments showed that ammonia content in the final waste water of Abu Qir fertilizer factory is not only poisonous but also destroys ionic regulation of heavy metals through the gills which implies the high toxicity of such waste water. On the other hand, the suspended matter in the final waste water of the Rakta paper factory, which adsorbs heavy metals, accumulates on the gills causing their fouling.

*
* *

Introduction

Most bioassay studies are mainly to estimate the biological effects of different concentrations of heavy metals in the aquatic environment.

JACKSON et al. (1980) explained that the biological effect of trace elements in the aquatic environment depended on the forms as well as the concentration of these elements.

RUDD et al. (1983) showed that suspended sediments are thought to play an important role in the bioaccumulation of toxic substances.

This study is an attempt to estimate the mortality rate of Mugil capito Cuv exposed to different types of final waste water, e.g. Abu Qir fertilizer factory, Rakta paper factory, El Seouf textile factory and an Egyptian plastic factory.

Materials and method

Six aquaria were used to test the toxicity of the final waste water of each factory at different dilutions.

Each aquarium contained 100 litres of clean water (tap water) and 10 Mugil capito Cuv ('X = 11 cm ± 1.3 cm TL). The aquaria were slowly aerated by air compressor. After the fish had acclimatized for two weeks, different dilutions of the final waste were effected: as 20 %, 40 %, 60 %, 80 %, 100 %, final waste water. The mortality rate of Mugil capito was recorded in the different aquaria after exposure to the contaminated water.

Cu, Zn and Pb as examples of heavy metals, were measured in the gills and flesh of the Mugil capito that had died due to water pollution. The above mentioned elements were also estimated in the final waste water of each factory using an AAS instrument.

Results and discussion

The physicochemical analysis of the final waste water of Abu Qir fertilizer factory showed its high content of ammonia, sulphates, and heavy metals. The final waste water of Rakta paper factory contained high amounts of suspended matter, sulphates, and heavy metals. The final waste water of El Seouf textile factory contained considerable amounts of ammonia, sulphates, suspended matter, and heavy metals. On the other hand, the final waste water of Egyptian plastic factory contained heavy metals in nearly normal water (table 1).

Parameters	mg/L			
	Abu Kir fertilizer factory	Rakta paper factory	El Seouf textile factory	Egyptain plastic factory
Temperature	33°C	24°C	27°C	25°C
pH	9.35	8.37	9.7	7.2
Nitrate (as N)	4.0	2.22	5.23	3.95
Nitrite (as N)	0.01	0.034	0.01	0.013
Ammonia (as N)	600	0.62	9.1	2.04
Phosphate	0.62	0.33	-	-
Sulphate	350	295	-	-
Chloride	750	376	260	750
Total Alkalinity	880	318	300	220
Total hardness	280	431	240	330
Calcium hardness	140	234	120	100
B.O.D.	70	964	850	160
C.O.D.	160	1430	1140	229
Dissolved oxygen	7.2	0.63	5.3	2.5
Total solids	2341	2842	1712	1466
Fixed solids	2259	1557	595	1165
Volatile solids	82	1285	1117	201
Settled solids	0.1	36.7	6.60	1.0
Oil & Greeze	16.	50	70.5	127
Copper*	0.096	0.140	0.092	0.052
Zinc *	0.176	0.138	0.168	0.168
Lead *	0.142	0.126	0.112	0.062

Cu, Zn and Pb were measured by AAS.

Table 1. - Physicochemical characteristics of the final waste water for Abu Kir fertilizer factory, Rakta paper factory, El Seouf textile factory, and Egyptian plastic factory (After the High Institute of Public Health, Alexandria, Egypt).

The mortality rate of Mugil capito inhabiting different dilutions of the final waste of the previous by mentioned factories showed that Abu Qir fertilizer factory waste water had the maximum toxic effect. The mortality rate of Mugil capito inhabiting different dilutions of the final waste water of Rakta paper factory or El Seouf textile factory were high but lower than that of Abu Qir fertilizer factory. The mortality rate of Mugil capito inhabiting different dilutions of the final waste water of the Egyptian plastic factory was, in comparison very low (table 2). The shorter periods of survival of Mugil capito inhabiting different dilutions of the final waste water of Abu Qir fertilizer factory, Rakta paper factory, and El Seouf textile factory could be considered as due to synergism i.e. a combination of factors causing the high toxicity of such industrial waste water. On the other hand, the low toxicity of the final waste water of the Egyptian plastic factory is attributed to the effect heavy metals only.

Factory	% of final waste water in aquarium water					
	Clean water (blank)	20% final waste water	40% final waste water	60% final waste water	80% final waste water	100% final waste water
Abu Kir fertilizer factory	0%	100% (during 15 minutes)	100% (during 12 minutes)	100% (during 8 minutes)	100% (during 5 minutes)	100% (during 5 minutes)
Rakta paper factory	0%	60% (during 24 hours)	90% (during 24 hours)	100% (during 24 hours)	100% (during 12 hours)	100% (during 6 hours)
El Seouf textile factory	0%	60% (during 24 hours)	80% (during 24 hours)	100% (during 24 hours)	100% (during 14 (hours)	100% (during 7 hours)
Egyptain plastic factory	0%	0%	0%	0%	10% (during 96 hours)	20% (during 96 hours)

Table 2. - Mortality rate of Mugil capito Cuvier inhabiting aquaria containing different dilutions of the final waste water, for Abu Kir fertilizer factory, Rakta paper factory, El Seouf textile factory, and Egyptian plastic factory.
Each aquarium contained 100 liters water + 10 Mugil capito of average T.L. = 11 ± 1.3 cm.

The content of heavy metals in Mugil capito that had died due to synergism, decreases with the increased contamination of water aquaria, while the reverse occurs in Mugil capito dead owing to heavy metals only (table 3). Such results probably indicate the marked effect of factors other than heavy metals e.g. ammonia and suspended matter, on the mortality rate of the fish. In other word heavy metals play only a partial role in the toxicity of such waste water.

Factory	Organ	20% final waste water in water aquarium			40% final waste water in water aquarium			60% final waste water in water aquarium			80% final waste water in water aquarium			100% final waste water in water aquarium		
		Cu	Zn	Pb	Cu	Zn	Pb	Cu	Zn	Pb	Cu	Zn	Pb	Cu	Zn	Pb
Abu Kir fertilizer factory	Gills	305.3	323.86	194.67	228.18	254.64	54.54	63.86	69.96	34.11	24.83	46.34	44.68	19.84	27.85	27.01
	Flesh	51.66	121.87	138.15	100.7	44.38	12.67	22.6	21.09	10.19	23.13	21.16	13.69	39.91	20.51	13.58
	Ratio	5.91	2.65	1.41	2.13	5.73	4.3	2.78	3.31	3.33	1.07	2.18	3.26	0.49	1.35	1.99
Rakta paper factory	Gills	81.86	162.35	173.15	79.14	155.12	168.11	52.33	153.12	153.07	50.14	151.21	101.33	38.63	146.7	91.64
	Flesh	50.14	71.12	44.14	48.11	64.51	41.14	36.15	50.32	29.35	16.63	29.42	15.07	12.21	29.11	12.23
	Ratio	1.63	2.28	3.92	1.64	2.4	4.08	1.44	3.04	5.21	3.01	5.13	6.72	3.16	3.04	7.49
El Seouf textile factory	Gills	161.14	177.11	159.91	148.14	141.11	152.14	56.35	120.13	147.12	38.42	103.12	85.14	36.10	63.14	75.15
	Flesh	81.11	45.26	36.85	59.14	37.88	27.84	36.27	38.15	22.11	26.14	28.56	16.28	9.84	25.14	10.15
	Ratio	1.91	3.91	4.33	2.5	3.72	5.46	1.55	3.14	6.65	1.46	3.61	5.22	3.66	2.51	7.40
Egyptain plastic factory	Gills										58.59	73.45	31.78	63.62	94.96	63.07
	Flesh										10.56	14.49	5.51	11.97	21.73	15.19
	Ratio										5.5	5.06	5.76	5.31	4.36	4.15

Table 3. - Content of Cu, Zn, and Pb in the gills and flesh of Mugil capito Cuvier died when exposed to different dilutions of the final waste water, for Abu Kir fertilizer factory, Rakta paper factory, El Seouf textile factory, and Egyptian plastic factory (mg element/kgm gills or flesh) and Ratio of heavy metals content in gills to that in flesh.

The rate of accumulation of heavy metals in the gills compared with that in the flesh of Mugil capito dead due to exposure to different dilutions of the waste water, as expressed by the ratio between the content of heavy metals in the gills to that in the flesh, decreases with the increase in water contamination in the case of the Abu Qir fertilizer factory waste water which is characterized

by the presence of high amounts of ammonia, while the reverse occurs in the water aquaria contaminated with the waste water of the Rakta paper factory which is characterized by the presence of a considerable amount of suspended matter (table 3). The above results probably indicate that ammomia is not only poison (VAMOS et al., 1967), but it also destroys the ionic regulation of heavy metals through the gills. On the other hand, the suspended matter which adsorbs heavy metals accumulates on the gills causing their fouling.

References

JACKSON T.A., G. KIPPHUT, R. HESSLEIN and D.W. SHINDLER (1980). - Water lakes at different pH levels. Can. J. Fish, Aquat. Sci. 37: 387-402.

RUDD J.W.M. and M.A. TURNER (1983). - The English-Wabigoon, II suppression of mercury and selenium bioaccumulation by suspended and bottom sediment Can. J. Fish. Aquat. Sci. 40: 2,218-2,227.

VAMOS R. and R. TASNAD (1967). - Ammonia poisoning in carp, 3. The oxygen content as a factor in influencing the toxic limit of ammonia. Acta Biol., 13: 99-105.

EFFECT OF INDUSTRIAL WASTE WATER FROM TABIA PUMPING STATION IN ABU QIR BAY, MEDITERRANEAN SEA ON MUGIL CAPITO CUV.

by

Hamed HAMED SALEH
Alexandria Institute
of Oceanography and Fisheries
Alexandria (Egypt)

Abstract

Laboratory experiments have proved that Tabia water at 100 per cent or 80 per cent concentration has a lethal effect on adult Mugil capito which is even more pronounced on the fish fry.

The feeding activity and growth rate of Mugil capito surviving in sublethal dilutions of Tabia water decreased considerably.

Such results may explain the decline of Abu Qir Bay as a spawning ground for emigrant fishes, e.g. Mugil species from lake Edku.

*
* *

Introduction

Bioassay studies are mainly aimed at predicting what would happen under field conditions on the basis of laboratory experiments (SINDERMANN, 1980).

Tabia station could be considered as the main station pumping the industrial and agricultural waste water into Abu Qir Bay, Mediterranean Sea. It is a non-tidal bay with restricted exchange with the open sea and is considered the main spawning ground for emigrant fishes e.g. grey mullet which migrate from lake Edku.

This study is an attempt to explain the marked decrease in Mugil species in the fish population of lake Edku.

Materials and method

Six aquaria were used. Each aquarium contained 100 litres of clean sea-water from the open sea and 15 immature Mugil capito Cuv ('X = 10 cm ± 1.3 cm TL). The aquaria were slowly aerated by an air compressor. Water temperature in the aquaria was 28 °C ± 3.

Parameters	Value in mg/L	Parameters	Value in mg/L
Temperature	27°C	Total solids	1420
pH	6.8	Fixed solids	1145
Dissolved oxygen	0.6	Volatile solids	275
Ammonia	0.02	Dissolved solids	1272
Nitrate	0.2	Suspended solids	148
Nitrite	5	Settled solids	13
Phosphate	0.31	Sulphides	9.2
Sulphate	208	Silver	0.0125
Biological oxygen demand	273	Cadmium	0.0034
Chemical oxygen demand	237.6	Copper	0.0116
Chloride	475	Iron	0.0402
Total alkalinity	28.0 as $CaCO_3$	Manganese	0.0984
Total hardness	420	Lead	0.0625
Calcium hardness	80	Zinc	0.0793
Magnesium hardness	340		

Table 1. - Physicochemical characteristics of the waste water pumped by Tabia station into Abu Qir Bay (after High Institute of Public Health, Alexandria, Egypt).

Two types of experiments were carried out:

a) a short-term experiment for 48 hours to show the lethality of Tabia water on adult Mugil capito and its fry;

b) a long-term experiment for three months to show the sublethal effect of Tabia Water on the feeding activity of Mugil capito using the method of BROWN (1957) and its growth rate using the method of GRAHAM (1923).

% of Tabia water in water aquarium	% of adult fish died during 48 h	% of fries died during 48 h
100% clean sea water	0%	4%
20% Tabia water	12%	90%
40% Tabia water	20%	100%
60% Tabia water	20%	100%
80% Tabia water	54%	100%
100% Tabia water	80%	100%

Table 2. - Mortality of adult and fries of Mugil capito during 48 hours placed in aquaria containing different dilutions of Tabia water.

Results and discussion

The physicochemical characteristics of Tabia water are summarized in table 1. EL SAMRA (1973) traced the extension of Tabia water into Abu Qir Bay by its chemical composition. He found that it can reach several miles into the bay and may even enter lake Edku.

% of Tabia polluted water in water aquarium	Weekly													
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week	12th week	13th week	Total
0% (clean sea water) Blank	2.61	3.85	3.14	2.00	2.03	2.83	3.03	4.11	2.77	5.35	4.73	2.81	3.8	41.7
10% Tabia polluted water	2.55	3.78	3.72	1.80	1.69	2.78	2.20	3.41	2.18	5.65	4.64	1.79	3.11	38.34
20% Tebia polluted water	2.45	3.86	3.57	2.14	1.21	2.85	1.94	2.19	2.55	4.39	4.78	1.83	3.02	36.78
30% Tabia polluted water	2.36	3.46	3.64	1.92	1.58	2.21	1.86	3.04	2.57	4.64	4.70	2.97	3.51	37.56
40% Tabia polluted water	2.24	3.48	3.5	1.82	1.41	2.34	1.64	2.55	2.57	4.56	4.73	1.73	3.42	35.94
50% Tabia polluted water	1.33	3.45	3.06	1.67	1.49	2.82	2.25	2.34	2.66	4.39	4.23	2.18	3.38	35.23

Table 3. - Dried shrimp meat (in grams) eaten weekly by 15 Mugil capito Cuvier living in different dilutions of the polluted water pumped by Tabia pumping station during three months (July-September).
% of water content in shrimp meat due to drying before feeding = 70.73 ± 1.33 %
after feeding = 70.22 ± 1.81 %.

Within 48 hours, 80 per cent of Mugil capito placed in 100 per cent of Tabia water aquaria died, while 54 per cent of the fish died in aquaria containing 80 per cent of Tabia water. Fish mortality was comparatively low in the other aquaria. However, the mortality rate of the fish fry was very high or total in the different dilutions of Tabia water (table 2).

The feeding activity of Mugil capito surviving in sublethal dilutions of Tabia water was considerably decreased. This decrease in feeding activity of the fish was proportional to the degree of water contamination with Tabia water (table 3).

BROWN (1957) explained that food supply is probably the most potent factor affecting the growth of fish. Accordingly, a considerable decrease in the growth rate of Mugil capito was recorded at the end of the experiment (table 4).

% of Tabia water in water aquarium	$K_f = \frac{\text{gutted weight of the fish x 100}}{(\text{standard length})^3}$
100% clean sea water (blank)	1.131 ± 0.090
10% Tabia water	0.981 ± 0.093
20% Tabia water	0.892 ± 0.087
30% Tabia water	0.886 ± 0.091
40% Tabia water	0.773 ± 0.095
50% Tabia water	0.758 ± 0.098

Table 4. - Growth rate of Mugil capito living in different sublethal dilutions of Tabia water for three months, as expressed by the condition of its flesh K_f.

LOVE (1970) suggested that the death of older fish may be attributed to the large amount of energy required from the body for gonad maturation and spawning. Similarly, a considerable amount of energy is required by the fish inhabiting a polluted water environment (CROGHAN, 1961). In the meantime, the components required to provide such energy becomes less available for fish inhabiting polluted water due to the decrease in its feeding activity which eventually leads to its death (chronic toxicity). The progressive turbidity of the water in Abu Qir Bay owing to its contamination with Tabia water depresses photosynthesis (GLOVE et al., 1972), an additional factor that should be taken into account when considering the condition of the fish.

Year	Total catch in tonns	Grey mullet catch in tonns	% of the grey mullet to the total catch.
1964	4787.5	581.9	12.15%
1965	4747.8	561.2	11.82%
1966	3539	419	11.84%
1975	1059.5	52.1	4.91%
1976	875.5	51.2	5.84%
1977	1279.4	60.1	4.69%
1978	632.2	38.2	6.04%
1979	780.5	50.3	6.44%
1981	807.5	40.9	5.08%
1982	442.9	8.7	1.96%

Table 5. - Annual total catch of lake Edku, and percentage of the grey mullet to the total catch (after Statistical Department in Alexandria Institute of Oceanography and Fisheries, Egypt) (Tabia station established in July 1965).

The high mortality rate and the bad condition of the grey mullet inhabiting the polluted water of Abu Qir Bay are clearly reflected in the considerable decline of Mugil species in the catch population of lake Edku efter the establishment of the Tabia pumping station (table 5).

References

BROWN M.E (1957). - Experimental studies on growth. In: Physiology of fishes, vol. I: 361-401.

CROGHAN P.C. (1961). - Competition and mechanism of osmotic adaptation., Sympos. Soc. Exper. Biol. 15: 156-167.

EL SAMRA M.I. (1973). - Chemical and physical properties of the water between lake Edku and the sea, M. Sc. thesis, Alexandria University (Egypt) 111 pp.

GRAHAM M. (1923). - The annual cycle in the life of the mature cod in the North Sea, Min. Agr. Fish. invest: Ser II. 6(6).

GLOVER R.S., G.A. ROBINSON and G.M. COLOBROCK (1972). - Plankton in the North Adriatic. An example of the problems of analysing variability in the environment. In: Marine Pollution and Sea life FAO: 439-445.

SINDERMANN C.J. (1980). - Pollution effects on fisheries potential management activities, Helgolander Meeresuntersuchungen 33: 674-686.

LOVE M. (1970). - The chemical biology of fishes, Acad. Press. Inc. London 547 pp.

TOXICITY OF CRUDE OIL AND CRUDE OIL PLUS DISPERSANT MIXTURES ON THE MARINE DIATOM PHAEODACTYLUM TRICORNUTUM*

by

Victor AXIAK and James L. SCHEMBRI
Mathematics and Science Department
University of Malta Msida (Malta)

Abstract

The effects of six different oil dispersants of the third generation on the toxicity of crude oil emulsions to the marine diatom Phaeodactylum tricornutum were investigated. Cultures were exposed to five different concentrations of oil or oil plus dispersant mixtures for 24 h and the algal cell counts relative to controls are presented. The higher concentrations of total petroleum hydrocarbons in the test mixtures changed from 145 to 48 ppm, while the lower concentrations changed from 2.7 to 1.7 ppm over the 24 h test period. Generally, the oil plus dispersant mixtures were more toxic than the oil alone especially for three particular dispersants at the higher concentrations. The effects of different temperatures (12°, 16°, 20° and 25° C) on the toxicity of one particular dispersant are reported, indicating that enhancement of the toxicity of crude oil by the dispersant is even more pronounced at the higher temperatures. The ecological significance of these results is discussed.

*
* *

Introduction

Evaluation of the toxicity of oil dispersants to marine life represents a basic component of licensing control tests carried out by national authorities (BLACKMAN et al., 1978). Ideally, such toxicity tests should be performed on organisms from the various trophic levels and the final evaluation should rest on all these data rather than on one set of data obtained by investigating one test species. In view of their importance as primary producers, marine algae are often chosen as one of such test species. Thus, the toxicity of dispersants to the freshwater green algae Chlamydomonas reinhardti, and the marine flagellate Dunaliella bioculata have been investigated (HELDAL et al., 1977; BRATBAK et al., 1982).

---

* This work has been carried out as part of the Joint FAO (GFCM)/UNEP Long Term Programme for Pollution Monitoring and Research in the Mediterranean Sea (Med POL Phase II).

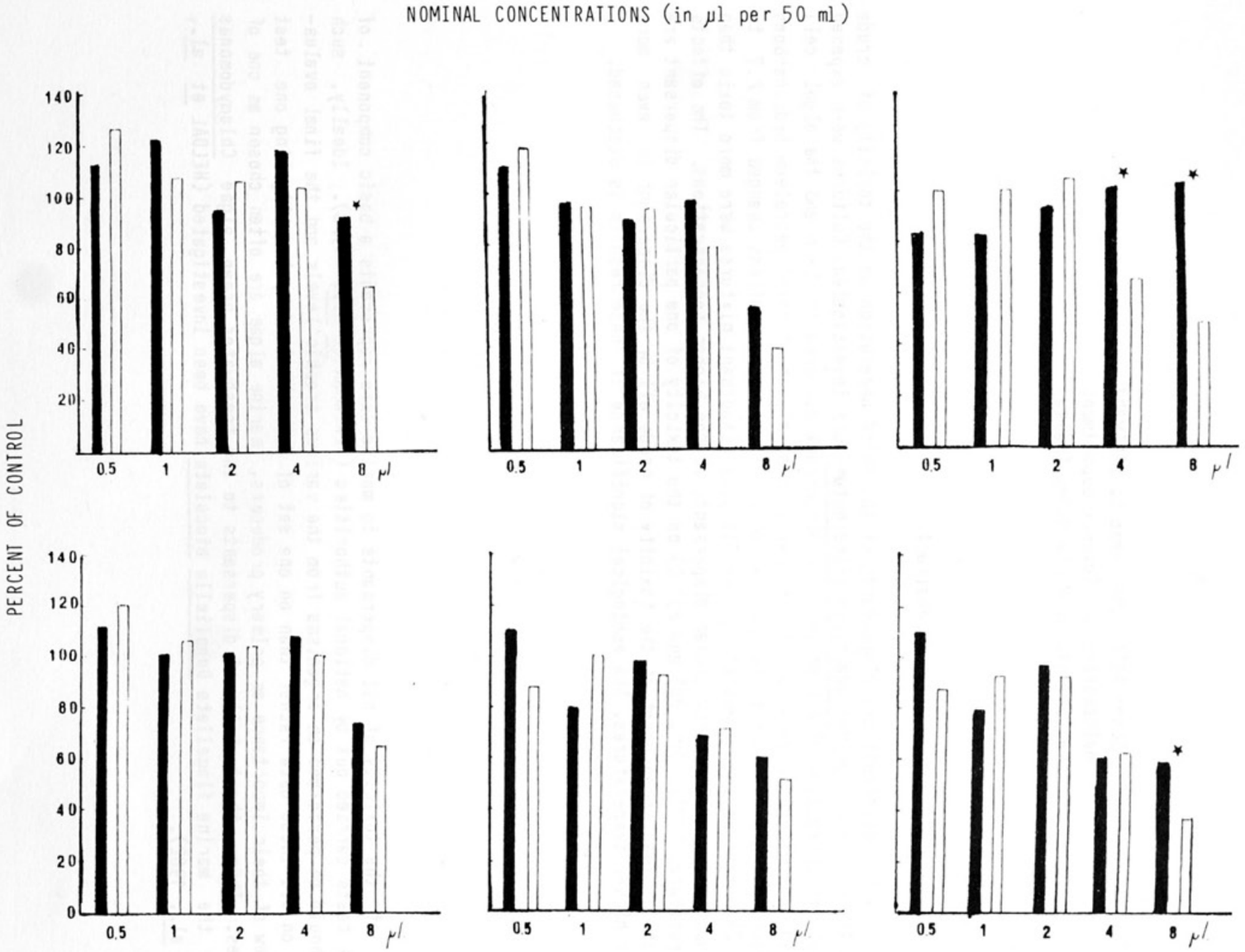


Figure 1. - Effects of different concentrations of oil (black bar) or oil plus dispersant mixtures (white bar) on the growth of Phaeodactylum tricornutum, expressed as percent of control cell counts after 24h exposure at 20° C to six different dispersants. * indicates significant difference (P = 0.05) between pair.

The aim of the present study was to investigate: the feasibility of utilizing marine diatoms for the toxicity assessment of oil dispersants: the relative toxicities of a range of oil dispersants which are at present in use in Mediterranean coastal waters: and the effect of temperature on such toxicities. As already indicated by several authors (BLACKMAN et al. 1977, 1978) the rationale of such toxicity tests should be based on the fact that marine organisms in the field are exposed to a mixture of oil and dispersants rather than to the dispersant alone. Therefore to evaluate the likely biological effect of the use of a dispersant, the toxicity of oil and dispersant mixtures should be compared to that of oil alone, under the same experimental conditions.

Due to the instability of oil or oil plus dispersant mixtures, a flow-through test should be preferred to a static toxicity test. But for obvious reasons, only static tests may be utilized for marine unicellular algae. The alternative is to expose the test organisms to the test mixtures for a relatively short period of time during which the characteristics of the mixtures may be maintained. Moreover, in the present study, preliminary microscopic investigations indicated that on exposure to the test mixtures a fraction of the diatoms were killed within the first 7 h, while the surviving cells grew and developed normally. The same observation was made by other authors (BRATBAK et al., 1982). Therefore, due to the unnaturally high nutrient levels present in the culture media, and the subsequent rapid growth of algae, any toxic effect on such growth would only be apparent during the first 24 h of exposure. Therefore a 24 h exposure period was chosen.

Materials and methods

Kuwait crude oil was used as the standard oil throughout the tests. Six oil dispersants which have been identified as being commonly in use in Mediterranean coastal areas were investigated and these were obtained from the manufacturers. All may be classified as third generation dispersants and three of them are concentrates. In this report they are not identified.

Phaeodactylum tricornutum (culture bath number 1052/1A obtained from the Culture Centre of Algae and Protozoa, Cambridge, UK) was cultured in marine algal maintenance medium (WARD and PARRISH, 1982) at 20° C with continuous 2,000 Lux illumination. Two weeks prior to use, the diatoms were transferred to a test medium (WARD and PARRISH, 1982) for acclimatization. This same test medium was used in all exposure experiments. All media and acid-washed glassware were sterilized by autoclaving prior to use. 1 ml of inoculum from actively growing stock cultures acclimatized to the test medium, were used for the experiments. Starting algal concentrations ranged from 10 to 15 x 10^4 cell ml^{-1}. Cultures were exposed to oil or oil plus dispersant mixtures for 24 h in 125 ml cotton-plugged Erlenmeyer flasks with a total volume of 50 ml test medium at 20° C, continuous 2,000 Lux illumination and continuous agitation maintained by placing flasks upright on a shaking platform with a circular motion of 100 revolutions per minute.

Immediately after inoculation, the experimental flasks were dosed with varying amounts (0.5, 1, 2, 4 and 8 µl) of oil or oil and dispersant (equal volumes) mixtures by means of a micropipette. The concentrate dispersants were diluted to 10 per cent by sea-water as recommended by their respective manufacturers. Three replicate flasks were used per treatment level as well as for the control. In all cases the experiment for each dispersant was repeated at least twice. After 24 h, cell concentrations were determined by a haemocytometer (Improved Neubauer Levy Ultra Plane). Quadruple counts were made for each flask.

The total petroleum hydrocarbon concentrations (PHC) at the start of the experiment and then after 24 h of exposure were determined by extracting 100 ml of test media (i.e. the contents of two experimental flasks with no diatoms) with two volumes of 10 ml dichloromethane and the extract was dried over anhydrous sodium sulphate. Fluorescence was measured on a Turner 430 spectrofluorimeter at 310 nm excitation and 360 nm emission wavelengths. The instrument was calibrated with Kuwait Crude oil standard concentrations.

To investigate the effect of temperature on the toxicity of oil plus dispersant mixtures, the above experiments were carried out at four different temperatures (12°, 16°, 20° and 25° C) for one particular dispersant. The exposure flasks were maintained at the required temperature by placing them in a circulating water bath on the shaking table.

Table 1. - Concentrations of petroleum hydrocarbons (aromatics) in mg/l in test mixtures at 0 and 24h.

Nominal concentrations in µl per 50ml of medium.			
Oil	Dispersant	0h	24h
1/2	0	2.72	1.7
1	0	3.77	2.04
2	0	22.53	3.66
4	0	29.05	14.95
8	0	37.85	29.05
1/2	1/2	3.17	1.87
1	1	14.90	2.57
2	2	26.40	7.03
4	4	60.75	21.12
8	8	144.40	47.56

Results

Results of analysis of PHC (aromatics) levels in the test mixtures at 20° C for one particular dispersant are given in table 1. These data indicate that the PHC concentrations were greater in the presence of the dispersant. As expected, there were significant losses in the test PHC concentrations over the 24 h exposure period, especially at the lower range of concentrations, mainly due to evaporation. Such losses were less pronounced in the presence of the dispersant.

The mean 24 h cell counts for each treatment level as expressed in mean percentage of control, are presented in figures 1 and 2. Any diffferences between means of pairs of oil and oil plus dispersant mixtures were statistically analysed by Duncan's multiple range test (ALDER and ROESSLER, 1977).

Generally a wide range of variability in response on exposure to both oil or oil plus dispersant mixtures was recorded. At the lower test concentrations, no consistent toxic effect on the growth rate could be detected for any of the dispersants investigated. Stimulation of algal growth in some cases was recorded. Similar algal growth stimulations by PHC were reported by other authors (KAUSS and HUTCHINSON, 1975). At the higher test concentrations, three of the dispersants investigated significantly increased the toxic effect of oil on algal growth (at $P = 0.05$).

Moreover, such increase in toxicity of oil by dispersant was rendered more significant at the higher experimental temperatures as seen from data presented in figure 2. At 12° and 16° C there was no significant difference between 24 h cell counts of algae exposed to oil as compared to those exposed to oil plus dispersant mixtures. At 20° C, there was such a difference at the higher concentrations tested. while at 25° C this difference in toxicity to algal growth was more pronounced even at lower concentrations.

Discussion and conclusions

The toxicity of PHC to marine microalgae has been studied by several authors (PULICH *et al.*, 1975, KAUSS and HUTCHINSON, 1975). More recently, the toxicity of oil plus dispersant mixtures on

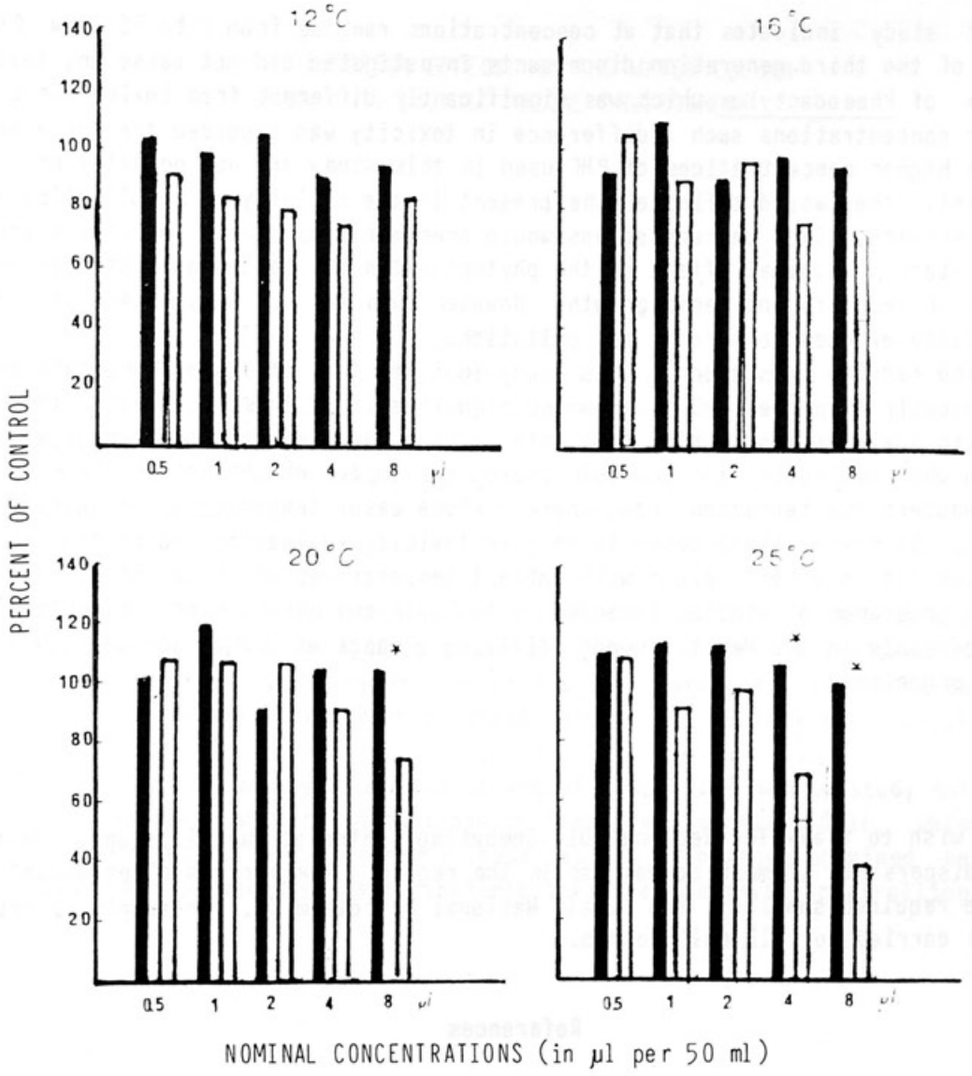


Figure 2. - Effects of different concentrations of oil (black bar) or oil + dispersant mixtures (white bar) on the growth of Phaeodactylum tricornutum, expressed as % of control cell counts after 24 h exposure at different temperatures. * indicates significant difference between pairs.

Chlamydomonas and Dunaliella have been investigated (HELDAL et al., 1977, BRATBAK et al., 1982). Experiments by PULICH et al. (1975) indicate that direct contact with 10 ml of Kuwait Crude oil per 25 ml of medium (more than twice the highest nominal concentrations used in the present study) inhibited growth of a green microalga while no such effect was apparent on exposure to water-accommodated fractions of the oil. The authors suggest that the toxic activity is mainly localized in medium and higher boiling fractions of the crude oil, i.e. oil components which remain in suspension and not in solution. The present study indicates that at lower nominal concentrations of the same test crude oil no significant toxic effects on growth of Phaeodactylum exist under the given experimental conditions, even at 25° C. However in the presence of three of the dispersants investigated, significant reductions in cell counts relative to controls were recorded. This increase in toxicity of chemically dispersed oil may probably be correlated with increased availability of the PHC to the test species in the presence of the dispersant. As indicated in table 1, the oil plus dispersant mixtures were more stable over the 24 h exposure period, than the oil mixture alone. BRATBAK et al. (1982) suggested that the toxicity of dispersant plus oil mixtures to marine algae may be correlated with the effectiveness of the dispersant so that with increasing effectiveness (i.e. greater availibility of PHC in the sea column) the toxic effects are more pronounced.

The present study indicates that at concentrations ranging from 2 to 30 ppm PHC chemically disperse by any of the third generation dispersants investigated did not cause any toxic effect on the growth rate of Phaeodactylum which was significantly different from toxicities caused by oil alone. At higher concentrations such a difference in toxicity was recorded for three of the dispersants. Though the higher concentrations of PHC used in this study are not normally encountered in the marine environment, they would definitely be present in the vicinity of an oil slick which has been treated with dispersants. Such concentrations would presumably be stable for only a brief period of time and the long-term biological effect on the phytoplankton may be insignificant ecologically, due to their powers of recovery and rapid growth. However such may not be the case in regions of poor nutrient availability exposed to chronic oil pollution.

Moreover, the results presented in this study indicate that at higher temperatures (25° C) the toxicity of chemically dispersed PHC is enhanced significantly. This illustrates the importance of designing specific toxicity tests which take into account the prevalent environmental stresses of a particular region when evaluating the possible biological impact of the use of dispersants. Thus, in the central and eastern Mediterranean areas where surface water temperatures in summer normally range from 25 to 27° C, it makes little sense to rely on toxicity evaluation and control criteria of oil dispersants carried out in other regions with ambient temperatures of 10 to 15° C. The present study forms part of a programme of studies intended to evaluate any possible environmental impact of the use of oil dispersants in the Mediterranean utilizing a range of lethal and sublethal tests on a number of marine organisms.

Acknowledgements

The authors wish to thank the Regional Oil Combating Centre at Manuel Island, Malta for help in identifying the dispersants in most common use in the region: the various dispersants' manufacturers for supplying the required samples: the Kuwait National Petroleum Co. for supply of crude oil and Mr Charles Galea who carried out all cell counts.

References

ALDER H.L. and E.B. ROESSLER (1977). - Introduction Probability and Statistics. W.H. Freeman, San Francisco 426 pp.

BLACKMAN R.A.A., F.L. FRANKLIN, M.G. NORTON and K.W. WILSON (1977). - New procedures for toxicity testing of oil slick dispersants. Fish Res. Tech. Rep., MAFF Direct. Fish. Res., Lowestoft, 39: 7 pp.

BLACKMAN R.A.A., F.L. FRANKLIN, M.G. NORTON and K.W. WILSON (1978). - New procedures for the testing of oil slick dispersants in the United Kingdom. Mar. Pollut. Bull., 9: 234-238.

BRATBAK G., M. HELDAL, G. KNUTSEN, T. LIEN and S. NORLAND (1982). - Correlation of dispersant effectiveness and toxicity of oil dispersants towards the alga Chlamydomonas reinhardti. Mar. Pollut. Bull. 13(10): 351-353.

HELDAL R., S. NORLAND, T. LIEN and G. KNUTSEN (1977). - Acute toxicity of several oil dispersants towards the green algae Chlamydomonas and Dunaliella. Chemosphere 6: 247-255.

KAUSS P.B. and T.C. HUTCHINSON (1975). - The effects of water soluble petroleum components on the growth of Chlorella vulgaris Beijernick. Env. Poll. 9: 157-173.

PULICH W.M.Jr., L. WINTERS and C. VAN BAALEN (1975). - The effects of a n° 2 fuel oil and two crude oils on the growth and photosynthesis of microalgae. Mar. Biol. 28(2): 87-94.

WARDS G.S. and P.R. PARRISH (1982). - Manual of methods in aquatic environmental research. Part 6, Toxicity Tests. FAO Fish. Tech. Pap. (185): 23 pp.

SEWAGE DIVERSION EFFECTS ON THE WATER COLUMN OF AN OLIGOTROPHIC MARINE ENVIRONMENT

by

N. FRILIGOS
Institute of Oceanographic and Fisheries Research
GR-166 04 Hellinikon (Greece)

Introduction

The Saronikos Gulf represents in many ways an excellent case for investigations of the effects of urban waste disposal into an oligotrophic marine environment. In the course of these investigations, measurements of nutrients, oxygen and hydrological parameters were carried out at various stations in the Inner Saronikos Gulf. Part of these results has already been published (FRILIGOS, 1976, 1981, 1982, 1984).

This work was carried out weekly from July to August 1982, in order to study nutrient levels and the hydrological parameters at certain stations in the Inner Saronikos Gulf, which are directly affected by the sewage outfall and to compare these results with those obtained between 1973 and 1976. These studies have provided valuable insights into the mechanisms controlling biomass in the bay.

Methods

Eight stations (Fig. 1) were chosen in the Inner Saronikos Gulf following the results and terminology used in the water masses report (COACHMAN and HOPKINS, 1975). The sampling was done at weekly intervals from July to August, 1982. Samples of water were collected from 1, 10, 20, 30, 40, 50, 60, 70 and 90 m using a Nansen reversing water sampler. Measurements of temperature, salinity, dissolved oxygen, chlorophyll-a and inorganic nutrients were made by methods described by FRILIGOS (1982 b).

Results and discussion

Hydrological parameters

COACHMAN and HOPKINS (1975) and FRILIGOS (1984) reported that the upper waters in the Inner Saronikos Gulf underwent a well-defined annual cycle of thermal stratification. From a minimum average temperature of about 14° C in February and March, temperature rose during the spring and summer to a maximum in July and August of about 25° C. Peak summer temperatures of the near surface waters of the Inner Saronikos Gulf recorded during the summer 1982 cruises again reached 26° C.

FRILIGOS (1984) reported that the salinity range was 38-39 ‰ in the Inner Saronikos Gulf and the density lay in the range 26-29 ot. The density increased with depth and the pycnocline was related to the thermocline. The situation was not the same during the 1982 summer cruises: the

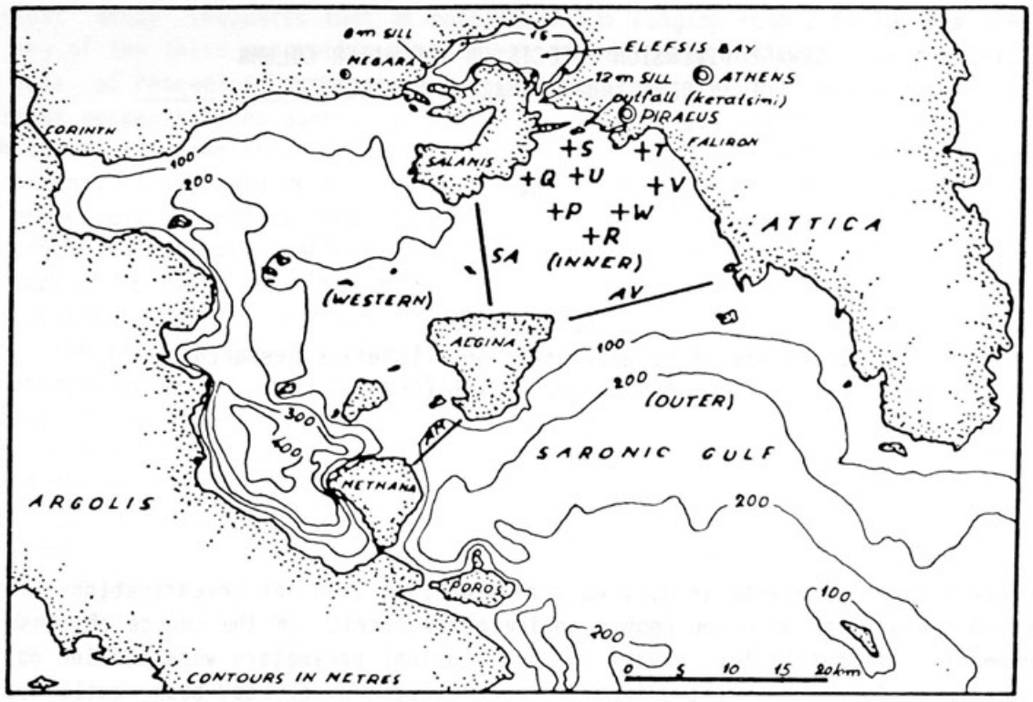


Fig. 1. - Location of stations, oceanographic subregions and bathymetry of the Saronikos Gulf. Main passages designated as follows : SA Salamis-Aegina; AV Aegina-Vouliagmeni; Am Aegina-Methana.

Ammonium	1580	$\mu g\text{-}at \cdot l^{-1}$
Nitrite	75	$\mu g\text{-}at \cdot l^{-1}$
Nitrate	136	$\mu g\text{-}at \cdot l^{-1}$
Phosphate	187	$\mu g\text{-}at \cdot l^{-1}$
Silicate	332	$\mu g\text{-}at \cdot l^{-1}$
Suspended matter	730	$mg.l^{-1}$
BOD_5	360	$mg.l^{-1}$
COD	820	mg.l-1

Table 1

Concentration of chemical parameters in a composite sewage sample. August 1982 (the composite sample was made up of 24 hourly samplings).

Depth interval (m)	V(x $10^6 m^3$)
0-37	15382
18.3-100	11995
100-104	45
Total	27422

Table 2

Surface area and volumes of Inner Gulf.

(A) Total surface area $468 \times 10^6 m^2$ (B) Volume

salinity range was 36.9-38.4 ‰ and low salinities were observed in the upper layers. These probably indicated changes of a regional nature in the Aegean source waters. Bottom water salinities were less variable and similar to those reported by COACHMAN and HOPKINS (1975) and FRILIGOS (1984). It should be noted that, with the decrease in salinity in the upper layers of the water column, the density difference increased (25-29 σt) between bottom and surface waters during the summer 1982 cruises. Measurements of dissolved oxygen taken during summer 1982 in the Inner Saronikos Gulf indicated a fairly constant level of about 5 ml.l-1 at all depths.

Nutrients and standing stock

Table 1 shows that the content of silicon, nitrogen and phosphorus of the inorganic nutrients in the Keratsini waste water is high, as expected. The inorganic nitrogen is almost all present as ammonia. The inorganic Ni: P ratio in Keratsini domestic sewage is typically low (Ni: P = 9.6).

The total nutrient of the Inner Saronikos Gulf was determined for nine cruises (July-September, 1982).

Current levels of circulatory nutrients were estimated by computing mean values for the depth intervals indicated in table 2, station by station S, T, Q, U, P, W, V, R and computing these to give mean values for a given cruise. The mean values were multiplied by the appropriate volumes to give quantitative estimates of total nutrients contained in the basin.

At the offshore stations of Saronikos Gulf, typical water values of phosphate, silicate, nitrite, nitrate and ammonia were 0.12, 1.22, 0.16, 0.42, 0.36 respectively (FRILIGOS, 1982 a). Mean nutrient concentrations for the Inner Saronikos Gulf, for all cruises, are shown in table 3. Mean values for various cruises as shown in table 3, were multiplied by the appropriate volumes given in

Date	PO_4^{3-}-P	SiO_4^{-4}-Si	NO_2^--N	NO_3^--N	NH_4^+-N
10-12 July	0.17	0.60	0.07	0.35	1.03
19-20 July	0.18	0.67	0.09	0.42	0.74
26-27 July	0.16	0.79	0.07	0.38	0.98
2-3 August	0.16	0.65	0.06	0.39	0.53
9-10 August	0.17	0.62	0.09	0.60	0.54
17-18 August	0.16	0.99	0.11	0.41	0.80
24-25 August	0.11	0.67	0.06	0.25	0.25
30-31 August	0.19	0.76	0.08	0.60	0.81
7-9 September	0.16	0.76	0.08	0.83	0.87

Depths > 40 m

Date	PO_4-P	SiO_4-Si	NO_2-N	NO_3-N	NH_4-N
10-12 July	0.20	1.79	0.10	0.98	0.82
19-20 July	0.17	2.18	0.10	1.01	0.47
26-27 July	0.18	2.52	0.10	1.43	1.25
2-3 August	0.13	2.33	0.07	1.07	0.36
9-10 August	0.16	2.55	0.11	1.60	0.35
17-18 August	0.15	2.18	0.09	1.04	0.44
24-25 August	0.11	2.39	0.11	1.29	0.25
30-31 August	0.12	1.23	0.10	0.98	0.51
7-9 September	0.12	1.53	0.09	0.96	0.43

Table 3
Mean nutrient concentration in the Inner Gulf during Summer 1982
water column averages in μg-at l^{-1}
Depths 0-40 m

table 2 to obtain an estimate of total nutrient held in dissolved form in the Inner Gulf. The results are presented in table 4 along with background values and the summer 1974-1976 values extract from FRILIGOS (1982 a). The phosphate content did not change significantly from year to year. This enabled FRILIGOS (1981 a) to use it as a conservative parameter and define through it as an index of marine pollution.

Date	PO_4^{-3}-P	SiO_4^{-4}-Si	NO_2^--N	NO_3^--N	NH_4^+-N	ΣN
10-12 July	5.02	30.78	2.28	17.18	25.75	45.17
19-20 July	4.82	36.56	2.58	18.62	17.04	38.24
26-27 July	4.63	42.49	2.28	23.07	20.12	45.47
2-3 August	4.03	38.05	1.76	18.88	12.48	33.12
9-10 August	4.54	40.24	2.70	28.49	12.52	43.71
17-18 August	4.27	41.48	2.77	18.83	17.61	39.21
24-25 August	3.01	39.09	2.24	19.38	6.86	28.48
30-31 August	4.36	26.50	2.43	21.03	18.60	42.06
7-9 September	3.90	30.11	2.31	24.33	18.56	45.20
Means	4.29	36.14	2.37	21.09	16.61	40.07
Means* (Summer 1974-1976)	5.25	40.88	4.69	44.25	23.12	72.06
Background*	3.28	33.45	4.39	11.52	9.87	25.78

* Values extracted from Friligos (1982a).

Table 4
Total nutrients by cruise in Inner Gulf in g-at X 10^6

The inorganic nitrogen falls by about half, owing to the diminution of nitrate and nitrite, since ammonia remains nearly constant. Also, silicate concentrations presented decreased values. The fact that the mean water column ammonia and phosphate concentrations were fairly constant between summers, and that nitrate plus nitrite was actually reduced, suggests that the phytoplankton stripped virtually all the added ammonia from the water, and then took up additional nitrite and nitrate, to compensate for the low Ni: P ratio in the sewage. Also, silicate concentrations were lower during the 1982 summer conditions, which is due to the fact that silicate is required to support the additional biomass produced from the N and P in the sewage. Eutrophication of sea-water in the vicinity of the sewage outfall was demonstrated by surface levels of chlrorophyll-a being forty to two hundred times above background. Mean values of the chlrorophyll-a for the outfall stations approached the highest values reached in the 1982 study, 48.0 mg.m-3 and 30.0 mg.m-3 at stations S and T respectively. Generally the distribution of nutrients and chlorophyll-a depends on the prevailing winds, i.e. under northerly or variable winds, the plume develops towards the south-west, and under southerly towards the south-east.

Considering the fact that inorganic nitrogen and silicate concentrations instead of rising decreased between the year 1974-1976 and 1982, it may be concluded that the sewage discharges did not adversely affect the water quality with respect to nutrient concentrations. This would seem to imply that the phytoplankton growth was sufficiently vigorous to deplete the waters of excess nutrients. The results indicated that the contribution of nutrients that might affect eutrophication, made by the Keratsini outfall in the area sampled, was minimal.

References

COACHMAN L.K. and T.S. HOPKINS (1975). - Description analysis and conclusions on water masses in the Saronikos Gulf. Interim Technical Report 2, Envir. Pollut. Cont. Proj., Athens, Greece.

FRILIGOS N. (1976). - Seasonal variation of nutrients around the sewage outfall in the Saronikos Gulf Thalassia. Yugosl. 12: 441-453.

FRILIGOS N. (1981 a). - An index of marine pollution in the Saronikos Gulf. Mar. Pollut. Bull. 12: 96-100.

FRILIGOS N. (1981 b). - Some chemical observations in the Saronikos Gulf, Greece Rev. Int. Oceanogr. Med. 63-64: 35-47.

FRILIGOS N. (1982 a) - Enrichment of inorganic nutrients in the Inner Saronikos Gulf Mar. Pollut. Bull., 13: 154-158.

FRILIGOS N. (1982 b). - Some consequences of the decomposition of organic matter in the Elefsis Bay, an Anoxic Basin Mar. Pollut. Bull., 13: 103-106.

FRILIGOS N. (1984). - Nutrients of Saronikos Gulf in relation to environmental characteristics. (1973-1976). Hydrobiologia, 112: 17-25.

EFFETS DE DETERGENTS SUR LE REGIME ALIMENTAIRE DE DEUX ORGANISMES APPARTENANT A UN RESEAU TROPHIQUE COURT

par

R.A. KAIM-MALKA et S.S.JACOB.
Station Marine d'Endoume - CNRS LA 41
rue Batterie des Lions
13007 Marseille (France)

Résumé

A chaque étape d'un réseau trophique court, constitué par l'Isopode Idotea balthica basteri et le poisson Scorpaenidae Scorpaena notata, sont étudiés les effets à long terme de détergents non ioniques.

Chez l'Idotée, les quantités de nourriture consommées sont très importantes, la consommation diminue en fonction de la durée d'exposition. Chez la Rascasse, on observe des variations de consommation dans trois directions différentes.

On peut envisager que des variations de métabolisme ou d'assimilation de la nourriture se produisent, ce qui peut avoir des conséquences sur l'équilibre de l'écosystème.

Abstract

The long term effects of non ionic detergents on the link of a short natural food chain, consisting of the Isopod Idotea balthica basteri and the Scorpaenid fish Scorpaena notata have been investigated.

In Idotea, the nourishment is very important, consumption decreases as a function of time. In the Scorpaenidae, results indicate that the consumption fluctuate in three different directions.

It could be envisaged that variations of metabolism or food assimilation is produced, and may have consequences on the equilibrium of ecosystem.

*
* *

Introduction

Au cours d'études antérieures, KAIM-MALKA (1980 a, 1981, 1983) a mis en évidence les conséquences de l'action à long terme des détergents sur diverses grandes fonctions de l'Idotée (Crustacés-Isopode) et l'influence sur la répartition de cette espèce dans le milieu naturel. Parmi les fonctions biologiques qui se trouvent affectées, la nutrition de cette espèce présente des modifications à la suite d'une telle exposition.

Le présent travail est une étude des effets à long terme de détergents non ioniques sur le régime alimentaire de deux organismes : l'Isopode Idotea balthica basteri et la Rascasse Scorpaena notata (poisson Scorpaenidae) qui constituent deux étapes d'un réseau trophique court existant dans le milieu naturel.

Protocole expérimental.

Choix des détergents et des concentrations.

Pour des raisons pratiques de marquage, deux détergents non ioniques proches ont été retenus :

- Pour l'Idotée : un acide oléique condensé sur 14 molécules d'oxyde d'éthylène aux concentrations de 5 et 30 mg/l.
- Pour la Rascasse : un monooleate sorbitol polyoxyéthylé aux concentrations de H 1 ; 5 ; 10 mg/l.

Les concentrations retenues permettent des survies assez longues, mais aussi de déterminer l'influence du facteur concentration.

Choix des animaux et conditions d'élevage.

Les deux espèces ont été élevées dans une salle dont la température est de 17° ± 0,5° à saturation d'humidité. Le rythme d'éclairage est calqué sur celui du milieu naturel.

- Pour les Idotées, le nombre d'individus est de 10 par sexe et par concentration. Les animaux sont maintenus séparément dans des bacs de 0,5 l contenant la solution renouvelée une fois par semaine.
- Pour la Rascasse, le nombre d'individus est de 21 par concentration. Les animaux sont maintenus par trois dans des bacs contenant 15 l de solution. Les bacs sont dans un circuit ouvert semi-continu dont le renouvellement complet se fait en 24 h.

Pour les deux espèces, les observations ont été faites sur une période maximale de trois mois.

Mesures de la nourriture consommée.

La nourriture des Idotées est constituée par l'algue Codium fragile dont elle se nourrit et qui représente un aliment de choix. Elle est fournie en faible quantité et seulement lorsque la ration précédente a été totalement ingérée. L'appréciation de la consommation de nourriture a été faite pour chaque intermue et sur une durée totale de trois intermues, ce qui correspond à trois mois environ pour les témoins. L'animal, quant à lui, est pesé 24 h après chaque mue.

Chez la Rascasse qui est prédateur d'Idotées, et constitue ainsi le second maillon de ce réseau trophique, la nourriture est fournie tous les trois jours. Elle est constituée par des Idotées mâle et femelle qui sont données jusqu'à satiété. L'ensemble des proies est pesé avant d'être donné aux Rascasses. Quand les animaux sont rassasiés, les proies restantes sont pesées et, par différence, on connaît la quantité de nourriture ingérée (en poids frais). Les Rascasses sont elles aussi pesées régulièrement.

Toutes les pesées ont été faites sur une balance de précision au 1/10 000 de gramme.

Il est à noter que chez l'Idotée, la gonade effectue un cycle complet d'activité dans le temps d'un intermue et les mesures de nutrition portent sur trois intermues. Les Rascasses quant à elles, se trouvent en période de repos sexuel. Par conséquent, dans les deux cas, les résultats obtenus ne peuvent être influencés par une variation du niveau d'activité de la gonade.

Résultats

Quantités de nourriture ingérée par l'Idotée.

Pour évaluer la consommation de nourriture chez l'Idotée, il est nécessaire de tenir compte du fait que l'animal grandit par mues successives et donc d'incorporer les exuvies au calcul de poids. Il est également nécessaire de faire un calcul de consommation à chaque mue, mais ausi un calcul cumulé. Ainsi, il est possible de voir l'effet du détergent à un instant donné, mais aussi de suivre l'évolution dans le temps pendant la durée de l'expérience.

Les principaux résultats figurent dans le tableau 1 : ils sont exprimés en mg de codium frais consommés par gramme d'animal et par jour (mg/g/j).

	1er intermue	2e intermue	3e intermue
Témoins	635,32	382,22	316,62
5 mg/l	830,77	384,59	328,86
30 mg/l	694,12	444,65	338,21
Témoins	939,33	684,67	427,77
5 mg/l	1575,79	903,78	665,30
30 mg/l	626,70	527,88	422,61

Tableau 1
Consommation de nourriture chez Idotea mâle et femelle soumises ou non au détergent

A partir du Tableau 1, en règle générale, on observe que :

- les femelles consomment plus de nourriture que les mâles. Chez les témoins, la consommation est supérieure d'environ un tiers.
- Chez tous les animaux (mâles et femelles), la consommation diminue avec le temps.
- Les quantités consommées sont très importantes, en particulier pour le premier intermue.

Chez les femelles, c'est à la concentration de 5 mg/l qu'elles consomment le plus quel que soit le stade d'intermue considéré. A 30 mg/l, la consommation est un peu inférieure à celle des témoins. Il est possible que pour chacune des concentrations considérées, les réactions de l'animal vis-à-vis du détergent différent. On peut envisager qu'à 5 mg/l, il y ait :

- un métabolisme plus élevé que chez les témoins, s'accompagnant d'une stimulation de la consommation,
- ou une consommation accrue du fait d'une assimilation moins bonne.

Par contre, à 30 mg/l, il est possible que ce soit le phénomène inverse qui se produise :

- soit un métabolisme plus bas que celui des témoins, avec une consommation moindre,
- soit une consommation moindre due à une meilleure assimilation.

Les mâles soumis à l'influence du détergent consomment plus que les témoins pendant le premier intermue. Pour les deux autres intermues, les quantités consommées sont légèrement supérieures à celles des témoins. Dans ce cas, on peut envisager soit un métabolisme plus élevé, soit une assimilation moins bonne que chez les témoins.

L'étude détaillée des résultats montre que, à chaque concentration, chez les mâles comme chez les femelles, il existe un faible lot d'individus qui ont une consommation extrêmement importante.

Consommation de nourriture chez la Rascasse

Pour l'ensemble des individus témoins nourris avec le régime et dans les conditions expérimentales décrits plus haut, il apparaît que la ration journalière pour couvrir l'ensemble des besoins de l'animal est de 4,68 mg de proie/g poisson/jour. Chez les animaux soumis au détergent, les résultats diffèrent en fonction de la concentration. L'ensemble des résultats sont résumés dans le tableau 2 :

Conc. détergent	Témoin	1 mg/l	5 mg/l	10 mg/l
Q nourr. mg/g/j	4,68	6,31	5,1	2,7

Tableau 2. - Consommation de nourriture chez la Rascasse soumise au détergent

Chez les animaux soumis au détergent, quand la concentration du milieu augmente entre 1-5 mg/l, la consommation de nourriture est plus élevée que chez les témoins. Entre 5-10 mg/l, elle est inférieure à celle des témoins.

Si on considère uniquement la série soumise au détergent, on observe que, lorsque la concentration augmente, la consommation moyenne diminue.

A la concentration de 1 mg/l, la consommation de nourriture est légèrement plus élevée que chez les témoins. Cependant, une observation plus détaillée permet de mettre en évidence la présence de trois lots :

- un lot dont la consommation est nettement supérieure à celle des témoins : 12,9 mg/g/j et qui représente 16,7 % des individus,
- un lot dont la consommation est un peu supérieure à celle des témoins : 5,3-6,8 mg/g/j ; il représente 50 % des individus,
- un lot enfin dont la consommation est inférieure à celle des témoins : 2-3,5 mg/g/j : il représente 33,3 % des individus.

Quand la concentration du milieu expérimental est portée à 5 mg/l, on observe que la consommation est inférieure à celle de la concentration précédente (1 mg/l) et proche de celle des témoins. Le faible nombre d'individus sur lesquels les observations ont pu être faites ne permet pas de mettre en évidence l'existence de plusieurs lots.

A la concentration de 10 mg/l, on observe que la consommation globale est nettement diminuée car elle est inférieure de moitié à celle obtenue à 5 mg/l. Cependant, comme pour la concentration de 1 mg/l, il est possible de mettre en évidence la présence de trois lots :

- un lot qui consomme nettement plus que les témoins et que la moyenne à 10 mg/l : trois fois plus avec 8,7 mg/g/j : il représente 5,56 % des individus,
- un lot qui a une consommation proche de celle des témoins, mais supérieure à la moyenne du lot. Il représente 27,78 % des individus, avec une consommation de 3,8-4,8 mg/g/j,
- un lot qui représente 66,67 % des individus et qui a une consommation nettement inférieure à celle des témoins. Cependant, il est possible d'y distinguer deux groupes dont la consommation est différente : 2,6-3,1 mg/g/j qui représente 22,22 % du total et 0-1,8 mg/g/j qui représente 44,44 % du total.

Il apparaît donc que, chez la Rascasse, le comportement nutritif est perturbé dans trois directions différentes simultanément. On peut émettre les mêmes hypothèses que pour l'Idotée, à savoir :

- un métabolisme plus ou moins élevé s'accompagnant d'une plus ou moins grande stimulation de la consommation,
- ou une consommation plus ou moins importante en fonction du degré d'assimiliation de la nourriture.

Discussion

Il existe peu de données sur le régime alimentaire d'organismes soumis à l'action de détergents. FORET-MONTARDO (1970) indique que des observations pharmacologiques sur des espèces non aquatiques montrent que les détergents agiraient en augmentant l'absorption intestinale. La nourriture serait

donc mieux utilisée. LACAZE (1972) a pu, quant à lui, observer des phénomènes de stimulation de photosynthèse sur une microcommunauté de diatomées du périphyton soumises pendant de brèves périodes à l'action du Gamosol. Par la suite, il observe une modification de la communauté qui se réduit à quelques espèces bien représentées et ayant une activité photosynthétique en augmentation. Ces observations peuvent être rapprochées de celles de AUCLAIR (1973) qui, dans le cas du grand émissaire de Marseille, précise : "L'abondance maximale de l'ensemble des individus ...correspond à une zone intermédiaire ...lieu d'accumulation d'un sédiment très fin, vaseux, riche en matières organiques, mais aussi en hydrocarbures et détergents". On peut donc penser dans ce cas que, si le phénomène de pollution et la présence de matières organiques jouent un rôle sélectif dans la localisation des espèces, il est possible qu'un facteur de stimulation des phénomènes d'assimilation intervienne dans le milieu naturel. LECLERC (1971) indique que les détergents non ioniques provoquent une prolifération très abondante des bactéries protéolytiques aérobies. On peut donc envisager que le détergent favorise un développement bactérien important de la flore intestinale des organismes permettant ainsi une meilleure assimilation de la nourriture.

A côté de ces effets favorables sur l'alimentation, d'autres observations faites sur le poisson par BARDACH et coll. (1965) et FOSTER et coll. (1966) ont montré que les détergents agissent à faible dose sur l'épithélium olfactif des capsules nasales. Les altérations provoquent des conséquences néfastes sur le comportement alimentaire des poissons. En fait, la Rascasse est un poisson qui chasse à vue et d'autres phénomènes doivent intervenir. Bien que les espèces et les fonctions en cause soient différentes, la perturbation du comportement nutritif dans trois directions différentes est à rapprocher de ce qui a été observé par KAIM-MALKA (1980a, b) à propos de la mue chez l'Idotée où, sous l'effet du détergent à la concentration de 5 mg/l, les durées d'intermue sont perturbées dans trois directions différentes. On retrouverait dans le cas présent pour le même type d'altéragène (les détergents), le même type de perturbation pour des fonctions différentes chez des espèces différentes. SWEDMARK et coll. (1971) a également observé des modifications de la réponse à la nourriture chez Leander.

Conclusion

Sur un réseau trophique court, il est possible d'étudier les effets des détergents sur le régime alimentaire des organismes qui le composent. Chez les animaux, les quantités moyennes consommées diminuent quand la concentration augmente. Ces valeurs peuvent être supérieures à celles des témoins aux faibles concentrations. Les poissons présentent des variations de consommation dans trois directions différentes. On peut envisager des variations de métabolisme ou d'assimilation de la nourriture pour tenter d'expliquer ces changements. Il est possible que de tels phénomènes existent dans le milieu naturel et influencent la répartition des espèces. D'autres études que nous avons menées avec le même type de détergent montrent que l'altération ne se limite pas à une fonction, mais affecte l'ensemble des organes et des fonctions de l'individu et provoquent de très nombreuses altérations tout au long du cycle vital. Si on met en relation les modifications du régime alimentaire avec d'autres altérations majeures observées, telle une baisse importante de fécondité (allant jusqu'au blocage de la reproduction), on peut penser que des conséquences importantes pourront se produire sur l'écosystème en provoquant des ruptures d'équilibre.

Références

D. AUCLAIR-DESSEMON, (1973).- Etude de la pollution des fonds marins dans le secteur de Cortiou (Marseille). Thèse Faculté Pharmacie Marseille, 90 P.

J.E. BARDACH, M. FUHIYA, A. HOLL, (1965). - Détergents : effects on the chemical senses of the fish Ictalures natalis (Le Sueur). Sciences, 148 : 1605-1609.

P. FORET-MONTARDO, 1970 (1971). - Etude de l'action des produits de base entrant dans la composition des détergents issus de la pétroléochimie vis-à-vis de quelques invertébrés benthiques marins : Téthys,2 (3) : 567-614. Thèse Spécialité. Université Aix-Marseille II.

N.R. FOSTER, A. SCHEIER, J.Jr. CAIRNS, (1966). -Effects of ABS on feeding behavior of flagfish, Jordanella floridae. Trans. Am. Fish. Soc. 95 (1) : 109-110.

R.A. KAIM-MALKA, (1980a). - Action d'altéragènes sur des fonctions biologiques et écologiques d'un maillon d'écosystème. (Action de détergents sur Idotea balthica basteri). Thèse Doctorat d'Etat. Université Aix-Marseille II. 165 p. + annexes

R.A. KAIM-MALKA, (1980b). - Altération du rythme de mue chez l'Isopode valvifère Idotea balthica basteri soumis en permanence à un détergent non ionique. Journal français d'Hydrologie. 11, fasc. 3 n° 33 : 189-202.

R.A. KAIM-MALKA, (1981). - Altérations biologiques chez Idotea balthica basteri (Isopoda) Rapport Comm. int. Mer Médit. 27 (2) : 209-210.

R.A. KAIM-MALKA, (1983). - Altération de la fonction de reproduction chez Idote balthica basteri (Crustacea, Isopoda) soumis à l'effet chronique d'un détergent non ionique. Marine Environmental Researh, 8 (1) : 41-62.

J.C. LACAZE, 1972 (1973). - Effets de trois agents émulsionnants antipétrole sur la productivité primaire d'une communauté expérimentale de diatomées benthiques. Vie et Milieu, 23 (1-B) : 51-67.

E. LECLERC, (1971). - Les détergents et la pollution des eaux. Méthodes d'analyse. Ed. CEBEDOC, Liège : 167 p.

M. SWEDMARK, B. BRAATEN, E. EMANUELSSON, A. GRANMO, (1971). - Biological effects of surface active agents on marine animals. Marine Biology, 9 (3) : 183-201.

ACTION D'UN DISPERSANT DU PETROLE SUR L'EPITHELIUM INTESTINAL DE DEUX SOUCHES D'ARTEMIA

par

J. CASTRITSI-CATHARIOS, M. MORAITI-IOANNIDOU,
TH. KATSORCHIS et V. KIORTSIS
Université d'Athènes
Laboratoire de Zoologie-Panepistimioupolis-157 71 Athènes (Grèce)

Résumé

Etude toxicologique des effets sublétaux d'un dispersant, fréquemment utilisé pour combattre la pollution marine par les hydrocarbures. Le dispersant, (Finasol OSR5), a été appliqué sur deux souches d'Artemia, l'une parthénogénétique (Grèce), l'autre amphigonique (U.S.A.), aux stades nauplien II-III. La durée des expériences était de 24 heures, à la température constante de 25°±1° C. Nous avons préalablement déterminé la dose létale (LD50), qui est cinq fois plus élevée pour la souche parthénogénétique que pour l'amphigonique.

L'action nocive du polluant a été observée, à l'aide du microscope électronique, au niveau de l'épithélium unistratifié de l'intestin moyen, avec notamment une détérioration des microvilli. Cet effet devient apparent avec une dose de 270 ppm (souche parthénogénétique) et 50 ppm (souche amphigonique).

*
* *

Introduction

L'étude expérimentale de la toxicité aiguë, provoquée par les hydrocarbures ou leurs dispersants, nous a permis de préciser leurs concentrations létales, qui occasionnent la mort de 50 % des individus testés.

Toutefois, après un accident en mer causé par du pétrole, surtout s'il a lieu près d'une zone littorale, très riche du point de vue écologique et par conséquent très sensible à la pollution, se pose le problème des doses sublétales et des dégâts dont souffriraient ces organismes, en particulier leurs systèmes digestif et reproducteur. Etant donné la multitude et la complexité des paramètres environnants sur le terrain, il nous a semblé indispensable d'étudier, d'abord au laboratoire, à des conditions standard, les effets toxiques sublétaux, avant de rechercher ces mêmes effets nocifs parmi les organismes du milieu.

Matériel et méthode

Pour nos expériences, nous avons utilisé comme organisme-test, des nauplii au stade II-III, obtenus par éclosion des kystes d'Artemia francescana (U.S.A.) et d'Artemia salina (Missolonghi, Grèce). Le choix a porté sur ces deux espèces pour les raisons suivantes : par des expériences préalables de toxicité, on a constaté une grande différence de sensibilité entre ces deux souches, (CASTRITSI-CATHARIOS et al. 1984).

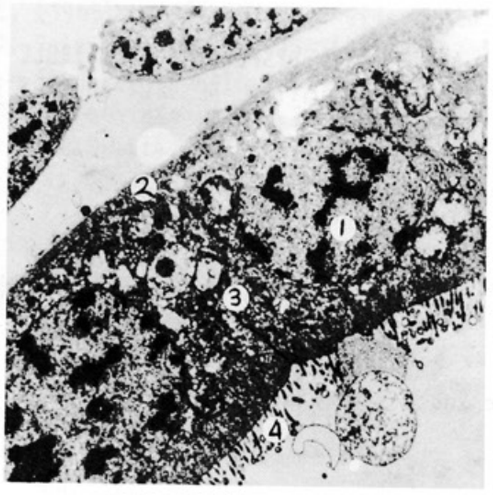


Figure 1.

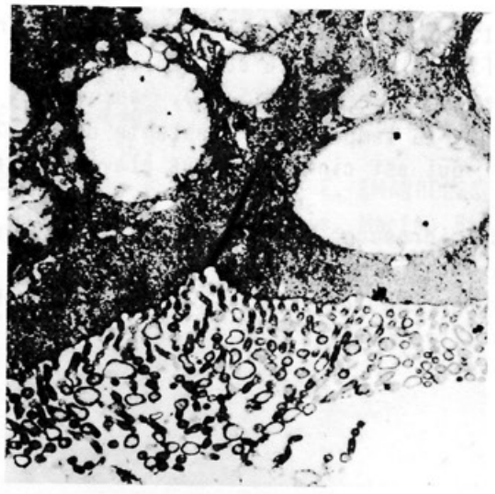


Figure 2a.



Figure 2b.

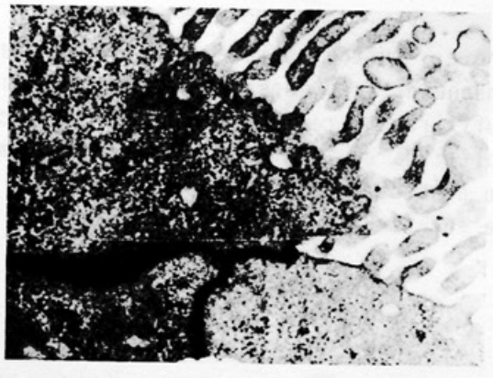


Figure 3a



Figure 3b

Un grand nombre de salines en Grèce se trouve au contact immédiat de la mer. Artemia, responsable de l'équilibre de l'écosystème des salines, présente un intérêt commercial important (MACDONALD, 1980), mais fait également partie de la faune littorale.

La durée des tests était de 24 heures, à une température constante de 25°±0,5° C. La substance toxique étudiée (Finasol OSR5) est un dispersant d'hydrocarbures composé d'un mélange d'esters du sorbitol (40 %), d'agents mouillants, "Sorbitan (20 %) et de solvants synthétiques (20 %). Le milieu est de l'eau de mer naturelle, filtrée deux fois, d'un pH égal à 8,1-8,2 et d'une salinité d'environ 3,2 %.

Les nauplii éclos, dès qu'ils atteignent le stade I, sont transférés dans des cristallisoirs, où ils séjournent pendant 72 heures, sans nourriture. Ensuite, à l'aide d'un microscope binoculaire, nous vérifions leur passage au stade II-III, la différence de sensibilité entre les stades I et II-III étant considérable, (VANHAECKE et al., 1980). On place alors les nauplii, par groupe de 10, dans des récipients contenant 50 ml de solution, avec ou sans dispersant, pendant 24 heures. Les nauplii sont ensuite fixés au glutaraldéhyde à 3 %, tamponné au cacodylate de sodium. La post-fixation s'effectue au tetroxyde d'osmium et les coupes, de 50-70 µm, sont contrastées à l'acétate d'uranyle et au citrate de plomb. L'étude ultrastructurale est réalisée au miscroscope électronique de transmission (Phillips E.M. 200).

Résultats et discussion

L'étude des stades de développement d'Artemia (WEISZ et al., 1947, ANDRESON, 1967, BENESCH, 1969), a montré que la perméabilité du tube digestif n'est pas complète avant la première mue larvaire. Etant donné que le but de ce travail était une investigation préalable des altérations probables du système digestif, dans des conditions défavorables du milieu, nous avons déterminé le stade II-III comme le plus approprié. En effet, à cet âge, il se forme un passage entre l'intestin antérieur et postérieur : le tube digestif devient perméable sur toute sa longueur et subit un flux continu du milieu extérieur.

Les trois séries expérimentales comprennent (a) des animaux sans dipersant qui servaient comme témoins ; (b) les survivants de la dose létale (LD 24/50 = 50 ppm) de la souche anphigonique : (c) ceux de la dose létale (LD 25/50 = 270 ppm) de la souche parthénogénétique. Nous avons choisi ces concentrations pour rechercher l'effet nocif éventuel le plus marqué.

L'épithélium de l'intestin moyen joue un rôle absorbant dans la physiologie du transport ionique et acqueux d'Artemia. Son ultrastructure a été étudiée en détail chez les nauplii par HOOTMAN, HARRIS et CONTE (1974). Ayant pu confirmer, nous-mêmes, la plupart de leurs observations, nous ne pensons pas qu'il soit nécessaire de nous y étendre. Tout au plus, devons-nous rappeler qu'entre les microvillosités, sans feutrage glucolique, qui caractérisent la surface apicale (libre) de cet épithélium cubo-cylindrique unistratifié, se trouve un grand nombre de vésicules, d'origine incertaine et de fonction inconnue.

On peut donc supposer que ces vésicules extra-cellulaires dérivent par exocytose des vésicules similaires et des corps multivésiculaires intracellulaires, qui se trouvent dans la zone corticale des cellules épithéliales.

Le but de nos recherches à l'aide du microscope électronique était de vérifier, sur ce système cellulaire sensible, les effets nocifs des doses létales du dispersant. Des expériences en cours permettront de préciser les effets nocifs du mélange dispersant - gas-oil dans des proportions 1:10.

Une série d'observations préalables nous a permis de constater qu'il n'y a pas de différences ultrastructurales notables entre la souche parthénogénétique (fig. 1, 2a) et amphigonique (fig. 3a). En revanche, les survivants des LD 24/50 montrent quelques altérations, aussi bien chez les nauplii amphigoniques que parthénogénétiques. Ainsi, les vésicules extra-cellulaires disparaissent, ou leur nombre y est très fortement réduit (fig. 2a-2b ; fig. 3a-3b). Une autre modification, moins évidente, concerne les microvillosités elles-mêmes. Leur densité et leur nombre semblent moindres chez les survivants des doses létales, par rapport aux témoins correspondants (fig. 2a, b, 3a, b).

Cette analyse montre que certaines des structures, directement liées au transport de l'eau et

des ions, sont affectées par le dispersant ; en revanche, le reste de la morphologie cellulaire de l'épithélium intestinal ne semble pas touché par l'action du dispersant.

Références

D.T. ANDERSON, (1967). - Larval development and segment formation in the branchiopod crustaceans Limnadia stanleyana King (Conchostraca) and Artemia salina (L.) (Anostraca) Aust. J. Zool. 15, p. 47-98

V.R. BENESCH, (1969). - Zur Ontogenie und Morphologie von Artemia salina (L.) Zool. Jb. Abt. Anat. u. Ontog. 86, 307-458.

J. CASTRITSI-CATHARIOS, M. MORAITI-IOANNIDOU, K. MAVRIKAKIS (1984). - Etude de la sensibilité de deux peuplements d'Artemia par rapport à un dispersant et son mélange avec du gas-oil. 30th International Congress on the Zoogeographie and Ecology. Greece, Patras.

S.R. HOOTMAN, P.J. HARRIS, F.P. CONTE, (1972). - Surface specialization of the larval salt gland in Artemia salina nauplii. J. Comp. Physiol., 79 p. 97-104.

P. VANHAECKE, G. PERSOONE, C. CLAUS and P. SORGELOOS, (1980). - Research on the development of a short term standard toxicity test with Artemia nauplii. The brine shrimp Artemia. Vol. I, p. 263-282. (Ed.) Universa Press.

P.B. WEISZ, (1947). - The histological pattern of metameric development in Artemia salina. J. Morph., 81, p. 45-95.

G.H. MACDONALD, (1980). - The use of Artemia cysts as food by flamingo (Phoenicopterus ruber roseus) and the shelduck (Tadorna tadorna). The brine shrimp Artemia. Vol: 3, Ecology (Ed.) Universa Press.

* Travail effectué avec l'aide financière de la F.A.O.

LABORATORY EXPERIMENTS ON THE EFFECT OF PULP MILL WASTES ON ALGAL GROWTH

by

Ramses REYAD ABDALLA
Institute of Oceanography and Fisheries
Alexandria (Egypt)

Abstract

Algal bioassay methods were used to evaluate the effect of the Rakta pulp mill wastes on the growth of the algae Selenastrum gracile, Ankistrodesmus falcatus, Dunaliella tertiolecta and Cheatoceros sp. Results indicate that Rakta wastes discharging in Abu-Kir Bay have both stimulative and inhibiting effects on the growth of freshwater algae. In the case of marine algae only the inhibition effect can be noticed. The effective concentration that would inhibit algal growth by 50 per cent "EC50" was 48 per cent for both freshwater algae and 22 per cent, 10 per cent for the marine species respectively.

*
* *

Introduction

Abu-Kir Bay is a semienclosed basin and recieves polluted land drainage coming through El-Tabia pumping station. This waste water comprises irrigation drainage from the neighbouring cultivated area in addition to the wastes from several major industrial plants. The pulp mill industry discharges about 80,000 m3/day of waste water into the Bay. The Rakta paper company, which discharges 50,000 m3/day of heavily polluted wastes, is also considered to be an important source of pollution in Abu-Kir Bay (SALEH et al., 1978). The purpose of this work is to evaluate the potential hazard of liquid wastes discharged by the Rakta paper factory to fresh water and marine algae.

Material and methods

The algae Selenastrum gracile, and Ankistrodesmus falcatus were used as test algae for the freshwater bioassay. For the marine water bioassay the algae Dunaliella tertiolecta and Cheatoceros sp. were chosen. Stocks of freshwater algae were maintained in a modified Chu n° 10 solution (CHU, 1942). Stocks of marine algae were maintained in natural sea-water of salinity about 36 ‰ after filtration and autoclaving. Erlenmayer flasks of 100 ml capacity were used as culture vessels. All tests were performed in triplicate. Twenty-five ml of waste dilution were transferred to each flask. The control flask in the case of freshwater algae contained Chu n° 10 solution, while for marine

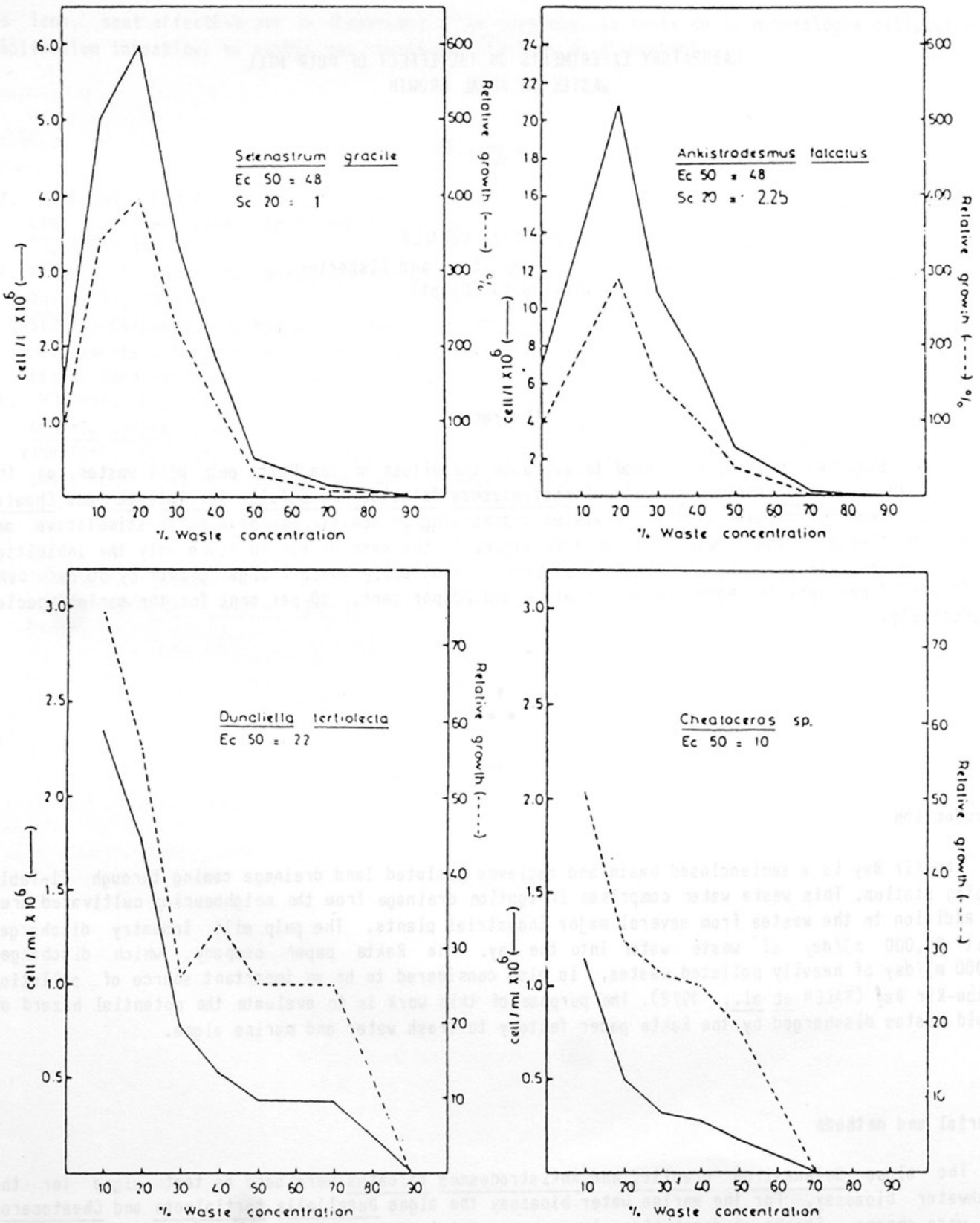


Figure 1. - The effect of Rakta paper factory waste concentration on the growth of the algae : Selenastrum gracile, Ankistrodesmus falcatus, Dunaliella tertiolecta and Cheatoceros sp.

species it contained sea-water diluted with distilled water equivalent to the amount of waste water added in every test. The pH was adjusted before adding the wastes to 7.3 for fresh water algae and 8.1 for marine algae using a pocket pH meter. Cultures were inoculated with actively growing algal cells to yield 10 x 103 cell/ml as the initial inoculum in all tests. Cultures were exposed to 5 K Lux day-light fluorescent lamps in an incubator at a temperature of 25 ± 1° C. The duration of the test was 96 hours. Cultures were agitated daily during the exposure period. About five litres of waste water were sampled, decanted and filtered through fine filter-paper to get rid of any suspended matter and then used in bioassay experiments.

% of waste conc.	Selenastrum gracile		Ankistrodesmus falcatus	
	algal growth cell/ml. 10^3	relative growth to Chu culture %	algal growth cell/ml. 10^3	relative growth to Chu culture %
00	1510 ± 157	100 %	7110 ± 900	100 %
10	5090 ± 330	337	13570 ± 1090	190
20	5960 ± 520	395	20670 ± 1260	291
30	3360 ± 320	223	10820 ± 870	152
40	1810 ± 130	120	7290 ± 930	103
50	480 ± 60	32	2570 ± 200	36
70	120 ± 30	8	185 ± 50	3
90	00	0	00	0

Table 1. - Effect of different waste concentration of Rakta paper factory on the growth of the fresh water algae : Selenastrum gracile and Ankistrodesmus falcatus.

Results

Fresh water bioassay

The waste water of Rakta paper factory was diluted with Chu n° 10 solution to obtain a series of dilutions from 10 to 90 per cent. The effect of these dilutions on the growth of S. gracile and A. falcacus is given in table 1 and figure 1. From these data, it appears that waste water has both inhibitory and stimulative effects on algal growth. The relative growth value attained 395 per cent and 291 per cent at 20 per cent waste dilution for S. gracile and A. falcatus respectively. The waste concentration that would stimulate growth of S. gracile by 20 per cent "SC 20" is 1 per cent while for A. falcatus 2.25 per cent. The effective concentration that would inhibit growth by 50 per cent "EC 50" is 48 per cent for the two algae. The "toxicity emission rate" (TER) for Rakta waste water calculated from the EC 50 for the freshwater species tested equals 72.34 tu x m3/min.

Marine water bioassay

The marine algae in the experiments are affected by salinity decrease and the toxic effect of the wastes. The effect of decreasing salinity as well as the effect of decreasing salinity combined with the toxicity resulting from the waste water are given in table 2 and shown in figure 1. The data

Table 2. - Effect of different waste concentrations of Rakta paper factory on the growth of the marine algae Dunaliella tertiolecta and Cheatoceros sp.

% of waste or water conc.	Dunaliella tertiolecta			Cheatoceros sp.		
	algal growth with different waste conc. cell/ml.10^3	algal growth with different water conc. cell/ml.10^3	relative growth %	algal growth with different waste conc. cell/ml.10^3	algal growth with different water conc. cell/ml.10^3	relative growth %
10	2345 ± 102	3190 ± 235	74 %	1152 ± 279	2246 ± 91	51 %
20	1760 ± 55	3152 ± 277	56	488 ± 69	1631 ± 88	30
30	782 ± 36	3030 ± 175	26	313 ± 63	1161 ± 78	27
40	520 ± 41	1570 ± 118	33	285 ± 52	1113 ±100	26
50	390 ± 35	1560 ± 118	25	175 ± 55	898 ± 72	19
70	390 ± 40	1560 ± 120	25	00	840 ±149	00
90	00	690 ± 100	00	00	820 ±150	00

N.B. The differences in cell counts of marine algae exposed to osmotic and osmotic plus pollutant stress are statistically significant at 5% level.

show that Rakta waste water has a strongly inhibitory effect on the marine algal species tested. The EC 50 for D. tertiolecta is 22 % while for Cheatoceros sp. it is 10 %. The "Toxicity emission rate" (TER) for Rakta waste water calculated from the EC 50 for the marine species tested equals 157.8 and 347.2 tu x m3/min respectively.

Parameter		Value	Parameter		Value
Temperature	°C	28.5	T.R. 105°C	mg/l	3674.00
pH		9.25	T.R. 600°C	"	1342.00
Alkalinity	mg/l	450.00	Organic volatile	"	2332.00
Total hardness	"	300.00	Dissolved solids	"	1828.00
Chloride	"	250.00	Suspended solids	"	1846.00
D.O.	"	0.60	Settleable solids	ml/l	50.00
B.O.D.	"	1602.50	Oil and grease	mg/l	195.00
C.O.D.	"	2428.80	Colour (W.L. 425)	unit	3000.00
T.O.C.	"	508.90			

Table 3. - The physico-chemical characteristics of the final waste water of Rakta paper factory (based on the information from the High Institute of Public Health, Alexandria, Egypt).

Discussion

The chemical analysis of the waste water discharged from the Rakta paper factory which is given in table 3 shows that it is heavily polluted water. It is mainly characterized by its high pH, alkalinity, BOD, COD values and high colour intensity, in addition to its high content of oil and grease. Accordingly its inhibitory effect on algal growth is a combined effect of several inhibitors. According to VON UNRUH 1980, the most dangerous pollutant in the pulp mill wastes is the black liquor which is distinguished by its high alkali concentration and its lignin content which is not biodegradaded. Both of them are extremely toxic to marine life. The toxic action on the marine ecosystem can be brought about either through their direct effect on fishes or through their effect on phytoplankton, zooplankton and benthos which constitute the main food of fishes (ZAVODNIK and ZAVODNIK 1978; OBIOLS et al:, 1980).

In view of the fact that the waste water sample was filtered before use in tests, the effect of both suspended matter and the toxicants absorbed on its surfaces were eliminated.

Practically all Rakta wastes are diluted with the drainage water of Abu-Kir drain before they reach Abu-Kir Bay, so their toxic effect on phytoplankton community has until now been restricted to a small area in the Bay.

Referring to the results of bioassay experiments and the phytoplankton survay in Abu-Kir Bay (SAMAAN, unpublished data), it is suggested that at least a preliminary treatment of pulp mill wastes must be carried out to reduce their pH,BOD, COD, alkalinity values and content of oil, grease and suspended matter.

References

CHU S.P. (1942). - The influence of mineral composition of the medium on the growth of phytoplanktonic algae. J. Ecol. 30, 284-325.

OBIOLS J., X. THOMAS and A. SAN MIGUEL (1980). Study of some pollutants on Mytilus species and Mugil sp. in the Barcelona coast area. Ves Journées Etud. pollution. Cagliari C.I.E.S.M. 395-398.

SALEH M.S.E., M.A. EL TRABOULIS, M.S. MAKKAR, M.S. GEMAI and H.A. FARAG (1978). - Pollution of the environment by wastes of pulp and paper industry and treatment for recovery of valuable chemicals. IVes Journées Etud. pollutions. Anatalya C.I.E.S.M. 267-272.

VON UNRUH P.B., B. BOURGAD-LE et R. KANTIN (1980). - Etude synécologique des fluctuations temporelles des rejets d'un égout urbain dans un bassin portuaire à Marseille. IVes Journées Etud. pollution. Cagliari C.I.E.S.M. 635-642.

ZAVODNIK D. and N. ZAVODNIK (1978). - The benthos of Rijeka Bay subject to stress of pollution. IVes Journées Etud. pollution Anatalya C.I.E.S.M. 405-410.

RELATION ENTRE POLLUTION ET BIOCENOSES EN BAIE D'AUGUSTA

par

I. DI GERONIMO, G. CANTONE, A. CASTAGNA,
M. CORMACI, S. FAILLA, G. FASSARI,
G. FURNARI, S. GIACOBBE, G. GIACCONE,
M. LEONARDI et F. SINATRA.
Istituto di Scienze della Terra, Università di Catania
Corso Italia, 55 - 95129 Catania (Italia)

Résumé

La baie d'Augusta est une zone sujette à un fort déséquilibre dû à une pollution très complexe : industrie pétrochimique, urbaine et portuaire. Pour essayer d'en évaluer l'impact une étude a été menée sur la composition et la structure des peuplements algaux et animaux (Mollusques, Polychètes) méso-infralittoraux de substrats dur et sur la teneur en métaux lourds de quelques espèces d'algues et de Mollusques. 12 stations à l'intérieur de la baie ont été sélectionnées et nous avons effectué 2 prélèvements, l'un en mai et l'autre en octobre 1983.

Une grande différence entre les stations orientales de la partie la plus externe de la baie, moins polluée, et celle de la partie interne occidentale plus polluée est apparue.

Les valeurs de la biomasse diminuent du nord au sud et elles sont plus basses en automne qu'au printemps. La biomasse animale d'automne est toujours plus basse que celle du printemps alors que celle des algues des stations externes est quasi constante ou plus élevée. En général, les peuplements du versant oriental de la baie sont mieux structurés que ceux du versant occidental.

Les peuplements algaux d'automne présentent une augmentation de leur couverture dans toute la baie, alors que le nombre d'espèces diminue sur le côté occidental et augmente dans la partie orientale.

Les peuplements animaux (Polychètes et Mollusques) présentent une inversion : dans le prélèvement d'automne on note un net appauvrissement tant en nombre qu'en espèce. Les valeurs moyennes de la teneur en métaux lourds (dans les mollusques et les algues) sont comparable à celles des autres milieux portuaires. On note que Brachidontes variabilis concentre Cu avec des teneurs plus fortes que Mytilus.

*
* *

Introduction

En Sicile, il y a trois aires côtières sujettes à une forte pollution principalement de type industriel : Milazzo, Augusta et Gela.

Pour l'aire d'Augusta, ces dernières années, un certain nombre de travaux ont été effectués tant sur la nature de la pollution que sur les modifications apportées au milieu marin.

Le port d'Augusta (Fig. 1) s'allonge en direction N-S ; au NE une petite bouche naturelle le met en communicatiion avec le port Xifonio : à l'E et au SE, il y a deux bouches artificielles entre la digue qui le sépare de la mer Ionienne et le Seno del Priolo. Le secteur occidental est intéressé par l'industrie pétrochimique, le secteur Nord-Oriental subit essentiellement des déversements urbains, le trafic portuaire, militaire ainsi que celui des bateaux de pêche.

La baie d'Augusta est une zone à fort déséquilibre dû à une pollution complexe : industrie pétrochimique, urbaine et portuaire.

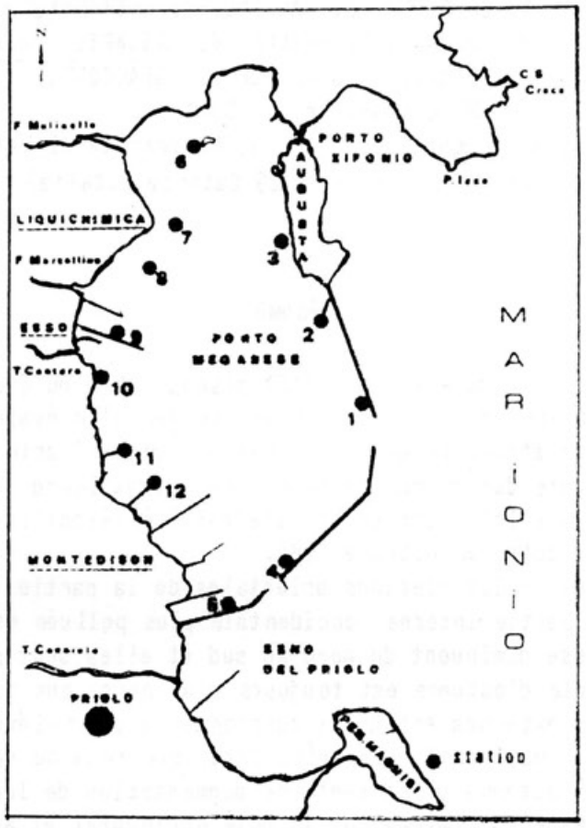


Figure 1. - Port d'Augusta : carte de situation des stations de prélèvements.

Matériel et méthodes

Pour essayer d'évaluer l'impact de la pollution une étude a été menée sur la composition et la structure des peuplements alguaires et animaux (Mollusques, Polychètes) méso-infralittoraux des substrats durs et sur la teneur en métaux lourds de quelques espèces d'Algues et de Mollusques. Douze stations ont été sélectionnées à l'intérieur de la baie sur lesquelles ont été effectués deux prélèvements : un en mai et l'autre en octobre 1983 (Fig. 1).

Les recherches ont été menées dans le cadre du projet F.A.O., plan d'action pour la Méditerranée (MED POL -Phase II).

Les prélèvements ont été effectués selon les méthodes utilisées par BELLAN-SANTINI (1969) et reprises par STIRN (1981) correspondant à un grattage d'une superficie de 400 cm2 (20x20 cm). Pour l'étude qualitative de la biocénose on se réfère à PERES et PICARD (1964) et GIACCONE (1972). Par contre pour l'étude quantitative c'est la méthode proposée par STIRN (1981) qui a été retenue, et pour les métaux lourds on a employé des échantillons animaux de 2 gr (poids sec à 50°C) et végétaux de 5 gr (poids humide) avec la méthode d'ADRIAN (1971) et BERNHARD (1976), à l'aide d'un spectrophotomètre d'absorption atomique (A.A.S.) Perkin-Elmer 50.000.

Résultats et discussion

Le stock végétal est composé de 92 espèces : Rhodophycées (68) et Chlorophycées (11) ont une valeur importante de couverture : les Pheophycées, sont représentées par 13 espèces, mais aucune d'entre elles ne forme une couverture appréciable.

Le rapport R/P (Rhodophycées/Phéophycées) utilisé par GIACCONE (1977) pour caractériser l'état de dégradation ambiental en Méditerranée a des valeurs comprises entre 2.5 et 4, alors que dans un milieu déstabilisé elles vont de 5 à 10. Dans la rade d'Augusta la valeur globale de R/P est de 5.2 : celle relative au versant industriel est de 6.2. celle du versant urbain est de 5.

Le tableau phytosociologique, synthétisé graphiquement sur le diagramme circulaire de la végétation, fait apparaître que l'analyse floristique est confirmée par les rapports en pourcents des unités de la végétation présente (Fig. 2).

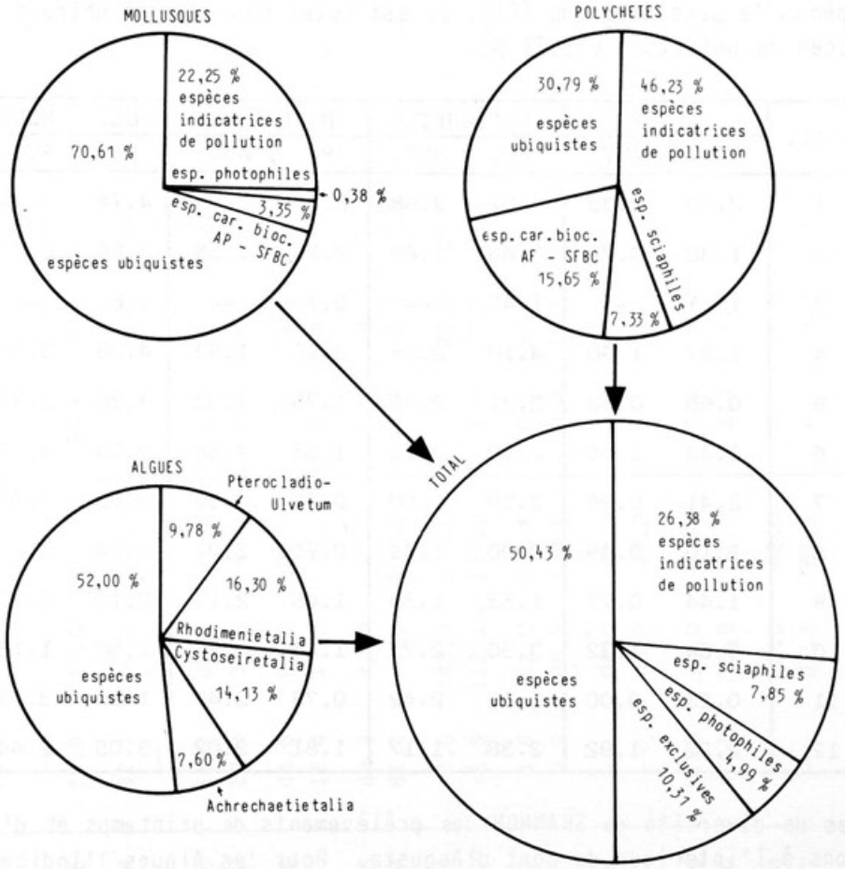


Figure 2. - Diagrammes circulaires des différents stocks biocénotiques dans le port d'Augusta pour chaque groupe d'organismes; le grand cercle représente la moyenne de tous les groupes.

La couverture varie de même notablement entre les deux secteurs avec une moyenne par station, au printemps 88,33 % dans le secteur oriental et de 35,16 % dans le secteur occidental et en automne respectivement de 94 % à 37,50 %.

Le même résultat est mis en évidence par l'examen des indices de diversité calculés pour les deux versants de la rade.

Les peuplements alguaires au cours de l'année conservent fondamentalement une même structure, même si l'on observe quelques variations physionomique (plus notables dans le secteur oriental) par renouvellement des espèces dominantes.

Les polychètes sont représentés par 71 espèces : l'espèce dominante tant dans les prélèvements printaniers (43,5 %) que dans les prélèvements d'automne (43 %) est Hydroides elegans espèce carac-

téristique des ports plus ou moins pollués. La présence dans les stations occidentales de Cirriformia tentaculata, espèce indicatrice de pollution, est aussi significative avec un pourcentage de 3 % à 5 %.

Le diagramme circulaire de la Fig. 2, met en évidence la structure bionomique globale des deux séries de prélèvements.

Les peuplements des stations orientales sont mieux structurés que ceux des stations occidentales. Dans ces dernières les indices de diversité sont nettement plus bas (Tab. 1), en outre les indices de diversité sont toujours plus bas que ceux du printemps.

Les mollusques sont représentés par 35 espèces : Bivalves (18 espèces), Gastéropodes (14), Polyplacophores (3).

Les espèces dominantes sont les mytilidés : Mytilaster minimus, Mytilus galloprovincialis et l'hôte lessepsien Brachidontes variabilis avec plus de 65 %, accompagnées du Bivalve Petricola lithophaga et de toutes les autres espèces avec des pourcentages très bas.

Le stock des espèces le plus nombreux (Fig. 2) est celui des espèces ubiquistes (70,61 %) suivit des espèces indicatrices de pollution (25,22 %).

STAT.	ALGUES		POLYCHETES		MOLLUSQUES		POL. + MOLL.	
	PR.	AUT.	PR.	AUT.	PR.	AUT.	PR.	AUT.
1	2.10	1.33	4.07	2.98	2.97	1.94	4.74	3.39
2	1.38	0.79	3.40	1.69	2.28	1.15	3.88	2.43
3	1.11	--	1.45	--	0.10	--	1.88	--
4	1.87	1.60	4.10	2.88	2.16	1.93	4.28	3.00
5	0.65	0.53	2.91	2.40	1.75	1.03	3.38	2.31
6	1.44	1.66	2.70	2.15	1.88	2.38	3.13	3.17
7	2.41	0.96	2.19	1.50	0.75	0.96	2.63	2.22
8	1.10	0.49	0.00	1.49	0.75	2.27	0.74	2.90
9	1.44	0.77	1.53	1.34	1.48	2.19	2.15	2.82
10	0.64	1.32	3.30	2.75	1.26	0.87	1.58	1.18
11	0.23	0.00	1.12	2.42	0.73	2.42	1.35	3.13
12	2.22	1.92	2.38	1.17	1.81	2.02	3.05	2.44

Tableau 1. - Indices de diversité de SHANNON des prélèvements de printemps et d'automne dans les 12 stations à l'intérieur du port d'Augusta. Pour les Algues l'indice a été calculé avec les modifications apportées par BOUDOURESQUE (1970) en remplaçant le nombre des individus avec la couverture en pourcentage des espèces : $H = \sum_{i=1} \frac{c}{c} \log_2 \frac{c_1}{c}$ dont : c = couverture % des espèces dans le prélèvement; c_1 = couverture % de chaque espèce.

Pratiquement, les espèces caractéristiques exclusives de la biocénose AP (3,55 %) et celles définies comme photophiles (0,83 %) comprenant des espèces de la biocénose AP et HP, sont disparues. Les filtreurs sont représentés par plus de 85 %, les détritivores, herbivores et carnivores sont insignifiants. Une telle structure anomalique est bien mise en évidence par les indices de diversité des prélèvements printaniers (Tab. 1) dans lesquels les stations occidentales et la A3 (située à quelques dizaines de mètres d'une bouche d'égout) présentent des valeurs plus basses de celles du secteur oriental.

STAT.		POIDS HUMIDES			POIDS DECALCIFIES			POIDS SECS			POIDS DE CENDRE			MATIERE ORGANIQUE		
		Vég.	An.	Tot.	Vég.	An.	Tot.	Vég.	An.	Tot.	Vég.	An.	Tot.	Vég.	An.	Tot.
1	PR.	62.9	417.5	480.4	36.7	80.1	116.8	4.7	15.1	19.8	1.15	7.35	8.50	3.55	7.75	11.30
	AUT.	297.1	57.4	354.6	133.6	15.8	149.4	18.5	2.1	20.6	11.78	1.64	13.42	6.72	0.46	7.18
2	PR.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	AUT.	99.6	835.0	934.6	53.9	17.6	71.5	10.6	41.1	51.7	2.08	33.64	35.72	8.52	7.46	15.98
3	PR.	51.1	1502.3	1553.4	32.1	250.2	282.3	4.0	67.5	71.5	0.77	43.40	44.17	3.23	24.10	27.33
	AUT.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
4	PR.	27.2	370.8	398.0	14.7	41.8	56.5	2.2	10.0	12.2	0.14	2.70	2.84	2.06	7.30	9.36
	AUT.	34.3	251.8	286.2	17.3	30.0	47.3	2.5	1.8	4.3	0.34	1.01	1.35	2.16	0.79	2.95
5	PR.	69.7	224.9	294.6	44.6	83.1	127.7	2.7	29.0	31.7	0.56	26.28	26.84	2.14	2.72	4.86
	AUT.	51.8	265.5	317.3	18.4	17.3	35.7	2.7	4.1	6.8	0.26	0.57	0.83	2.44	3.53	5.97
6	PR.	72.6	189.0	261.6	48.3	42.0	90.3	8.0	8.2	16.2	1.39	2.88	4.27	6.61	5.32	11.93
	AUT.	94.9	210.4	305.4	61.4	41.8	103.2	4.9	8.1	13.0	0.63	2.73	3.36	4.27	5.37	9.64
7	PR.	5.2	808.7	813.9	3.0	143.0	146.0	0.5	29.2	29.7	0.24	8.59	8.83	0.26	20.61	20.87
	AUT.	37.8	496.9	534.7	3.9	113.1	117.0	1.6	20.7	22.3	0.56	4.56	5.12	1.04	16.14	17.18
8	PR.	3.5	612.7	616.2	1.6	152.0	153.6	0.1	24.0	24.1	0.04	2.41	2.45	0.06	21.59	21.65
	AUT.	7.8	509.5	517.3	2.9	105.7	108.6	0.4	18.5	18.9	0.11	3.20	3.31	0.29	15.30	15.59
9	PR.	--	674.0	674.0	--	98.0	98.0	--	21.9	21.9	--	4.42	4.42	--	17.48	17.48
	AUT.	--	547.3	547.3	--	43.2	43.2	--	10.7	10.7	--	2.75	2.75	--	7.95	7.95
10	PR.	78.2	127.4	205.6	31.5	20.0	51.5	4.0	6.0	10.0	0.35	2.64	2.99	3.65	3.36	7.01
	AUT.	88.8	131.8	220.6	45.1	15.9	61.0	3.3	4.5	7.8	0.19	2.06	2.25	3.11	2.44	5.55
11	PR.	--	506.4	506.4	--	62.0	62.0	--	15.5	15.5	--	7.56	7.56	--	7.94	7.94
	AUT.	--	611.7	611.7	--	67.2	67.2	--	9.4	9.4	--	2.00	2.00	--	7.40	7.40
12	PR.	7.3	396.8	404.1	2.7	75.9	78.6	0.4	10.9	11.3	0.05	1.02	1.07	0.35	9.88	10.23
	AUT.	--	409.2	409.2	--	20.2	20.2	--	5.0	5.0	--	1.05	1.05	--	3.95	3.95

Tableau 2.

L'indice de diversité, contrairement à ce qui a été enregistré pour les algues et les polychètes et seulement pour ce qui concerne les stations occidentales, augmente de valeur dans les prélèvements d'automne et dépasse considérablement celui des stations orientales.

Les valeurs de la biomasse (g pour 1/25m2) rencontrées (Tab. 2) sont parfaitement identiques à celle des peuplements de facies semblable en milieu portuaire ou pollué (BELLAN-SANTINI, 1969).

Les valeurs moyennes de la teneur en métaux lourds sont confrontables avec celles des autres milieux portuaires. En particulier on enregistre une différence notable pour les valeurs de concentration dans les prélèvements printaniers et de l'automne. Les organismes végétaux, dans l'ensemble montrent des concentrations légèrement inférieures à celles des organismes animaux.

Les stations les plus contaminées sont celles du secteur méridional et ceci en accord avec la direction principale de circulation de l'eau à l'intérieur du port du N vers le S.

	Conc.max ppm	St.	Org.	Conc.min ppm	St.	Org.
Cd	6.72	12	P.coerulea	0.02	10	Corallina sp.
Cr	6.64	12	P.coerulea	0.32	5	Ulva sp.
Cu	81.53	10	M. trunculus	0.47	5	Ulva sp.
Fe	5217.00	12	P.coerulea	12.23	10	Ulva sp.
Zn	434.67	10	M. trunculus	0.09	6	Enteromorpha
Ni	7.52	12	P.coerulea	0.41	10	M.trunculus
Pb	45.30	12	P.coerulea	0.90	2	Codium sp.
Hg	3.13	4	B.variabilis	0.02	3	Gracilaria sp.

Tableau 3. - Valeurs minimales et maximales (ppm) en métaux lourds rencontrés dans les algues et les animaux du port d'Augusta.

Conclusion

On a noté une nette différence entre les stations du secteur oriental du port soumis aux apports urbains uniquement sur la portion centro-septentrionale et les stations du secteur occidental où il y a décharges de l'industrie et les pontons d'amarrage des navires.

Les données issues de l'analyse biocoenotique concordent parfaitement pour ce qui concerne la structure des peuplements. Par rapport à un milieu "normal" on constate un appauvrissement de stocks des espèces exclusives de la biocénose AP et des espèces photophiles, parallèlement on a une forte augmentation des espèces sciaphiles, ubiquistes et indicatrices de pollution. Les peuplements du secteur oriental industriel sont moins structurés que ceux du secteur occidental : et les indices de diversité de SHANNON ont une valeur toujours plus basse. D'intérêt particulier l'indice R/P, avec sa valeur élevée (plus de 5), est indicatif d'une zone perturbée pour la Méditerranée.

Les espèces dominantes, comme dans de nombreuses zones similaires, sont les mollusques Mytilus galloprovincialis, le Polychète Hydroides elegans et l'algue Ulva rigida.

Les valeurs de la biomasse (constituée en grande partie par Mytilus), élevées dans la partie centro-septentrionale du port, et moins dans le secteur méridional, laissent penser à une concentration anomalique de polluants dans la partie méridionale du port, attribuable à la circulation N-S des eaux. Une telle situation est confirmée par la présence dans les stations 4,10 et 12 d'organismes à taux élevés en métaux lourds. Patella caerulea et Murex trunculus, espèces comestibles souvent vendues sur le marché, semblent être les organismes qui concentrent le plus les métaux lourds. Au contraire, les Algues sont ceux qui ont le taux d'accumulation le plus faible. On note que Brachidontes variabilis concentre le cuivre en quantité importante.

En première comparaison avec les données plus anciennes, il ressort que la situation générale du

port d'Augusta est en légère amélioration. Ce fait est probablement dû d'une part à la réduction du trafic portuaire et d'autre part à un ralentissement des polluants, dont les causes sont dûes respectivement à la crise italienne de l'industrie pétrochimique et à l'entrée en fonction de la centrale d'épuration.

Références

D. BELLAN-SANTINI, (1969). - Contribution à l'étude des peuplements infralittoraux sur substrat rocheux (Etude qualitative et quantitative de la frange supérieure). Rec. Tr. Stat. Mar. Endoume, v.63, f. 47, pp. 1-294.

M. BERNHARD, (1976). - Manual of methods in aquatic environment research. Part 3. Sampling and analysis of biological material. F.A.O. Fish. Techn. Pap. N° 158, Rome.

G. GIACCONE, (1972). - Elementi di botanica marina. Parte prima : Bionomia bentonica e vegetazione sommersa del Mediterraneo. Pubbl. Ist. Bot. Univ. Trieste, 41 pp.

G. GIACCONE, (1977). - Effetti sul fitobenton smaltimento a mare della acque di fogna. Ingegneria ambientale, v. 6. f. 1, pp. 37-43.

J.M. PERES & J. PICARD, (1964). - Nouveau manuel de bionomie benthique de la Mer Méditerranée. Rec. Trav. Stat. Mar. Endoume Bull., 31 (47), pp. 1-137.

J. STJRN, (1981). - Manual of methods in aquatic environment research. Part 8 - Ecological assessment of pollution effects. F.A.O. Fish. Techn. Paper. n. 209, pp 1-70.

MERCURY-BINDING PROTEINS OF THE GILLS AND DIGESTIVE GLAND OF MYTILUS GALLOPROVINCIALIS

by
M. TUSEK-ZNIDARIC, M. SKREBLIN*, J. PAVICIC*
I. KREGAR, P. STEGNAR and A. PROSENC
"J. Stefan" Institute
"E. Kardelj" University of Ljubljana and
* Center for Marine Research, Rovinj (Yugoslavia)

Abstract

The distribution of Hg-binding proteins from unpolluted, naturally Hg polluted and laboratory highly contaminated mussels (Mytilus galloprovincialis) were compared. The soluble fraction of gill and digestive gland homogenates were chromatographed on a calibrated Sephadex G-75 column and fractions were analysed by UV absorbance at 280 and 254 nm, and for mercury content. Differences found in the distribution of mercury between the protein fractions from the two types of tissue homogenate are discussed.

*
* *

Introduction

Mussels (Mytilus galloprovincialis, Lam.) are able to concentrate heavy metals without obvious signs of distress. In recent years they have been recognized as suitable organisms for monitoring trace metal pollution of coastal marine water (1, 2). Like some other marine invertebrates, they possess metal-binding proteins which serve to bind intracellular mercury, cadmium, copper and zinc as a metal storage and detoxification compartment. Synthesis of these metallothionein - like proteins appears to be induced by exposure to the appropriate metal (3, 4) in both natural conditions and in the laboratory.

The aim of the present work was primarily to study differences in the binding of Hg in the mussel Mytilus galloprovincialis exposed to high levels of inorganic Hg in the laboratory and in the marine environment in comparison with non-exposed control organisms.

Materials and methods

Mussel (Mytilus galloprovincialis, Lam.), 4-7 cm long, used for analyses, lived in different conditions:

- a non-contaminated area; mussel were sampled at Lim fjord, Istria, Jan. 1984;
- a very contaminated area polluted with inorganic mercury; mussels were sampled in Kastela Bay, Dec. 1983;

- laboratory conditions: mussels were placed in an aquarium with a flow system for 33 days and the mussels were exposed to 60 ppb of inorganic mercury ($HgCl_2$).

Only two organs were used:
- the gills; they represent an organ which functions as a site of uptake for mercury and as an important reservoir for total mercury body burden;
- the digestive gland which is known to function in the storage of trace metals.

4-10 g of tissue from 20-30 animals were cut in pieces and homogenized in 3 vol. of ice cold buffer (20 mM Tris-HCl, pH = 8.6 and 0.1 mM phenylmethylsulphonyl fluoride). A glass homogenizer with a teflon pestle was used. The homogenate was centrifuged at 27,000 g for 1 hour at 4° C immediately. The supernatant from the digestive gland was filtered (black ribbon filter paper) to remove fats. 3.5 ml of clear supernatant were chromatographed on a 2.5 x 70 cm column of Sephadex G-75 equilibrated with 20 mM Tris-HCl, pH = 8.6 at a flow rate of 19.2 ml/h, maintained at 4° C. Aliquots of 5-7 ml were collected and monitored for absorbance at 280 nm and 254 nm. In homogenates, supernatants and fractions total Hg was analysed.

Total mercury was determined by two different methods:
- neutron activation analysis using a pyrolysis technique to separate ^{197}Hg by volatilization and selective trapping. (5, 6)
- flameless atomic absorption technique using tin (II) chloride reduction followed by volatilization of the mercury and amalgamation on elemental gold. (7, 8)

In a few selected fractions, both analytical methods were used and good agreement was found.

Results and discussion

All samples of digestive glands and gills were prepared in the same way. The total mercury content and the percentage of extractable Hg for the different sample types are shown in table 1.

Table 1

Sample	Hg(µg/g)	% of extraction
Control organisms		
gill homogenat	0.08	
supernatant	0.003	3.75
Mussels from natural contaminated area		
gill homogenat	2.07	
supernatant	0.08	3.87
Digestive gland homogenat	3.83	
supernatant	0.16	4.12
Laboratory exposure mussels		
gill homogenat	46.53	
supernatant	20.55	44.2
Digestive gland homogenat	19.51	
supernatant	6.72	34.4

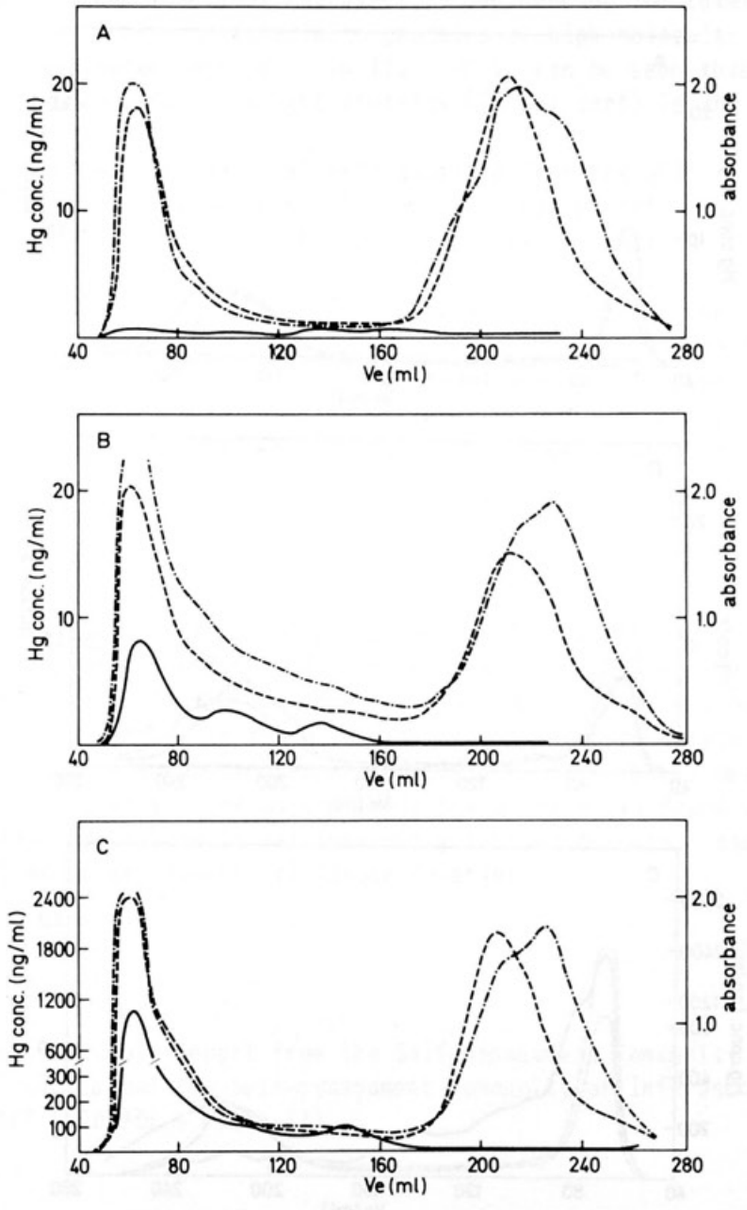


Fig.1: Sephadex G-75 chromatograms of supernatants of mussel digestive glands. In 5-7 ml fractions total mercury (——), absorbance at 280nm (- - - -) and 254nm (-·-·-·-) were measured. A: control organisms; B: mussels eksposured to high levels of mercury in the marine environment; C: mussels exposured to high levels of mercury in laboratory conditions.

Although the concentrations of total mercury in the tissue homogenates from mussels from nature (uncontaminated and contaminated) are different, the percentage extracted from the homogenates is similar. The percentage of mercury extracted from tissue homogenates of laboratory exposed mussels is different and runs up to 30-44 per cent. In these tissues of these mussels the subcellular localization of the Hg-binding proteins is supposed to be different because of the relatively short exposure time.

Figures 1 and 2 show the Sephadex G-75 elution profiles of supernatants. The absorbance at 280 nm and 254 nm and the mercury concentrations were determined in each fraction.

Elution profiles on Sephadex G-75 obtained from all supernatants have two separated peaks measured in UV spectrum at 280 and 254 nm. The first one corresponds to high molecular weight

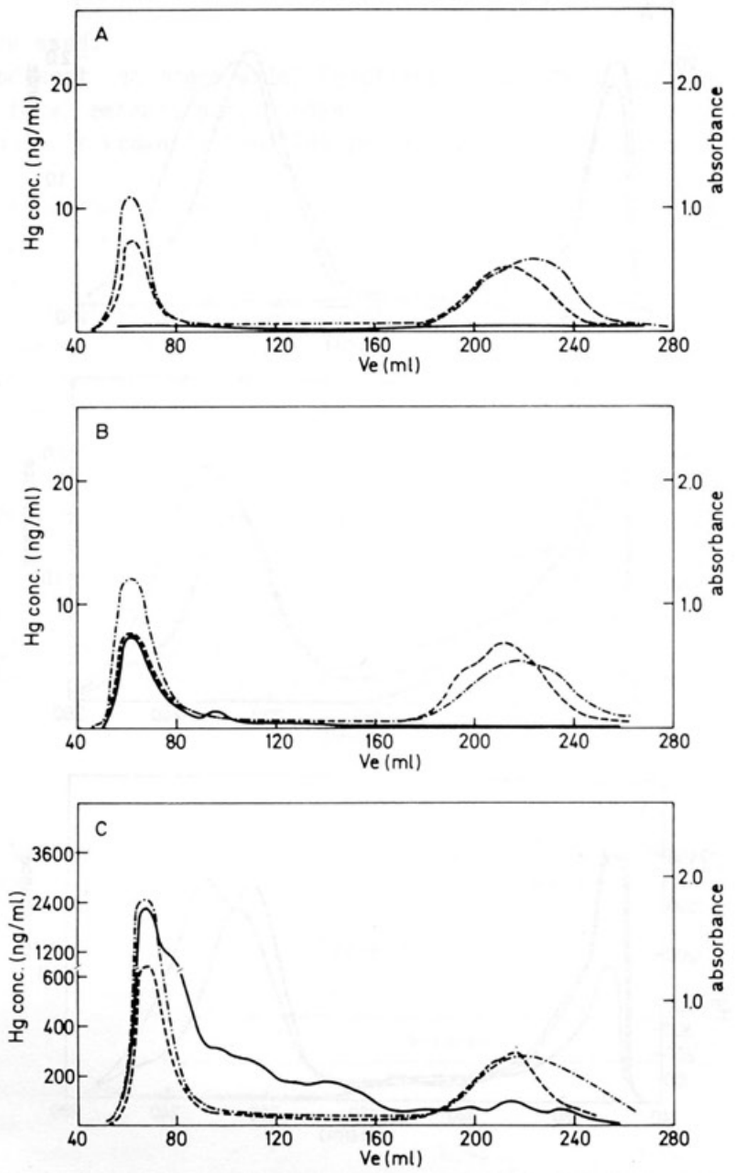


Fig. 2: Sephadex G-75 chromatograms of supernatants of mussel gills. In 5-7ml fractions total mercury (——), absorbance at 280nm (- - - - -) and 254nm (— · — · —) were measured. A: control organisms; B: mussels exposured to high levels of mercury in the marine environment; C: mussels exposured to high levels of mercury in laboratory conditions.

proteins (> 80,000 Daltons), while the second one corresponds to low molecular weight compounds. The second peak contains low levels of total mercury, in some cases under the detection limit of analytical technique. In contrast to other metals which can induce the synthesis of metal-binding proteins (Zn, Cu, Cd) (9, 10, 11, 12), mercury does not associate with low molecular weight compounds.

Fig. 1 shows elution patterns on Sephadex G-75 of water soluble digestive gland extracts. The fractions obtained from the extract of control organisms contain very low mercury concentration (less than 1 ng/ml). Fig. 1B demonstrates the separation of the soluble fraction of digestive glands of mussels living in a contaminated area. The first peak at 254 nm which corresponds to high molecular weight proteins has a shoulder. It is possible that the protein fractions in the area of intermediate

molecular weight proteins (the position of metallothionein-like proteins) have not separated. The two Hg-peaks corresponding to these fractions represent 34 per cent of the total mercury in the extract, and they follow the first Hg peak associated with proteins of high molecular weight. This contains 66 per cent of the total supernatant mercury. In fig. 2C it can be seen that the major part of the supernatant Hg binds to high molecular weight proteins (76 per cent) in the case of the laboratory-exposed animals.

Chromatographic analysis of soluble extracts obtained from the gills of control animals demonstrates that Hg is not found bound to proteins of high molecular weight (Fig. 2A). The Hg concentration in the extract is only 0.003 ug/ml and Hg concentrations in fractions from gel filtration are at the detection limit.

In the extracts obtained from the gills of Hg contaminated mussels, mercury is mostly associated with proteins of high molecular weight (69.8-87.6 per cent of total supernatant mercury): very little mercury is associated with intermediate molecular weight proteins (corresponding to metallothionein-like proteins) in the case of naturally polluted mussels. In the laboratory-exposed animals, however, appreciable amounts of Hg are associated with proteins of a wide range of molecular weights.

Conclusions

Mussels, Mytilus galloprovincialis, exposed to high levels of inorganic mercury bind it mostly to high molecular weight proteins (65.8 - 87.6 per cent of total supernatant Hg). This is true for the gills and for the digestive glands. Our results show that in these organs 21.7 - 34.2 per cent of supernatant Hg is bound to proteins in the molecular weight range associated with inducible metallothionein-like proteins found in marine vertebrates exposed to other heavy metals.

It might be instructive to examine more closely the differences found in the intermediate range mercury-binding proteins for naturally and laboratory exposed mussels, and to see whether these differences are maintained in experiments of longer duration.

Acknowledgements

This work received financial support from the Self-management Community of Interest for Scientific Research of S.R. Slovenia and the Self-management Community of Interest for Scientific Research of S.R. Croatia, and UNEP (MED POL - Phase II).

References

1. PHILLIPS D.J.H. (1976). - Marine Biology 38, 59-69.
2. DAVIES L.K. and J.M. PIRIL (1978). - Marine Pollution Bulletin 9, 128-132.
3. ROESIJADI G. (1980): - Marine Environmental Research 4, 167-179.
4. NOEL-LAMBOT F. (1976). - Experientia 32, 324-325.
5. KOSTA L. and A.R. BYRNE (1969). - Talanta 16, 1,296.
6. BYRNE A.R. and L. KOSTA (1974). - Talanta 21, 1,083.
7. KONISHI T. and H. TAKAHASHI (1983). - Analyst 108, 827.
8. WITTMANN Z. (1981). - Talanta 28, 271.
9. VIARENGO A., M. PERTICA, G. MANCINELLI, S. PALMERO, G. ZANICCHI, and M. ORUNESU (1981). - Marine Pollution Bulletin 10, 347-350.
10. KOHLER K. and H.U. RIISGARD (1982). - Marine Biology, 66, 53-58.
11. GEORGE S.G. and B.J.S. PIRIL (1979). - Biochimica et Biophysica Acta, 580, 234-244.
12. BRAY J.T., L.A. WEBB and F.J. REILLY (1983). - The Science of the Total Environment, 28, 367-374.

BENTHOS OFF THE PO RIVER MOUTH- SUBJECT TO STRESS CONDITIONS ?

by

Jasna VIDAKOVIC and Dusan ZAVODNIK
Center for Marine Research Rovinj
"Rudjer Boskovic" Institute
52210 Rovinj (Yugoslavia)

Abstract

Environmental analyses showed that in the area off the Po River mouth, benthic communities suffer seasonal fluctuations of several variables, of which oxygen deficiency and sedimentation rates seem to be the most important. Although macro-and meiobenthic populations are obviously not directly affected by the Po River discharges, several alterations in macrobenthic communities have occurred over the past fifty years.

*
* *

Introduction

The wide-scale influence of the Po River discharge on the general oceanographic conditions and dynamics of the pelagic ecosystem have been continuously studied in the northern Adriatic Sea for more than two decades (STIRN, 1968: REVELANTE and GILMARTIN, 1976: DEGOBBIS et al., 1979: SMODLAKA and REVELANTE, 1984). On the contrary, only scanty information on macrobenthos assemblages in the area offshore of the Po River mouth are available (VATOVA, 1949: AMBROGI et al., 1983). The meiofauna "in toto" has never been studied in this area.

Area investigated

Three stations were established in international waters off the Po River mouth, along a permanent transect for continuous oceanographic surveys between Rovinj and the Po River (table I). The area is characterized by large seasonal fluctuations in temperature, salinity and dissolved oxygen contents, and an annual change from a stratified to a vertically well mixed water column (GILMARTIN et al., 1972). The bottom consists of sandy-silty and clayey-silty sediments.

Methodology

At all three stations samplings and measurements were performed at about two month intervals from November 1982 to October 1983. Six surveys were completed.

Table I
Basic data on locations and sediment characteristics at stations investigated in 1982-1983.

Station	SJ-7	SJ-8	SJ-9
Geographical position			
Latitude N	45°01.0'	44°59.8'	44°45.4'
Longitude E	12°59.7'	12°49.8'	12°45.0'
Depth (m)	33	31	31
Sediment granulometry			
Median diameter (um)	16.66	11.80	6.20
Mean size (um)	16.20	14.90	6.30
Sorting coefficient	2.66	2.47	1.60
Silt content (%)	43.30	52.53	62.84
Organic matter content (%)	12.45	11.30	15.55

Basic oceanographic, sedimentological and bacteriological parameters were studied using standard sampling and analytical methods. Macrobenthos was collected by a van Veen 0.1 m^2 grab. Usually five tows (i.e. total from 0.5 m^2 per station) were accomplished on each survey. From an undisturbed grab sample, four subsamples were taken for meiofauna studies with a plastic hand corer (inner diameter of 3.5 cm), stained with Rose Bengal, and processed by elutriation method.

Results

1. Environmental research. During the period covered by this research, basic oceanographical parameters showed seasonal fluctuations within the ranges previously noted for the area (GILMARTIN et al., 1972) (table II).
2. Sediment heterotrophic bacteria were rather low in numbers and showed local and temporal variations (table II).
3. Meiofauna in the sediments is characterized by nematodes and copepods. Other groups like Polychaeta, Archiannelida, Kinorhyncha, and Ostracoda, were represented by much lower abundances (table III).

Temporal variations in abundance compositions were noted at each station. ANOVA and LSD tests on meiofauna showed that significant differences in abundance existed between station SJ-9, and the other two stations.

Table II

Ranges of selected oceanographic parameters in bottom layers of sea-water, and means of sediment heterotrophic bacteria at stations investigated in 1982-1983.

Station	SJ-7	SJ-8	SJ-9
Temperature (° C)	9.29-19.42	9.17- 16.01	9.60-16.54
Salinity ($S \times 10^{-3}$)	38.14-38.33	38.03- 38.33	37.99-38.42
Oxygen saturation (%)	49.1 -99.0	31.2 -100.3	24.3 -99.7
Reactive phosphorus (m mol m^{-3})	0.05- 0.53	0.05- 1.05	0.06- 1.68
Nitrates (m mol m^{-3})	0.52- 2.91	0.76- 11.07	0.63-10.80
Ammonia (m mol m^{-3})	0.60- 3.97	0.34- 4.99	0.23- 1.29
Silicates (m mol m^{-3})	4.20-24.20	2.97- 32.60	2.30-34.60
Chlorophyll a (mg m^{-3})	0.30- 1.39	0.32- 1.27	0.22- 1.18
Heterotrophs ($\bar{X}$ n/1 g sediment)	2.08×10^5	2.42×10^5	4.05×10^5

Table III

Mean abundances of sediment meiofauna ($\bar{X} \pm$ S.E. per 10 cm^2) and analysis of nematode population.

Station	SJ-7	SJ-8	SJ-9
Nematoda	1,129±172	1,024±110	467±81
Copepoda	135± 27	149± 25	96±41
Total meiofauna	1,327±201	1,137±147	612±99
Nematode population			
Species diversity (H')	3.152	2.939	2.955
Dominance diversity (J)	0.879	0.692	0.705
Interspecific Encounter Probability (PIE)	0.859	0.810	0.811
N°. Species (S)	16	19	18

Special attention was paid to an analysis of nematodes which in total number of individuals share by 75-90 per cent (Table III). Dominant species (*Rhabdodemania* sp., *Sabatieria punctata*, *S. ornata*, *Dorylaimopsis punctatus*) belong to the groups of non-selective deposit feeders and omnivores.

4. *Macrofauna*. At all three stations, most abundant were polychaetes (especially *Notomastus latericeus*) and echinoderms. In echinoderms, the only dominating species is *Amphiura filiformis*, of

which a calculated maximum density of population (1,136 spm m^{-2}) was noted at station SJ-8 in October 1983. Other fairly abundant groups were the bivalves (especially Corbula gibba) and sipunculans which were almost exclusively represented by Aspidosiphon kovalevskii inhabiting abandoned Turritella shells. At all stations, gastropods were few in number. Turritella communis, a species reported as characteristic of this area, was never found in densities greater than 40 specimens per square metre. A biomass mean of about 60 g (w.w.) m^{-2} was established at stations SJ-8 and SJ-9.

Discussion and conclusions

The assumption that the benthic organisms inhabiting areas off the Po River mouth are subjected to stress conditions of several environmental factors was raised long ago. Northern Adriatic organisms are by origin adapted to variations of some climatic factors, such as temperature. But variations of other parameters, such as oxygen saturation, or increased eutrophication, seem to be much more important to benthic life in the area. In the area investigated, in the bottom layer of seawater, oxygen concentrations clearly indicated anoxic conditions in November 1982 and from August to October 1983, which were obviously related to phytoplankton blooms (Smodlaka, pers. comm.). At that time, no mass mortality of benthic organisms was noted at our stations, such as previously reported in Rijeka Bay (ZAVODNIK, 1977) and the Gulf of Trieste (Stachowitsch, pers. comm.). But it is possible that anoxic conditions in the past could be one of the reasons for a decrease in population densities of any species dominant about fifty years ago (i.e. Turritella communis). On the other hand, a population explosion of the rheophile suspension feeding Amphiura filiformis suggested the increased input of particulate organic matter in the area, which is probably related to the repeated strong phytoplankton blooms.

It is well known that fine sediments contain a higher percentage of organic material to which also the abundances of meiofauna are generally positively correlated (GRAY, 1981). A low meiofauna abundance at station SJ-9 is probably related to a high percentage of clay materials and to the restriction of meiofauna to the thin surface layer of the sediment. A possible influence of any toxicants discharged by the Po River could also be considered. However, the high PIE index established by our analyses means that the sediment nematodes community in the area investigated has not suffered stress conditions.

Acknowledgements

The authors express their warmest gratitude to Mr. Juracic, Zagreb, for the execution of granulometric analyses, to Miss D. Fuks, Rovinj for providing bacteriological data, to Prof. Dr. L. Amoureux, Angers, for identification of polychaetes, and to Prof. Dr. M. Gilmartin, Orono, for reading the manuscript.

The support of the Self-management Community of Interest for Scientific Research of S.R. Croatia, and of F.A.O./U.N.E.P. (MED POL - Phase II) is acknowledged.

References

AMBROGI R., L. AMOUREUX and D. BEDULLI (1983). - Contribution à l'étude des peuplements infralittoraux face au delta du Po. Rapp. Comm. int. Mer Médit. 28 (3): 189-190.

DEGOBBIS D., N. SMODLAKA, I. POJED, A. SKRIVANIC and R. PRECALI (1979). - Increased eutrophication of the northern Adriatic Sea. Mar. Pollut. Bull. 10: 298-301.

GILMARTIN M., S. KVEDER, D. DEGOBBIS, N. REVELANTE and N. SMODLAKA (1972). - Hydrographic data collected during approximately bi-weekly cruises in the northern Adriatic. Thalassia Jugosl. 8: 149-213, 331-438.

GRAY J.S. (1981). - The Ecology of Marine Sediments. An Introduction to the Structure and Function of Benthic Communities. Cambridge Studies in Modern Biology 2. Cambridge University Press: 185 pp.

REVELANTE N. and M. GILMARTIN (1976). - The effect of Po River discharge on phytoplankton dynamics in the northern Adriatic Sea. Mar. Biol. 34: 259-271.

SMODLAKA N. and N. REVELANTE (1984). - The trends of phytoplankton production in the northern Adriatic Sea: A twelve year survey. Rapp. Comm. int. Mer Médit. 28 (9): 89-90.

STIRN J. (1968). - Pelagial Severnega Jadrana. Razprave - Dissert. Slov. Akad. zn. Umet., cl. IV, 12 (2): 1-132.

VATOVA A. (1949). - La fauna bentonica dell'Alto e Medio Adriatico. Nova Thalassia 1 (3): 116 pp.

ZAVODNIK D. (1977). - Benthic communities in the Adriatic Sea: Reflects of pollution. Thalassia Jugosl. 13: 413-422.

ELECTROPHORESIS AND CHARACTERIZATION OF FISH ESTERASES A METHOD FOR THE STUDY OF THE EFFECTS OF POLLUTION BY CERTAIN PESTICIDES AND METALS

by

A.A. HARITOS, S.S. SALAMASTRAKIS
and M. VEINI
Zoological Laboratory
University of Athens
GR 157 71 Athens (Greece)

Abstract

Fish esterases are classified into 9 categories according to their substrate specificities and inhibition patterns to diisopropylfluorophosphate (DFP), eserine (physostigmine) and parahydroxymercuribenzoate (PHMB). An electrophoretic method is described for the resolution of the multiple molecular forms of esterases in fish extracts as well as a procedure for their classification in the above mentioned categories. Muscle extracts of Trachurus trachurus were used in this study.

DFP, eserine and PHMB represent three categories of marine pollutants i.e. of organic phosphates, carbamates and mercury compounds respectively. The possibility of the study of the in vivo effects of these pollutants in fish at the molecular level (i.e. inhibition of esterase activity), is discussed.

*
* *

Introduction

Esterases are a complex group of proteins catalysing the hydrolysis of organic ester bonds. They are usually classified into four main categories according to their inhibition patterns (AUGUSTINSSON, 1961): carboxylesterases (CE), sensitive only to diisopropylfluorophosphate (DHP); arylesterases (Ar), sensitive only to parahydroxymercuribenzoate (PHMB) and/or parachloromercuribenzoate (PCMB); cholinesterases (CH), sensitive to eserine sulfate and DFP; and acetylesterases (Ac), not sensitive to any of the above inhibitors but preferentially hydrolysing acetate esters. Later on three new categories of fish esterases were introduced by HART and COOK, 1976, (ER, ESe and ESdp). In table I we show the classification of fish esterases according to their inhibition by DFP, eserine and PHMB/PCMB, including all possible combinations of inhibition.

The electrophoretic patterns of soluble esterases of the teleost genera Barbus, Brachydanio, Catostomus, Clupea, Cnidoglanis, Cyprinodon, Electrophorus, Erimyzon, Fundulus, Gambusia, Katsuwonus, Lepomis, Notropis, Platypoecilus, Pomoxis, Poeciliopsis, Thunnus, Trachurus and Xiphophorus have been reported. (For relevant references see HARITOS and SALAMASTRAKIS 1982).

Table 1
Characterization of fish esterases according to their inhibitor specificities.

	Esterases	DFP	Eserine	PHMB PCMB
(CE)	Carboxylesterases	+[a]	-	-
(ESe)	Esterases sensitive only to eserine	-	+	-
(Ar)	Arylesterases	-	-	+
(CH)	Cholinesterases	+	+	-
(ESdp)	Esterases sensitive to DFP and PHMB/PCMB	+	-	+
	Not detected	-	+	+
(ES)	Sensitive esterases	+	+	+
(ER)	Resistant esterases	-	-	-
(Ac)	Acetyl esterases[b]	-	-	-

[a] + for inhibition and - for no inhibition

[b] Ac have a preference for acetyl esters as substrate while ER not.

For the study of the effect of inhibitors, gels after electrophoresis of tissue extracts were incubated in solutions containing the inhibitors at low concentrations and subsequently stained for enzymic activity. Inhibited esterases appeared as fainter bands or the bands disappeared for partial or complete inhibition respectively. It is interesting that the three inhibitors used for the classification of esterases are also representing three categories of pollutants of marine waters: DFP of organic phosphate pesticides, eserine of carbamate pesticides and PCMB or PHMB of mercury compounds. Organic phosphate (Parathion, Malathion etc) and carbamates (Carbaryl, Methomyl etc.) are used for the control of softbodied insects in agriculture. The organic phosphates inhibit certain esterases by forming very stable covalent phosphoryl-enzyme complexes (at a serine amino acid residue at the active center of the enzyme). Carbamates act by the same molecular mechanism on some esterases, creating carbamoyl-enzyme intermediates that are subsequently hydrolysed at very slow rates. Mercury and its salts are used in the manufacture of thermometers, felt, paints, explosives, lamps, electrical apparatus and batteries. The volatile diethyl and dimethyl mercury compounds are used in treating seeds. Mercury depresses cellular enzymatic mechanisms by combining with sulfhydryl groups (-SH) of proteins, including certain esterases, and for this reason mercuric salts are toxic to all cells.

We present a procedure for extraction, electrophoresis and characterization of fish esterases by using the Mediterranean Sea fish Trachurus trachurus and pollution related esterase inhibitors.

Materials and methods

Adult specimens of Trachurus trachurus were cought in Saronic Gulf. They were stored at -20° C. Muscle tissue posterior to the anus was homogenized in two volumes of 0.1 M Tris-HCl buffer, pH 7.2 and the homogenate was centrifuged for 30 min at 12,000 g and 4° C.

The supernatant was electrophoresed by vertical slab gel electrophoresis with a Shandon model 200 apparatus. The polyacrylamide concentration was 7.5 %. Tris-HCl buffer, pH 8.9 (0.48 moles/l HCl and 366 g/l Tris) was used for the preparation of the gel and Tris-glycine buffer, pH 8.5 (3 g/l Tris and 14.4 g/l glycine) for the electrodes. To a small volume of protein solution an equal volume of sample buffer was added and 50 µl applied to each well of the gel. The sample buffer contained 2 ml glycerol, 0.5 ml 0.1 % (w/v) bromophenol blue solution, 0.1 ml Tris-HCl buffer, pH 6.7 (0.45 moles/l HCl and 50 g/l Tris) and 7.5 ml water. Electrophoresis was carried out for about 2.5 hr at a constant current of 19 m A at 3° C and was terminated when the marker dye migrated to a point 9 cm from the origin.

The staining solution contained substrate (dissolved in 1 ml acetone) and 30 mg Fast Blue BB salt in 40 ml of 0.1 M Tris-HCl buffer, pH 7.2. The substrates used were 20 mg α-naphthyl acetate, 20 mg β-naphthyl acetate, 20 mg α-naphthyl propionate and 50 µl α-naphthyl butyrate. The stain was completed in 30 min at room temperature.

Inhibitors employed were 10^{-4} M diisoprophylfluorophosphate (DFP), 10^{-4} M eserine sulfate and 10^{-3} M parahydroxymercuribenzoate (PHMB). The gels were preincubated for 45 min at room temperature in 0.1 M Tris-HCl buffer, pH 7.2, containing the above inhibitors. Controls were incubated in the buffer alone. Subsequently the gels were immersed in the staining solution containing α-naphthyl acetate and the inhibitor at the same concentration. For the study of the heat lability of the esterases, samples were incubated for 15 min at 50 and 60° C in a water bath, prior to electrophoresis. All reagents were purchased from Sigma Chemical Co.

Results and discussion

Soluble muscle esterases of Trachurus trachurus were resolved by vertical slab polyacrylamide electrophoresis into seven bands (E1 to E7). The staining intensity of the bands was maximum when α-naphthyl acetate, β-naphthyl acetate or α-naphthyl propionate esters were used as substrates (Table 2).

Three of the esterases bands were inhibited by DFP (E4, E5, E6) and two by eserine (E6, E7) while one of them (E6) by both. Three bands were not inhibited by any of the three inhibitors (DFP, eserine, PHMB). On the basis of their inhibitor specificities bands E1 to E3 were characterized as acetylesterases: bands E4 and E5 as carboxylesterases: band E6 as cholinesterase; and band E7 as esterase sensitive only to eserine (Tables 1 and 2). Two of the three acetylesterases (E1 and E2) accounted for about half of the total soluble esterase activity of the muscles.

Carboxylesterases appeared as a group of heat sensitive bands (E4 and E5) of intermediate electrophoretic mobility. Upon treatment of samples with neuraminidase prior to the electrophoresis, bands E4 and E5 were shifted together for 1 cm towards the cathode suggesting the removal of almost equal amounts of negatively charged sialic acid from each of the carboxylesterases by neuraminidase. The presence of sialic acid attached to fish muscle carboxylesterases has been reported (ECOBCHON and ISRAEL, 1967; HODGES and WHITMORE 1977). A single rather faint band was identified as cholinesterase (E6). The same enzyme was found in the muscle tissue of Brachydanio (HART and COOK, 1976) with medium to low relative activity, but not in the muscles of other teleosts. Obviously the presence or absence of low activity electrophoretic bands depend on the concentration and volume of the samples applied for electrophoresis. Band E7 was shown to be sensitive only to eserine sulfate (ESe). Bands with similar inhibitor specificity have also been detected in tissues of the teleosts Brachydanio (HART and COOK, 1976) Barbus (VARMA and FRANKEL 1980) and Katsuwonus (McCABE and DEAN 1970) but not in the muscle tissue.

It is suggested that electrophoresis followed by esterase staining of tissue extracts of fish from clean and polluted waters could reveal qualitative and quantitative changes in the latter. These changes could be further related to the in vitro inhibition of esterases by the organic phosphates, carbamates and mercury compounds described in this communication.

Table 2

Differential properties of muscle esterases of Trachurus trachurus

Esterases	E1	E2	E3	E4	E5	E6	E7
Relative mobility	89	83	78	42	40	13	6
Substrate [a] specificity							
α-naphthyl acetate	4+	3+	2+	+	+	+	2+
β-naphtyl acetate	4+	3+	2+	+	+	+	2+
α-naphthyl propionate	4+	3+	-	+	+	+	2+
α-naphthyl butyrate	3+	2+	-	+	+	+	-
Inhibitor [b] specificity							
DFP (10^{-4} M)	-	-	-	2+	2+	2+	-
Eserine (10^{-4} M)	-	-	-	-	-	3+	3+
PHMB (10^{-3} M)	-	-	-	-	-	-	-
Heat lability [a]							
15 min at 50° C	2+	2+	-	-	-	+	-
15 min at 60° C	-	-	-	-	-	-	-
Classification [c]	Ac	Ac	Ac	CE	CE	CH	ESe

[a] Degree of activity: Activity varies from 4+ (high activity to + (low activity), and - (absence of activity).

[b] Degree of inhibition: Inhibition varies from 3+ (complete inhibition) to + (little inhibition) and (no inhibition).

[c] As described in table 1.

References

AUGUSTINSSON K.B. (1961). - Multiple forms of esterases in vertebrate blood plasma. Ann. N.Y. Acad. Sci. 94, 844-860.

ECOBICHON D.J. and Y. ISRAEL (1967). - Characterization of the esterases from electric tissue of Electrophorus by starch-gel electrophoresis. Can. J. Biochem. 45, 1,099-1,105.

HARITOS A.A. and S.S. SALAMASTRAKIS (1982). - A comparison of muscle esterases in the fish genus Trachurus by vertical gel electrophoresis. Comp. Biochem. Physiol. 72B, 477-480.

HART N.H. and M. COOK (1976). - Comparative analysis of tissue esterases of the zebra danio (Brachydanio rerio) and the pearl danio (B. albolineatus) by disc gel electrophoresis. Comp. Biochem. Physiol. 54B, 357-364.

HODGES D.H. Jr and D.H. WHITMORE Jr (1977). - Muscle esterases of the mosquitofish Cambusia affinis. Comp. Biochem. Physiol. 58B, 401-407.

McCABE M.M. and D.M. DEAN (1970). - Esterase polymophisms in the skipjack tuna. Katsuwonus pelamis. Comp. Biochem. Physiol. 34, 671-681.

VARMA A.K. and J.S. FRANKEL (1980). - A comparison of tissue esterases in the genus Barbus by vertical gel electrophoresis. Comp. Biochem. Physiol. 65B, 267-273.

CHARACTERISTICS OF FOULING COMMUNITIES IN PORTS OF THE EASTERN ADRIATIC COAST

by

Ljubimka IGIĆ
Center for Marine Research Rovinj
"Rudjer Bosković" Institute
52210 Rovinj (Yugoslavia)

Abstract

Investigations of fouling communities on glass, wood, metal and polypropylene test plates, relatively close to sewage outfalls (about 30-50 m, stations 1, 2, 4: about 200 m station 3) in ports which are mostly insignificantly inhabited (about 500-700 inhabitants - station 1: 10,000 - stations 2 and 4), except Pula (about 60,000 inhabitants - station 3), have been undertaken.

The fouling communities, in respect of their qualitative-quantitative composition, grade of stability, total number of species, etc., show that the test sites of ports (with urban pollution) are less polluted, and they belong to "semi-healthy" (station 1 - North Adriatic) and to "semi-polluted" (stations 2, 3 - North Adriatic: station 4 - South Adriatic) categories of pollution.

*
* *

Introduction

As fouling communities are mostly composed of cosmopolite and unbiquitous they are, because of that, very favourable for studying impacts of pollution of different quality and grade. Considering that effluents of urban origin could have multiplex influences (on inorganic and organic nutrient concentrations, on primary production level, on dissolution of O_2, turbidity, deposition of particulate organic matter, etc.) we tried to observe the fouling at particular sites in ports with the highest urban-pollution impact.

Sites, material and methods

In ports situated in the north-east (Rovinj - stations 1, 2; Pula - station 3), and in the south-east (Kotor - station 4) of the Adriatic coast, the following are the numbers of inhabitants: 500-700 (station 1), 10,000 (stations 2, 4), 60,000 (station 3). The number of inhabitants increases

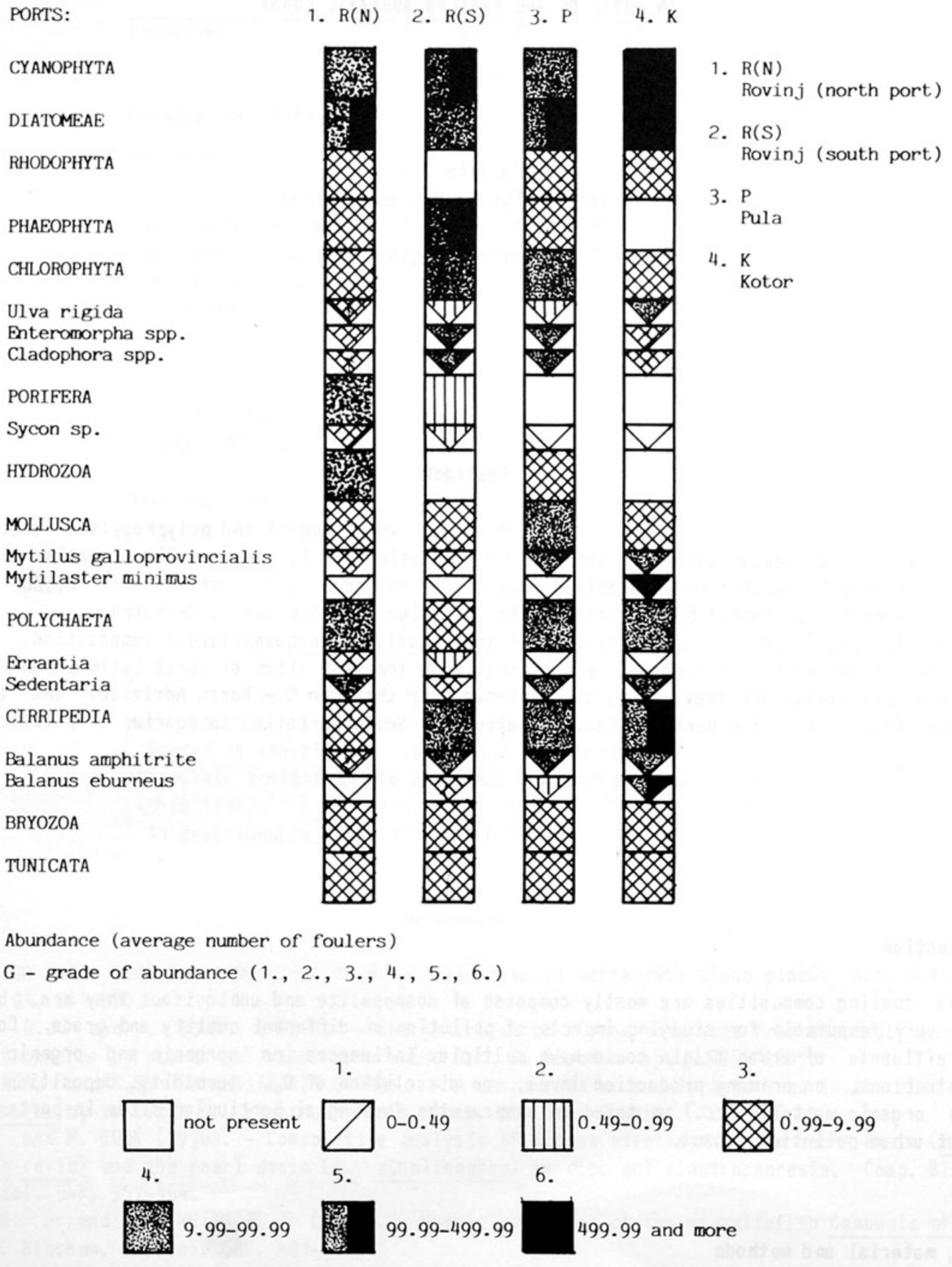


Figure 1. - Urban-pollution impact on grades of abundance of common fouling groups from North-East and South-East Adriatic ports

from three to four times, especially in Rovinj, during the tourist season. The stations tested were located in the inner parts of the ports relatively close to sewage outfalls (about 30-50 m - stations 1, 2, 4, 200 m - station 3).

Test substrata (glass - stations 1, 3; wood, metal - station 2: polypropylene - station 4) were immersed at depths of 0.5-2 m and about 4-7 m above the sea bottom. The investigations were carried out for 6 months (station 4), one year (station 2), and two years (stations 1, 3).

The biological materials were worked out according to methods of PERES and GAMULIN-BRIDA (1973) (grade of abundance), while hydrographic factors (T° C, Sal., pH, O_2) and nutrients (reactive phosphorus) according to standard methods.

Results and discussion

The range of values for temperature, salinity, pH and oxygen saturation generally corresponds to the fluctuation of these factors in our areas (North, South Adriatic). Only periodically is salinity lower at the surface of station 3 (4.46×10^{-3}) and station 4 ($12.45\text{-}34.20 \times 10^{-3}$ - 0.5 m; $20.72\text{-}35.79 \times 10^{-3}$ - 2 m) because of the freshwater inflows. There are insignificant concentrations of reactive phosphorus (0.045-0.98 µg-at/l), which is a general characteristic of our coast, especially when compared with the nearby port - Venice (2.01-4.5 $P\text{-}PO_4$ µg-at/l) (RELINI et al., 1972).

Particular fouling groups are higher or lower port-pollution bio-indicators. Macroflora is slightly represented, and that is generally typical for fouling communities. In our ports the nitrophile facies (Ulva-Enteromorpha-Cladophora), is not significantly represented (figure 1), although it increases in port waters as a result of lower salinity and enrichment of nutrients.

Concerning the fauna, sponges show the highest sensitivity to pollution (figure 1), and only the genus Sycon is more tolerant to the grade of urban pollution. Hydroids are slightly represented (figure 1), but for our areas the cosmopolite genus Obelia is the most characteristic, and was present at stations 1 and 3. Molluscs, polyzoans and tunicates groups play no special part in the fouling of our port waters. For example: the mussel - Mytilus galloprovincialis, generally, is not the main fouler in our ports, but it is in almost all Mediterranean ports. Only the polyzoan Cryptosula pallasiana is almost always present in the fouling of ports that are under the influence of urban pollution, in our ports, as well as in the whole Mediterranean. But the polyzoan Bugula (especially Bugula neritina) is fairly tolerant to this kind of pollution, and it does not reach a prevalent position in fouling communities.

The most tolerant organisms in ports which are especially rich in organic waste-waters, are the barnacles. This is particularly true of Balanus amphitrite, which is highly represented in our ports, where, because of the higher density it is often a real "nursery-garden", e.g. at stations 2 and 3. In the port of Kotor (station 4) Balanus eburneus is very characteristic as a result of a lower salinity. Also, the tubeworm, Hydroides elegans distinctly prefers port-waters rich in organic waste-waters. It is almost always in a dominant position in the fouling complexes of Mediterranean ports, and periodically in our ports as at station 3. The most characteristic fouling species in our sea is Pomatoceros triqueter which can be found in all environments, although it prefers cleaner waters. Therefore, the tubeworms known as bio-indicators of very intensive urban-industrial-pollution (Capitella capitata, Polydora ciliata, etc.) have never been met in our waters, i.e. in fouling communities.

The differences between fouling communities are almost exclusively the "reflex" of environments' characters. All communities in respect of their qualitative-quantitative composition, total number of species (105 - station 1, 33 - station 2, 37 - station 3, 25- station 4), grade of stability (more rapid development of communities - stations 2, 3, 4), etc., show that the testing sites could be divided into two categories of pollution: "semi-healthy" (station 1) and "semi-polluted" (stations 2, 3, 4). These terms and divisions were made by KOCATAS (1981) on the basis of almost identical criteria for the communities of benthos.

The harmful effect of effluents of a sewage outfall is lower, especially at station 1. But, directly at a sewage outfall, favourable sea currents and toxic effluents (detergents and pesticides), in a higher concentration, could possibly be expected to have a very harmful effect even on the euribiont fouling organisms.

Acknowledgements

The financial support for this study was provided by the Self-management Community of Interest for Scientific Research of S.R. Croatia.

References

KOCATAS A. (1981). - Evolution cyclique du benthos dans les zones de pollution du golfe d'Izmir (Turquie). Ves Journées Etud. Pollutions, Cagliari, C.I.E.S.M. (1980): 643-648.

PERES J.M., H. GAMULIN-BRIDA (1973). - Bioloska oceanografija. Skolska Knjiga, Zagreb: 494.

RELINI G., A. BARBARO, A. FRANCESCON (1972). - Distribuzione degli organismi del fouling in relazione all'inquinamento urbano di Venezia. Anno acc. 1971-72, 80: 433-448.

CANCERISATION DU FOIE PAR LA DIMETHYLNITROSAMINE CHEZ LE MUGE, MUGIL CAPITO L.

par

Bernadette LEVRAT, Jean-Philippe BREITTMAYER*
et Marc LAFAURIE
Laboratoire de Pathologie Cellulaire et Génétique.
U.E.R. Médecine,
Avenue de Valombrose, 06340 Nice (France)
* INSERM U40, U.E.R. Médecine,
Avenue de Valombrose, 06340 Nice (France)

Résumé

Des hépatocarcinomes sont induits chez le muge, Mugil capito L., par l'addition de 100 mg/l de dimethylnitrosamine dans le milieu d'élevage.

L'administration de l'hépatocancérogène entraîne l'apparition d'îlots d'hépatocytes déficients en certaines enzymes dont l'ATPase et la Glucose-6-Phosphatase. Ces îlots altérés sont la première étape de l'hépatocancérogénèse et ces variations histoenzymologiques sont corrélées avec les modifications histopathologiques qui conduisent au développement d'un hépatocarcinome.

L'hépatocancérogénèse chimique chez les poissons marins est peu étudiée. Le muge, apparemment très sensible aux composés cancérogènes dissous dans le milieu, représente un bon modèle de détection histo-cyto-physiologique de ces polluants et/ou peut être utilisé dans des tests de cancérogénicité à court terme.

*
* *

Introduction

Les données expérimentales concernant l'action des nitrosamines dans le milieu marin se limitent à quelques études de toxicité (FERRARO et al., 1977). Dans le cadre du programme commun coordonné sur la pollution en Méditerranée, FAO (CGPM)/PNUE, il a paru intéressant d'étudier l'hépatocancérogénicité de la dimethylnitrosamine (DMN) chez une espèce représentative des zones polluées, le muge (Mugil capito L.).

L'évolution du processus de cancérisation est décrit par une étude anatomopathologique séquentielle des foies de muges exposés à la DMN. De plus. certaines techniques histoenzymologiques, qui n'ont jamais été utilisées chez les poissons marins, permettent d'étudier les stades précoces de l'hépatocancérogénèse et d'évaluer le pouvoir d'initiation des nitrosamines. Les marqueurs suivants ont été plus particulièrement analysés : la glucose-6-phosphatase (G-6-Pase), la gamma-glutamyl-transpeptidase (Y GTase) et l'adénosine triphosphatase (ATPase).

Matériel et Méthodes

De jeunes muges, capturés dans le port de Monaco, sont répartis dans des aquariums en circuit fermé. Après une période d'acclimatation de 4 semaines, la dimethylnitrosamine (DMN) est administrée par dilution dans le milieu à la concentration de 100 mg/l d'eau de mer.

Les autopsies sont effectuées à 7, 15, 30, 50, 60, et 90 jours d'esposition à la DMN. Le foie, rapidement prélevé et pesé, est divisé en deux fragments : l'un est destiné à l'étude histopathologique, et l'autre à l'étude histoenzymologique.

DMN CONTINUE		CLARIFICATION	STÉATOSE	CÉROÏDES	NÉCROSE	RÉACTION INFLAMMATOIRE	PROLIFÉRATION DES CANAUX BILIAIRES	CELLULES NÉOCANALAIRES	RÉGRESSION CANALAIRE	CANAUX DYSTROPHIQUES	CELLULES BASOPHILES	HÉPATOCYTES HYPERTROPHIÉS	DILATATIONS KYSTIQUES	HÉPATOCARCINOME
7 JOURS	P 1	*		*		*								
	P 2	*		*	*									
	P 3	*	*	*		*								
	P 4	***	**	*										
	P 5	*	*	*	*	*								
15 JOURS	P 6	***	*	**	*	*	*	*			*			
	P 7	**	**	***		**	*	*	*		*			
	P 8	*	**	***	**	**	**	**	*	*				
	P 9			**	*	*	**	*	*	*				
	P 10		*	*		*								
30 JOURS	P 11	**		***	*	*	**	*	**					
	P 12			***	*	*	**	**	*					
	P 13	***		***	*	*	**	*		*				
	P 14	**		***	*	**	**	*	*	*	*			
50 JOURS	P 16			***		**	***	**	**	*				
	P 17	**		*		**					**			
	P 18	**	**	***	*		*	**	**	**		*	*	*
60 JOURS	P 20	**	**	***	*		*	**	**	**		**	**	*
	P 21	**	**	***	*	*	*	**	*	**	*	**	**	*
75 JOURS	P 22	**	*	***	**		*	**	*	**	**	**	**	*
	P 23	**	*	***	*		*	**	*	**	**	**	**	*
	P 24	**	*	***	*	*	*	*	*	**	**	**	**	*
90 JOURS	P 25	**	*	***		*	*	**	**	**		**	**	*

Figure 1. - Etude anatomopathologique et histologique du foie de Mugil capito L. (Exposition continue à 100 mg de DMN/ litre d'eau de mer).

Résultats

1. Etude anatomopathologique séquentielle du foie (Figure 1) :

Témoins : Chez les muges témoins, aucune modification du tissu hépatique n'a été observée.

Traités : Dès les premiers jours d'exposition à la DMN, une clarification surtout périportale s'observe dans le parenchyme. De nombreux hépatocytes contiennent des pigments céroïdes. L'aspect congestif du foie et les réactions inflammatoires caractérisent la souffrance cellulaire. Simultanément, des zones de stéatose se développent et traduisent un dérèglement du métabolisme lipidique. Parallèlement à ces lésions dégénératives, les premiers signes de régénération hépatique apparaisent au quinzième jour avec une prolifération des canaux biliaires. Les cellules néocanalaires se différencient en néohépatocytes constituant des images de régression canalaire. L'hyperbasophilie caractérise certains hépatocytes à 50 jours. C'est à cette date que le premier hépatocarcinome est observé (échantillon P 18).

Au cours des trois dernières autopsies (60, 75, et 90 jours) tous les individus sont porteurs d'hépatocarcinomes.

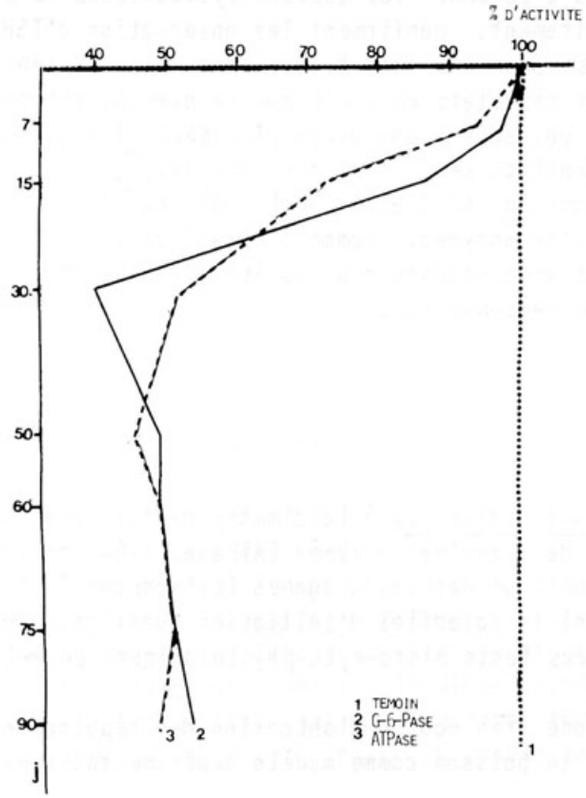


Figure 2. - Etude histoenzymologique. Variations des activités de la G-6-Pase et de l'ATPase du foie de Mugil capito L. (Exposition continue à 100 mg de DMN/ litre d'eau de mer).

2. Etude histoenzymologique (Figure 2)

Témoins : Avec la coloration spécifique de l'adénosine triphosphatase (ATPase), le tissu hépatique montre une réaction homogène granulaire des hépatocytes et une coloration très positive de la paroi des canaux biliaires. L'activité de la glucose-6-phosphatase (G-6-Pase) est observée uniformément et exclusivement dans les hépatocytes. La gamma-glutamyl-transpeptidase (γ GTase) n'a pas été mise en évidence dans le foie des muges témoins.

Au cours de cette expérimentation, aucune modification de l'activité de ces enzymes n'a été révélée dans le lot des témoins.

Traités : Dès la première semaine d'administration de la DMN, des groupes d'hépatocytes déficients en ATPase et en G-6-Pase sont observés. Le nombre de zones déficientes en activité enzymatique augmente rapidement après le huitième jour avec une intensité de coloration allant de légère à nulle. L'augmentation de ces zones, réparties dans tout le parenchyme, se poursuit jusqu'au trentième jour pour atteindre une valeur relativement constante, voisine de 50 % (Figure 2), jusqu'à la fin de l'expérimentation.

La technique de RUTENBURG (1969) qui met en évidence la γ GTase d'une manière spécifique chez les mammifères, n'a pas permis de révéler la présence et/ou l'activité de cette enzyme chez le Mugil capito L..

Discussion

Chez les poissons témoins aucune modification du tissu hépatique n'a été observée.

Chez les poissons exposés à la DMN, les lésions cytotoxiques du parenchyme hépatique, décrites dès les premiers jours de traitement, confirment les observation d'ISHIKAWA et TAKAYAMA (1979) et de SIMON et coll. (1980). Les premiers hépatocarcinomes apparaissent rapidement après 50 jours d'exposition à la DMN. Ces résultats montrent que la dimethylnitrosamine, qui est un puissant hépatocancérogène chez divers poissons d'eau douce (ISHIKAWA et al., 1975), induit chez le poisson marin, Mugil capito L., une hépatocancérisation très rapide.

En accord avec les travaux de LAPIS et SIMON (1983) chez le poisson d'eau douce Lebistes reticulatus, l'activité de certaines enzymes, comme l'ATPase et la G-6-Pase, a été mise en évidence. Au contraire, la gamma-glutamyl-transpeptidase n'a pas été détectée et ce résultat correspond à l'observation de SIMON (communication personnelle).

Conclusion

L'exposition du muge, Mugil capito L., à la dimethylnitrosamine montre que :

- d'une part, l'activité de certaines enzymes (ATPase, G-6-pase), auxquelles il faudrait ajouter les enzymes d'activation métabolique des cancérogènes (cytochrome P450 dépendantes, ...) peut permettre d'évaluer expérimentalement le potentiel d'initiation tumorigène de tel ou tel composé naturel ou synthétique et/ou d'établir des tests histo-cyto-physiologiques permettant de détecter la présence d'un polluant dans le milieu.

- d'autre part, la période très courte d'obtention des hépatocarcinomes, entre 50 et 90 jours, souligne l'intérêt d'utiliser le poisson comme modèle expérimental dans des tests in vivo de cancérogénicité à court terme.

Références

L.A. FERRARO, R.E. WOLKE, P.P. YEVICH, (1977). - Acute toxicity of water-borne DMN to Fundulus heteroclitus. J. Fish Biol., 10.

T. ISHIKAWA, S. TAKAYAMA, (1979). - Importance of hepatic neoplasms in low vertebrate animals as tool in cancer research. In : "Liver Carcinogenesis". (K. LAPIS, J.V. JOHANNESSEN, eds.) HPC, 367-381.

K. LAPIS, K. SIMON, G. TESTIN (1983). - Environmental carcinogens in aquarium fishes. Med. Biol. Environ. n° 13, Mai-Décembre.

A.M. RUTENBURG, H. KIM, J.W. FICHBEIN, J.S. HANKER, H.L. WASSERKRUG, A.M. SELIGMAN, (1969). - Histochemical and ultrastructural demonstration of gamma-glutamyl-transpeptidase activity. J. Histochem. Cytochem. 17 : 517-526.

K. SIMON, L. TAJTI, K. LAPIS (1980). - Hepatic lesions in Lebistes reticulatus induced by N-nitrosodiethylamine. In "N-Nitroso-compounds : Analysis, Formation, and Occurence". (E.A. WALKER, L. GRICIUTE, M. CASTAGNERO, and M. BORZSONYI, eds.) IARC Sci. Publi. Monograph. N° 31, Lyon.

T. ISHIKAWA, T. SHIMAMINE, S. TAKAYAMA (1975). - Histologic electron microscopy observations on DENA-induced hepatomas in small aquarium fish (Oryzias latipes). J. Natl. Crucer Inst. 55.

ASSESSMENT OF THE VALUE OF FISH SPECIES DIVERSITY AS INDICATOR OF POLLUTION IN THE N. EVOIKOS GULF (GREECE)

by

C. PAPACONSTANTINOU
Institute of Oceanographic and Fisheries Research
GR-166 04, Hellinikon (Greece)

Abstract

Samples of demersal fish were taken every three months in a polluted sea area in N. Evoikos gulf (Greece) between June and December 1983. The pollution is due to slag particles of various sizes coming from the smelting of iron-nickel alloy. To compare habitats the study area was divided into three ecological regions according to the distance from the location where slag is discharged. Measurements were taken as regards the number of fish species and that of individuals, fish species diversity indices, species richness, evenness, and the findings reinforce the hypothesis that the three regions could be regarded as an ecological unity. In general the layer of the slag had no environmental impact on the fish fauna of the area, though it seemed to have affected fish production.

*
* *

Introduction

There are few published accounts of studies using fish diversity as a pollution indicator (KATZ and GAUFIN 1953; TSAI, 1968; BECHTEL and COPELAND, 1970) although fishes have been used to provide information about water quality for many years. The purpose of this contribution is to study the pollutional effects of discharge of the slag particles coming from the smelting of iron-nickel alloy in the N. Evoikos gulf, investigating the fish fauna by utilizing various indices of community structure.

Material and methods

To compare habitas, the study area was divided into three ecological areas A, B and C, according to the distance from the location where slag is discharged. A (st. 1, 2 and 4) is characterized by the presence of a layer of slag, C (st. 7, 9 and 10) is far away from the first and not polluted, whilst B (st. 3, 5 and 6) lies between them (figure 1). The samples were taken at three-month intervals, from June to December 1983, using a 400 HP fishing trawler. Each station was trawled for 30 min. The study was based on the demersal fish fauna which are strongly influenced from the sea

bottom where slag is discharged. Computations were made at each station as regards the number of fish species (S) and individuals (N), the Brillouin (H) and Shannon-Weaver (H-) species diversity index, the evenness factor (J) and the species richness (SP) (PIELOU, 1966).

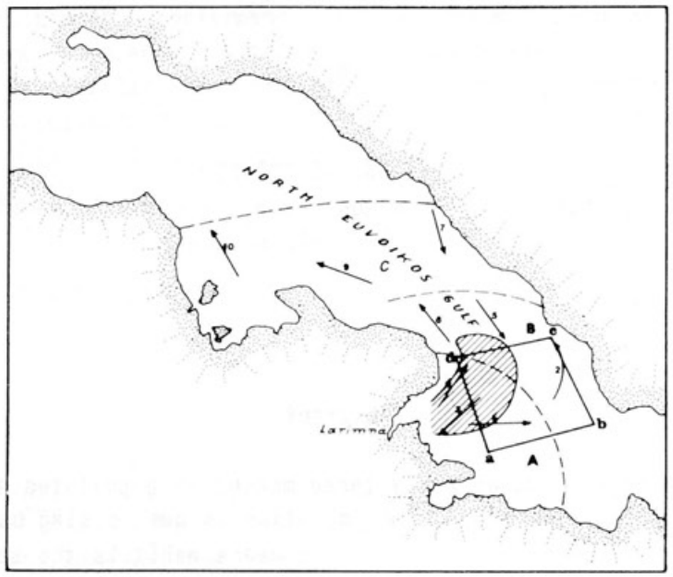


Fig. 1. N. Evoikos gulf and sampling stations. Letters indicate ecological areas (A, B and C), a, b, c, d region where the discharge of slag is allowed. [////] presence of slag on the sea bottom.

Results

The fish collected during this study totaled 17,566 individuals, representing 52 demersal species, weighing 656.6 kg. The June catch consisted of 2,771 individuals weighing 98.7 kg. Twenty-six species were taken. The September catch showed a marked increase in both number of fish and total weight, with 45 species. The number of individuals was 6,840, of weight 203.4 kg. The November catch was larger in number of fish and total weight, 7,955 individuals and 354.5 kg, respectively. Forty-five species were present in the catch.

The number of fishes (N) taken from area A were substantially lower than in the area B and C in June and September and increased abruptly in November (figure 2 b). The number of species (S) in area A was longer than in C and greater than in B (figure 2 a). A detailed breakdown by season of the N and S data for all stations during the whole year of trawling is shown in figure 2 a, b. Peaks of N and S at the majority of the stations occurred during November, with seasonal changes in the number of individuals and species related to the fishing status of the gulf: the trawl fishing in the N. Evoikos gulf is prohibited between the middle of November and the end of March. Winter and autumn peaks of S were evident at all stations. Area A stations had consistently higher numbers of S than in areas B and C (except st. 10), although such differences were minimal during early summer (June). The reduction of S at st. 7 throughout the whole year is due to its greater depth (75 m). On the other hand, the N taken from the stations of area A were substantially lower than those from stations B and C. Comparing the sampling CPUE (catch per effort, kg/h) for each station investigated, it was concluded that these data would indicate that the environmental stress due to the slag particles has a negative effect on the fish catches, whilst its influence on the fish fauna is not certain (figure 2c).

The Shannon-Weaver and Brillouin index at each station exhibited a "dampened" seasonal cycle, reflecting the changes in relative abundance of species (figure 3). Peaks usually occurred at the stations of area A all the year round. Consistent reduction in both parameters was noted at st. 7,

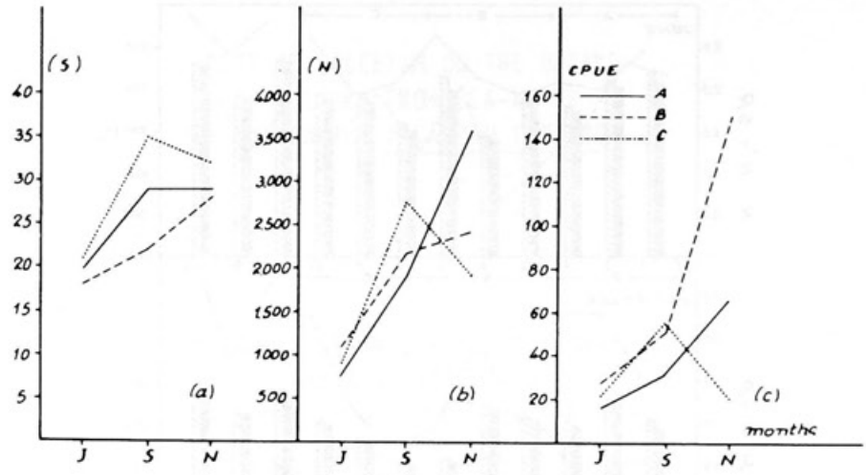


Fig. 2. Three-seasons comparisons of number of species (a), number of individuals (b) and CPUE (c), of fish taken from three areas.

together with a periodic reduction at st. 3, 5 and 6. Mean values suggest these relationships, but the statistical analysis does not confirm them.

The general behaviour of the evenness factor was consistent with the Shannon-Weaver and Brillouin index at the majority of the stations. Its mean value presented a slight increase in areas not severely affected by slag (area B). At most stations, there were seasonal and geographical changes in species richness. Peaks usually occurred in November, whilst reductions appeared in June. The maximum values of species richness were found at the slag-affected st. 3, 4 and 5.

Discussion

No single index is sufficient to indicate water quality impact on estuarine and benthic communities. It is usually acknowledged that stress conditions in benthic systems are related to low species diversity. Such areas are also characterized by a high level of dominance and high productivity (BOESCH, 1972).

In the most severely affected areas in the N. Evoikos gulf, there were relative reductions of N, CPUE, and a low increase of H, H-, J and SR, although species diversity indices were generally reduced in such areas. In areas between polluted and unpolluted systems, the relationship became far more complex. Species diversity was no longer useful as a measure of impact and actually showed moderate increases. In our case, there was a reduction of S, H, H- and a low increase in CPUE. On the other hand was a reduction of almost all the diversity indices in the unpolluted area (area C), except in those of st. 10. Species diversity (H, H-) and evenness (J) comparisons were of limited use in the analyses of data. The lack of significant statistical correlation between areas A, B and C, suggests that the study area must be regarded as an ecological unity.

Because of the reduced regional distribution of the slag in the N. Evoikos gulf, the mobility of fishes and their acknowledged tendency to avoid certain forms of pollution, it is possible that the distribution of fish in the gulf is partly dependent on their behavioural reaction to the slag.

In general, there was a relative consistency in the quantitative and qualitative aspects of the fish distribution that reflected not only the levels of the slag, but also various natural factors and the fishing status of the gulf. The latter parameter is very important, because the N. Evoikos gulf is a closed sea area in which the use of all fisheries techniques is allowed, despite the negative results on the fish populations.

However, despite the previous findings about the ecological status of the gulf, many of the fish

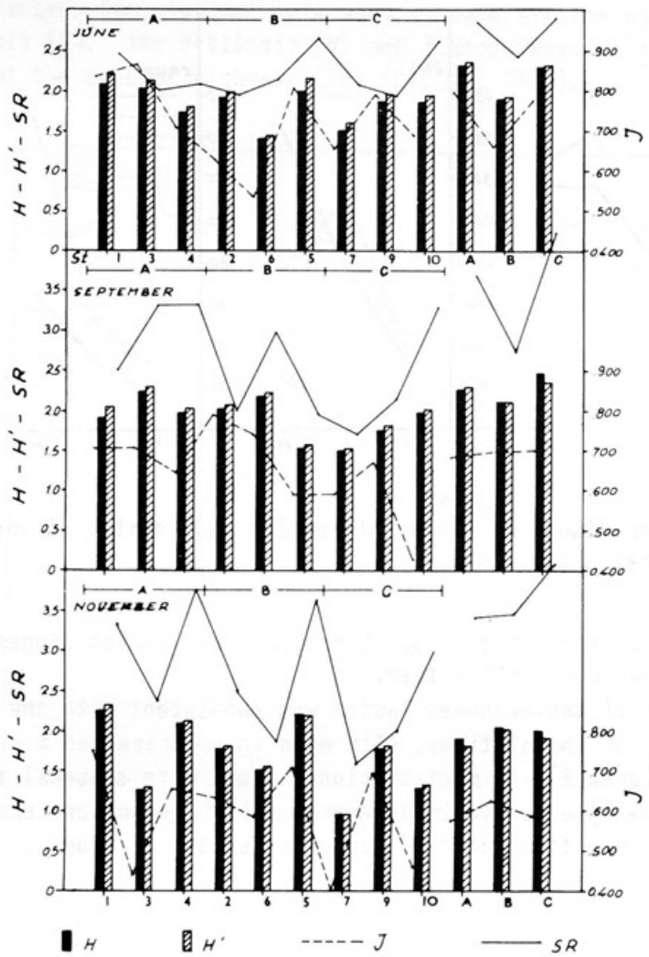


Fig. 3. Brillouin (H) and Shannon-Weaver (H-) species index, evenness factor (J) and richness index (SR) for each station for three seasons.

captured in areas A and B (especially in A) were in poor physical condition. In many of them, the caudal fin was partially damaged, and in some, the filamentous portions of the pectoral and pelvic fins were also severely affected. These effects are due to the mechanical action of the slag.

References

BECHTEL T.J. and B.J. COPELAND (1970). - Fishes species diversity indices as indicators of pollution in Galveston bay, Texas. Contr. Mar. Science 15: 103-132.

BOESCH D.F. (1972). - Species diversity of marine macrobenthos in the Virginia area. Chesapeake Sci. 13: 206-212.

KATZ M. and A.R. GAUFIN (1953). - The effects of sewage pollution on fish population of a midwestern stream. Trans. Am. Fish. Soc. 82: 156-165.

LIVINGSTON R.J. (1975). - Impact of kraft pulp-mill effluents on estuarine and coastal fishes in Apalachee Bay, Florida, U.S.A. Mar. Biology 32: 19-48.

PIELOU E.C. (1966). - The measurement of diversity in different types of biological collections. J. Theor. Biol., 13: 131-144.

TSAI, CHU-FA (1968). - Effects of chlorinated sewage effluents on fishes in Upper Patuxent river, Maryland. Chesapeack Sci. 9: 83-93.

EFFECT OF SELENIUM ON THE UPTAKE OF MERCURY FROM SEA-WATER BY THE SHRIMP PALAEMON ELEGANS

by

Mirjana SKREBLIN*, Peter STEGNAR**
and Ana PROSENC**
* Center for Marine Research Rovinj
"Rudjer Boskovic" Institute
52210 Rovinj, Yugoslavia
** "Edvard Kardelj" University
"Jozef Stefan" Institute
61000 Ljubljana, Yugoslavia

Abstract

Recently it has been found that selenium exerts a protective effect on mercury toxicity and that these elements coaccumulate in some marine organisms.

In the present study the effects of selenium on the uptake of ^{203}Hg from sea-water and on mercury release from the organism were investigated in the shrimp Palaemon elegans in laboratory conditions.

Shrimps which were previously treated with an elevated concentration of stable selenium in sea-water have shown significantly lower concentration factors for mercury than those from the control group during the first phase of accumulation preceding the steady state.

Selenium pretreatment had no effect on the ^{203}Hg release rate.

Contents of mercury and selenium in animals were determined by the method of neutron activation analysis.

*
* *

Introduction

There is evidence that selenium can interact with other elements and may have a detoxifying effect on certain heavy metals. notably mercury. In the marine environment, selenium and mercury have been found to coaccumulate in mammalia, birds and fish. Not very much data have been published on the interaction between these two elements in marine invertebrates. The purpose of this study was to determine the effect of elevated selenium concentration on mercury accumulation rate in the shrimp Palaemon elegans.

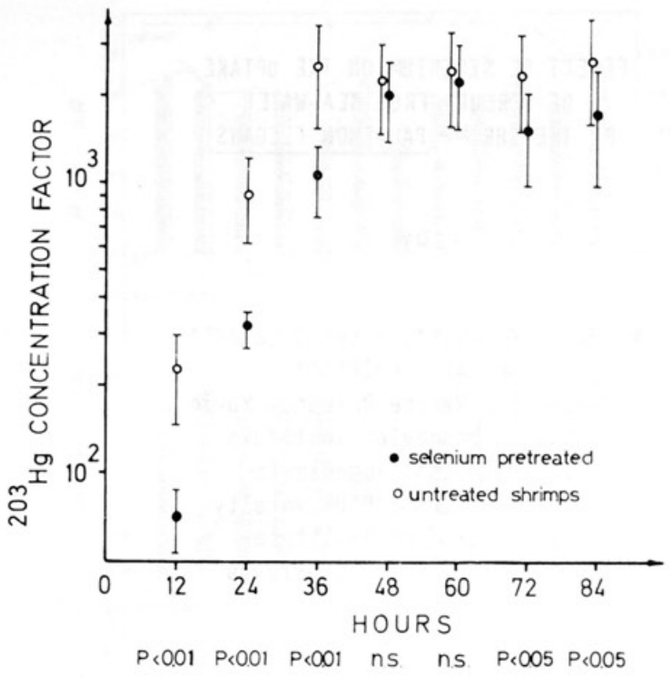


Fig 1. Accumulation of ^{203}Hg by Palaemon elegans.
Points represent a mean ±1 G of 9 to 12 individuals.

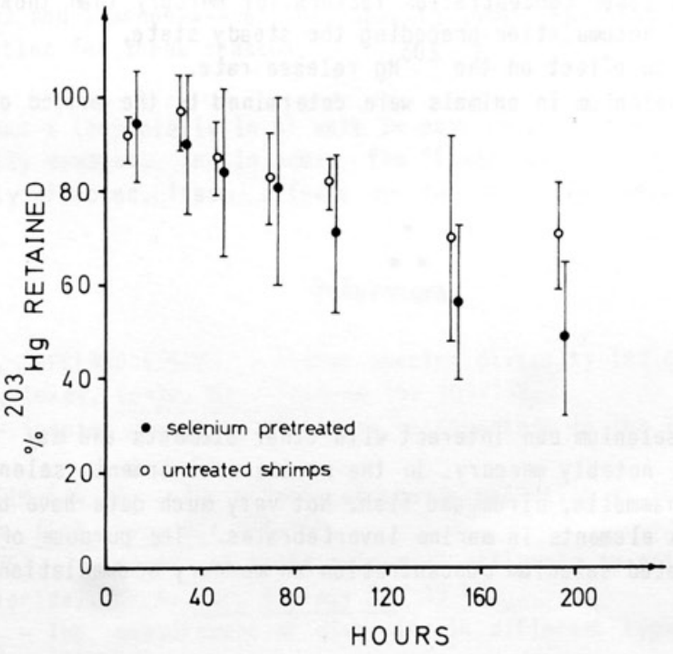


Fig 2. Elimination of ^{203}Hg from Palaemon elegans.
Points represent a mean ± 1 G of 3 to 10 individuals.

Material and methods

Adult, intermoult shrimps Palaemon elegans were collected on the Istrian Adriatic coast near Rovinj.

The accumulation of mercury ($^{203}HgCl_2$; NEN) was carried out in glass beaker containing 2 l aerated sea-water (12° C), 5.6 kBq ^{203}Hg/ml (specific activity = 44.8 kBq/ug) and 9-12 specimens (0.75±0.20 g) each. The isotope was added to the medium 24 hours before the animals were put in. Two groups of shrimps were used: one was kept for a month in running sea-water containing 300 ug Se/l, while the other was control. Before the uptake experiment started five animals from each group were homogenized and analysed for mercury and selenium contents by NAA method. Following the 86 hours of ^{203}Hg bioaccumulation phase, both groups of shrimps were transferred to clean running sea-water to measure the loss of incorporated ^{203}Hg.

Radiometric determinations were made with a Nuclear Chicago gamma scintillation counter connected with a Hershaw well type crystal.

Total mercury and selenium were determined simultaneously by NAA using a volatilization technique (BYRNE and KOSTA, 1974) after samples had been irradiated at the Triga Mark II reactor of the "Jozef Stefan" Institute, Ljubljana.

The statistical analysis was carried out using Student's t-test or the method by Cochran and Cox.

Results and discussion

Background mercury and selenium concentrations for the control group of shrimps were for the whole body 0.053 ug Hg/g and 0.44 ug Se/g (fresh weight), while higher values for both elements were found in the selenium pretreated shrimps: 0.11 ug Hg/g and 0.76 ug Se/g.

Figure 1 shows the accumulation of ^{203}Hg in these two groups of shrimps. During the first 36 hours the uptake of mercury by the pretreated shrimps was significantly reduced in comparison with the control group. This phase was followed by 24 hours' accumulation where differences between the two groups disappared, and finally, in the last 24 hour period of bioaccumulation a steady state was reached in the control group, while a decline of the concentration factor was observed in the pretreated group. These data suggest that the interaction of mercury and selenium in the organism is a time-dependent process and consequently different results may be expected at different uptake intervals. In this context, our results agree with those of KIM et al. (1977) who found that fish which were pretreated with selenium had accumulated significantly less mercury than those which were not pretreated. RUDD et al: (1980) suggest that an elevated concentration of selenium in an experimental aquatic ecosystem appeared to retard the rate of mercury bioaccumulation by fish and crayfish.

Reduced mercury toxicity in the presence of selenium could be partially explained by a retarded rate of mercury accumulation from the medium containing high levels of mercury. Thus, median lethal time for the shrimps Palaemon elegans pretreated for four days with an elevated concentration of selenium was delayed compared with the group which was not pretreated (LUCU and SKREBLIN, 1981).

Results of the ^{203}Hg loss experiment are expressed by the percentage of retained ^{203}Hg in organisms and presented in figure 2.

Statistical analysis indicated that the differences between the two groups were not significant at $P = 0.05$. On the contrary, a significantly decreased release of ^{203}Hg compared with the control group was observed in selenium-pretreated P. elegans, when shrimps had been successively injected with SeO_2 and $^{203}HgCl_2$ (LUCU and SKREBLIN, 1981). By comparing both experiments, the lack of effect in the present study has probably resulted from the difference in the intake route of the elements.

Acknowledgements

This work received financial support the Self-management Community of Interest for Scientific Research of S.R. Croatia and the Self-management Community of Interest for Scientific Research of S.R. Slovenia, and U.N.E.P. (MED POL - Phase II).

References

BYRNE A.R. and L. KOSTA (1974). - Simultaneous neutron activation determination of selenium and mercury in biological samples by volatilization. Talanta, 21, 1,083-1,090.

KIM J.H., E. BIRKS and J.F. HEISINGER (1977). - Protective action of selenium against mercury in northern creek chubs. Bull. Environ. Contam. Toxicol 17, 132-136.

LUCU C and M. SKREBLIN (1981). - Evidence on the interaction of mercury and selenium in the shrimp Palaemon elegans. Mar. Environ. Res. 5, 265-274.

RUDD J.W.H., M.A. TURNER, B.E. TOWNSEND, A. SWICK and A. FURUTANI (1980). - Dynamics of selenium in mercury contaminated experimental freshwater ecosystems. Can. J. Fish. Aquat. Sci. 37, 848-857.

MONITORING LETHAL AND SUBLETHAL EFFECTS OF WASTE WATER FROM EL-TABIA (ALEXANDRIA) ON THE CRUSTACEA ARTEMIA SALINA

by

R. LAENGE and Y. HALIM
University of Alexandria Research Center
Alexandria (Egypt)

Introduction

More than 2 million m^3/day of mixed industrial waste water from paper mills, food canning and textile industry, together with domestic waste water and agricultural drainage are released from El-Tabia pumping station into Abu-Kir Bay east from Alexandria.

The effluent water strongly affects life in the coastal zone of the Bay (HALIM, 1984 ; HALIM et al., 1985). For the study of the environmental effects of this waste water the crustacea Artemia salina was used. Its relatively short life cycle and ease in culturing give it great advantages for investigations of lethal and sublethal effects of water pollution.

Artemia nauplii have been used as test organisms for toxicity measurments by various authors SORGELOOS et al., 1975, 1978 ; VANHAECKE et al., 1980) and in particular for detergents and dispersents (CASTRITSI - CATHARIOS et al., 1980, 1982). The rate of hatching from eggs was found to show too great a variability to be of any use in toxicity measurements, as also found by ourselves. In the present work, the life-cycle of Artemia salina has been followed over three generations raised in three dilutions of waste water. This work was done within the frame of the Aquatic Environment Pollution Project (EGY/073/058).

Material and methods

Water from the "El-Tabia" pumping station near Alexandria was sampled and adjusted to 36,5 ‰ salinity. The water contains mainly a heavy load of particulate materials, like cellulose and lignin particles, together with high phosphate, ammonia, COD, BOD and alkalinity. A detailed analysis is given in HALIM et al. (1985). 80 adult Artemia salina were taken from a stock, which has been developed from dried eggs in clean seawater, and equally distributed into glass jars. The glass jars contained a mixture of sea water and effluent water up to different proportions, 0 %, 30 % and 60 % all of them adjusted to a salinity of 36.5 ‰. For each concentration one jar with 200 ml and one with 400 ml containing 10 specimen each used. The jars were moderately aerated without disturbance of the swimming performance of Artemia, and as a food source, phyto-plankton from cultures containing mainly one species of a green algae and a flagellate was added. The water temperature fluctuated between 25° and 27° C.

Results

a. - With regards to the short term effect of the effluent water on Artemia salina, the death rate has been monitored over a time of five days. There were distinct and significant differences between the various dilutions of effluent water (Fig. 1).

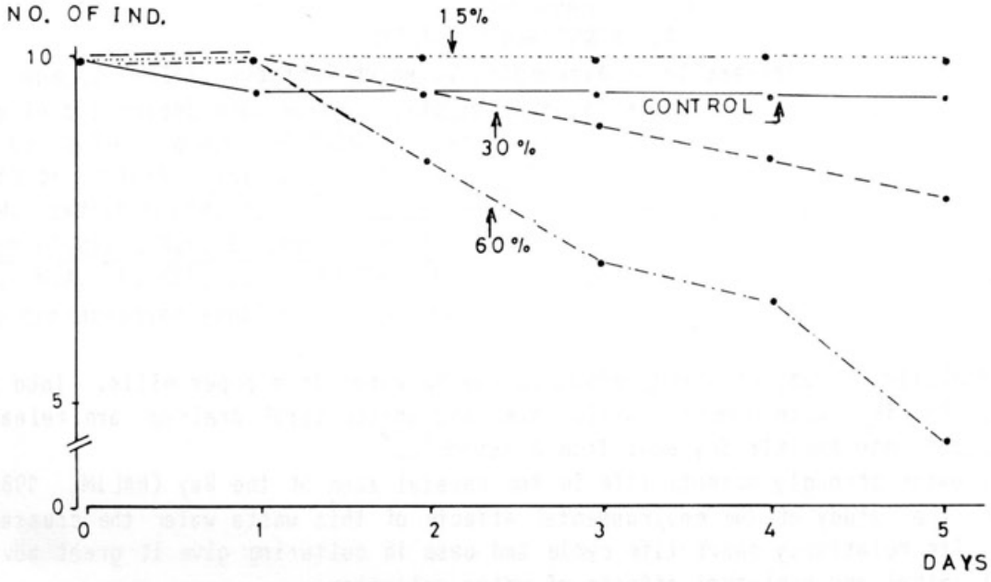


Figure 1. - Death rate of A. salina in different waste water dilutions over 5 days.

The control and the 15 % concentration showed no significant death rate differences, while the 30 % dilution killed more than 25 % of the introduced organisms in five days. The concentration of 60 % had a lethal effect on 50 % of the animals within five days.

b. - With regards to the long term effects of the effluent water, the development and fertility of Artemia salina has been studied over a period of two months. The following aspects have been observed in this study:

I. - Hatching and development of eggs and larvae in the different concentrations of effluent water.

II. - Frequency of fertility of one generation of adults and the survival rate of the larvae.

III. - Development of a new generation until it became fertile.

I. - Hatching and development of eggs in the different dilutions of effluemt waters.

Eggs hatched in all different dilutions of the effluent waters. No significant differences could be observed in the number of developed larvae compared to the number of introduced eggs.

II. - Frequency of fertility of one generatiom of adults and the survival of the larvae.

In the control (0 %), in the 15 % and in the 30 % concentration the frequency of reproduction was Ca. 25 days. There has been no significant differences during the 2 months observation period between those effluent water concentrations. Since after 20 days no adult Artemia survived in the 60% concentration no reproduction took place here.

The survival rate was proved by leaving 40 larvae in each jar. In the control about 25 % of them survived until the end of the study (Fig. 2). In the 15 % dilution 37,5 % survived, while the 30 % concentration showed a similar number of individuals as the control.

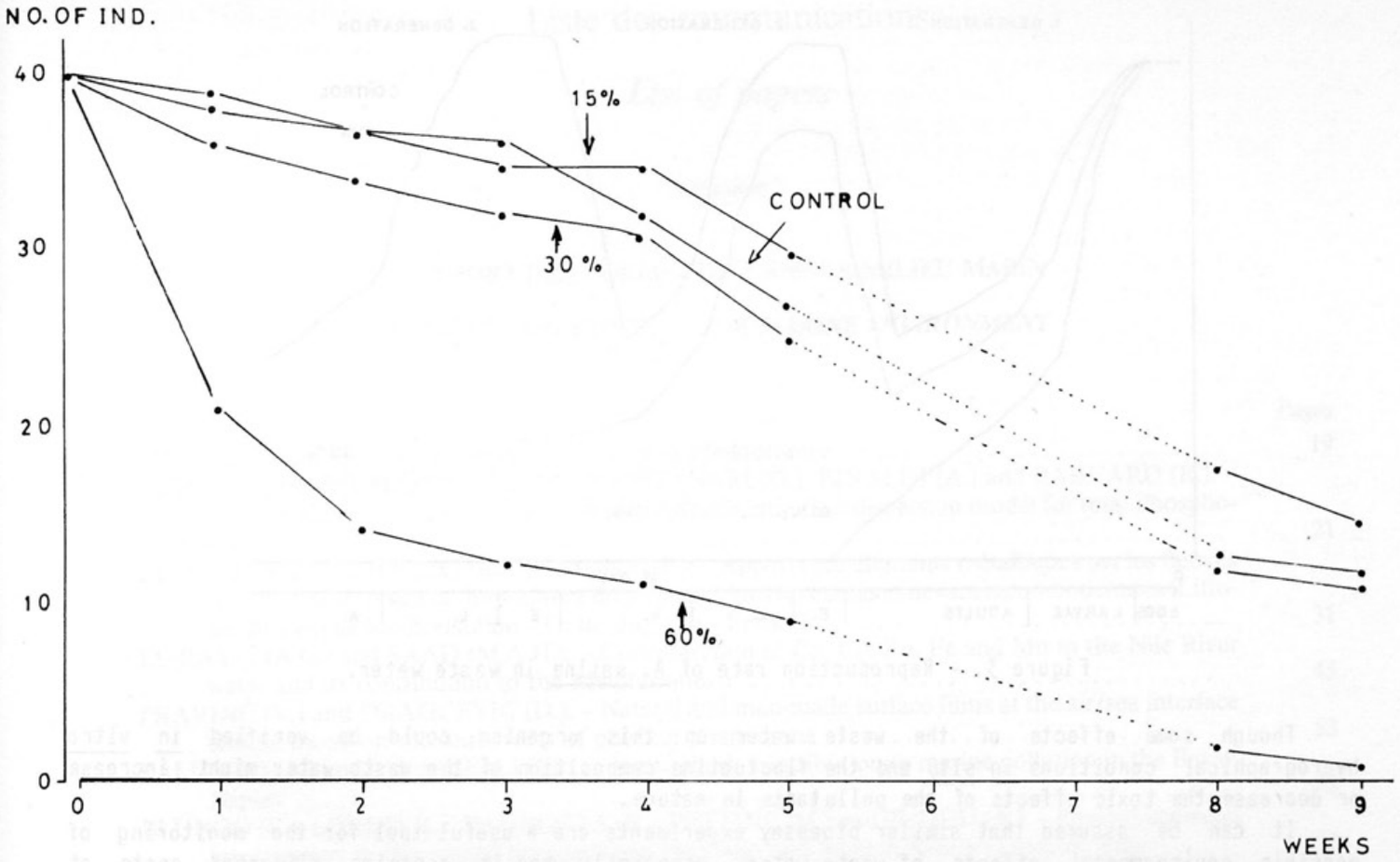


Figure 2. - Survival rate of Artemia nauplii in different waste water dilutions over 3 months.

From the 40 larvae introduced into the 60 % dilution only 1 survived until the end of the experiment.

III. - Development of a new generation until it became fertile.

Developing Artemia larvae, which have been raised from the eggs laid by the first generation raised in different dilutions of effluent water (expect the 60 % concentration) were separated from their parent generation and introduced into 0 % , 15 % and 30 % concentration of effluent water.

They became fertile in the control and in the 15 % concentration but not in the lower dilution. The larvae hatched mainly 42 to 46 days after the hatching of their parent generation in both jars. No distinction could be made of the development between the control and the 15 % concentration (Fig. 3).

Conclusions

The results of these experiments can be summarized as follows : the short term effects of the effluent water can be measured by the LD50 rate, which occured in our case in the 60 % concentration after 5 days. That means only a relatively high concentration of this effluent water from El-Tabia has lethal effects on the test organisms in a short time periods

Nevertheless longer term observations show clearly that even lower concentrations of the effluent water have severe lethal and sublethal effects.

The fertility of Artemia was significantly affected, and exposure times of several weeks or months to medium level concentrations of the waste water had finally lethal effects too.

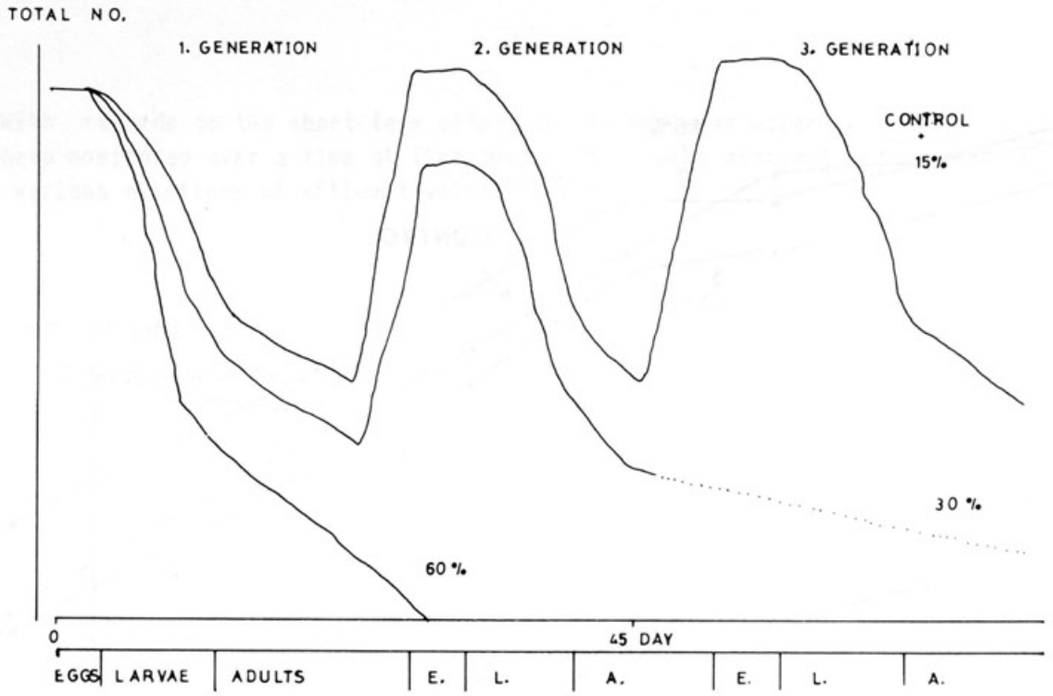


Figure 3. - Reproduction rate of A. salina in waste water.

Though some effects of the waste water on this organism could be verified in vitro hydrographical conditions in situ and the fluctuating composition of the waste water might increase or decrease the toxic effects of the pollutants in nature.

It can be assumed that similar bioassay experiments are a useful tool for the monitoring of possible environmental effects of waste water, especially when it contains different sorts of pollutants, which may have no clear hazardous influence on the environment when taken single, but do so when combined with other substances.

References

1. - CASTRITSI - CATHARIOS J., A. KARKA and A. MORAITI, (1980). - Toxicité de détergents et d'un dispersant sur l'Artemia salina L. Rev. Trav. Inst. Pêches Marit. 44 (4) :355-364.
2. - CASTRITSI - CATHARIOS J., V. KIORTSIS et M. MORAITI-IOANNIDOU, (1982). - Influence toxique de trois détergents et d'un dispersant sur Artemia (DL48/50). VIes Journées Etud. Pollutions, Cannes, C.I.E.S.M., 1982.
3. - HALIM Y., (1984). - First technical report. Aquatic Environmental Pollution Project EGY/73/058. UNARC, University of Alexandria.
4. - HALIM Y., H.H. SALEH and A. SALEM, (1985). - Environmental conditions in Abu-Kir Bay East of Alexandria Downstream from "El-Tabia" effluent. Impact on the Fish associations in the coastal zone. Proc. FAO/UNEP Meeting on the Effect of Pollution on Marine Ecosystems. Blanes, (Spain) 7-11 Oct. 1985.
5. - SORGELOOS P. and G. PERSOONE, (1975). - Technological improvements for the cultivation of invertebrates as food for fishes and crustacea. II. Hatching and culturing of the brine shrimp Artemia salina L. Aquaculture, 6 : 303-317.
6. - SORGELOOS P., C. REMICHE, VAN DER WIELEN and G. PERSOONE, (1978). - The use of Artemia nauplii for toxicity tests - a critical analysis. Ecotoxicology and Environmental Safety 2 : 249-255.
7. - VANHAECKE I., G. PERSOONE, C. CLAUS and P. SORGELOOS, (1980). - Research on the development of a short term standard toxicity test with Artemia nauplii. In : The brine shrimp Artemia. Vol. I, Wetteren, Belgium, 263-285.