1. INTRODUCTION

The workshop was held in Marseilles, France, from 18 to 20 April 2002, in the splendid Napoleonic Palais du Pharo overlooking the harbour's entrance. Twenty-two scientists from eighteen countries, all volunteer to join the project, participated in the meeting, an encouraging sign considering the rather extensive geographic distribution of the initial core group (see Fig. 1). The list is not exhaustive, and additional institutes are expected to join the project in the future, completing the geographic coverage.

In his welcoming remarks, the Director General of CIESM, Frédéric Briand, presented the scope and objectives of the meeting, stressing the needed development of synergies based on the use of common procedures and methods. He noted with appreciation the participation of the current and former Chairmen of the CIESM Committee on Marine Biogeochemistry, Nicholas Fisher and Scott Fowler, who would soon follow with keynote presentations on central biomonitoring issues. He then introduced with much appreciation the initiator and coordinator of the workshop, Hervé Thébault, who recalled the long path which had led to the present meeting.

At the origin, in the aftermath of the Chernobyl accident, a coordinated research program called GIRMED (Global Inventory of Radioactivity in the MEDiterranean Sea) was launched by CIESM during its 31st Congress held in Athens in 1988 (see Thébault *et al.*, 1998a). As other international programs at the time, it aimed at gathering data sets collected from many scientific institutions.

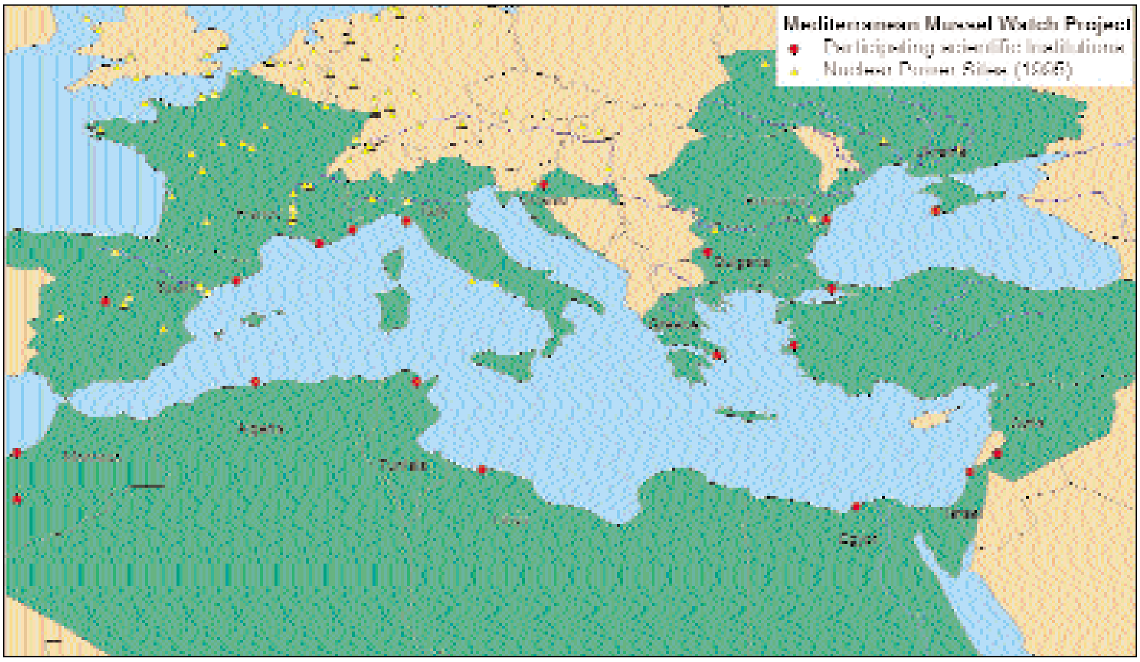


Fig. 1. Scientific institutions participating in the initial Mediterranean Mussel Watch monitoring network.

However, as the data were usually obtained from various indicator species, procedures and methods, this made direct comparison of results on spatial or temporal scales difficult. In addition interpretation of findings is sometimes unclear because baseline levels of some radionuclides are still missing or scarce, in particular in the eastern and southern parts of the Mediterranean.

Taking note of this context, Hervé Thébault, Scott Fowler and Frédéric Briand discussed in early 2001 the idea for CIESM to go further by organizing the production of monitoring data on a common basis and to set up an effective observation tool on an extended scale. This project was first presented and consolidated during a round-table on "Biomonitoring of marine trace contaminants" held in the course of the 36th CIESM Congress (Monte Carlo, September 2001). There followed a proposal to study the implementation requirements of a permanent Mediterranean Mussel Watch dealing with radionuclides and other trace contaminants. At the invitation of the CIESM Director, this technical and scientific workshop was called to discuss all aspects and feasibility of a project, which would serve as a starting point for a unique regional monitoring network in the Mediterranean basin.

The workshop unfolded as an open, interactive debate, allowing sharing of ideas and biomonitoring experience between participants; it progressively led to a consensus on the project issues and a technical scheme. The reader will note that the monitoring of conventional chemical contaminants, while discussed in the meeting, will become a central topic only in the second phase of the program.

2. RATIONALE AND BACKGROUND

2.1 An auspicious context

With increasing public awareness of the degradation of the marine environment comes increasing demand for appropriate remedial actions. Intolerance for possibly hidden or underestimated risks of contaminants for human health is growing, prompting considerable concern beyond those associated with the preservation of natural resources, habitats and biological diversity. Among the persistent pollutants present in marine waters, radionuclides invariably raise a strong emotional response from the public, although those scientists familiar with the issues usually consider that much of the worry over this problem is somewhat unwarranted (GESAMP, 2001). To address the increasing institutional demands for marine environmental quality standards, one thing at least is certain: coastal management requires more reliable, more accessible information on the existing baseline levels of trace contaminants as an essential tool for bottom-up control and regulation of emission rates.

The need for efficient monitoring of persistent contaminants is even more acute in closed systems like the Mediterranean and the Black seas, that are characterized by a long residence time of water masses. Among other things, the Chernobyl accident revealed that regional observations are critical, and that baseline levels of many important radionuclides were missing or scarce in large areas of the Mediterranean.

Successful monitoring networks are those attached to well-identified management objectives. The recent EU Water Framework Directive (OJEC, 2000) provides a favorable context as it projects in its first article "*for hazardous substances, the cessation of discharges, emissions and losses within 20 years, with the ultimate aim of achieving concentrations in the marine environment approaching background values for naturally occurring substances and close to zero for man-made synthetic substances*".

Although there is still room for debate (see Fowler, this volume), this implies that monitoring networks of trace contaminants must be established in european coastal waters – at least – even if there are only sub-minimal concentrations to be measured and even if, due to insufficient ecotoxicological knowledge, no associated risk can be well-defined at present.

2.2 Time for a change in radioecology

The Mediterranean Mussel Watch (MMW) project is designed to proceed with the monitoring of radionuclides first, integrating other trace contaminants next. This approach will mark a rupture with previous practices on at least two accounts. In the past, monitoring of radionuclides was performed by separate institutions, keeping the environmental assessment process quite distinct from other pollutants. It will be very valuable instead to track radionuclides as just another group of environmental contaminants that could be included in a common approach of risk assessment of chronic low-level exposure in a multipollution context (Hunter, 2001).

Further, until recently the main radiological protection paradigm has been that man is the most radiosensitive species in situations of long term exposure: if human health is adequately protected from ionizing radiation then the flora and fauna are assumed to be also protected. The principle of radiation protection is essentially based on dose to man with a strong focus on an energy-transfer model. But, at least for the marine environment, careful analysis indicates that this fundamental statement is based on little, if any, available scientific evidence (Baxter and Strand, 2000). Accordingly, there is now pressure to broaden the basis of radiological protection to include consideration of the effects on the flora and fauna as well (GESAMP, 2001).

2.3 From pioneer surveys to environmental quality standards

"Mussel Watch" programs obviously aim to monitor the pollution of coastal waters by taking samples from exposed biological "sentinels" (Goldberg *et al.,* 1978). This is based on the assumption that levels of trace contaminants accumulated in mussel tissues represent the time and space integrated value of these contaminants in the surrounding waters. Just as importantly, it is self-evident that concentrations of contaminants in mussel tissues reflect the biologically available (bioavailable) forms of these contaminants in a given region. Variations of contaminant levels in mussels may reflect the variations in water and particulate contaminant concentrations, although biological variability can also affect tissue concentrations. The kinetic parameters of the bio-accumulation process will usually attenuate the 'noise' generated by short term variability in the surrounding waters, which is advantageous for the detection of long-term trends.

Many reasons favour the selection of the mussel as a bio-indicator: mussels are widespread in coastal environments, sessile, easy to collect, tolerant to changes in environmental conditions and to high concentrations of many contaminants, rendering the measurement of contaminant concentrations technically simple.

This accounts for the use of mussels in monitoring programs worldwide since the 1970s (Jernelov, 1996), and in certain Mediterranean countries for more than 20 years (see extended abstracts, this volume). A rapid search in scientific literature databases using the "mussel watch" keywords shows that more than 50 papers per year still relate to this topic, which appears in more than 1000 web pages. Clearly the use of the mussel as a biological monitoring indicator has become standard practice in many national and international programs (UNEP, 1992).

As filter-feeder animals with gills that pump a lot of water, mussels can accumulate pollutants from both food and from the dissolved phase. For each contaminant, the final body burden is dependent on uptake from both sources, although the relative amount accumulated from each source can vary considerably among contaminants, including radionuclides (see Fisher, this volume). There are numerous factors that influence molluscan concentrations, including contaminant speciation, salinity, temperature, food abundance and composition, mussel size, reproductive state, etc (see Philipps and Segar, 1986). Although the standardization of sampling procedures in many monitoring programs limits these variations, the concentrations in mussel tissue may not give a true picture of the existing ambient levels in the surrounding waters. But if the concentration in water is important information for reduction and control of pollutant releases, the bioavailable fraction, which refers to the quantity that effectively enters the biological system, may be the most relevant information for coastal sustainable management. In that sense, "bio-indicator" means, through one representative species, how much a functional group of the ecosystem, here the benthic filter feeders, can be impacted. Indeed, the challenge for (radio)ecotoxicology will be to understand the link between the concentration of contaminants and their effects on organisms at the cellular, individual and population level, especially under chronic exposure. The risk assessment of a potential irreversible ecological drift requires the determination of end-points like the "Non Effect Concentration" (NOEC) for any hazardous substance (Chapman, 1995).

3. CURRENT BIOMONITORING ACTIVITIES

The first half of the workshop was devoted to presentations of current biomonitoring activities in the Mediterranean and Black Seas, focusing on regular surveys in each area. Participants were requested, in turn, to address the following issues:

- the objectives, strategy, organization and major results of existing monitoring networks and typical surveys;
- a summary of existing knowledge on trace contaminant inventories and inputs;
- natural and cultivated mussel bed distribution along the coastline with identification of possibly less documented areas;
- drawn from each field study, their propositions, in the perspective of a future Mediterranean Mussel Watch, such as a selection of relevant sampling stations, special events to monitor and routinely measured parameters (contaminants, biometrics, and biochemical).

As the communications made clear (see extended abstracts, this volume), a substantial effort in monitoring trace contaminants is already well engaged along the coasts of the Basin. Among the 18 institutions/countries represented, seven have already established permanent monitoring networks to detect radionuclides in mussels, which is the core component of the proposed MMW program. Five other countries have initiated monitoring of radionuclides using indicators other than mussels (other mollusks, algae, fish, seawater, and sediment).

Almost all participants reported on regular surveys of chemical pollutants in their area of concern, usually heavy metals and POPs (PCBs, PAHs and pesticides). In several cases monitoring of radionuclides and conventional chemical contaminants is conducted within the same group/institution. In other cases, appropriate collaborations allow to cover field and laboratory operations for a complete range of contaminants. This situation will certainly make it easier to implement the second phase of the project. In the southern and eastern parts of the region, these monitoring activities are usually performed within the framework of the MEDPOL program (UNEP/MAP) and great care will be taken not to duplicate efforts in this area.

Overall, the data presented in this Workshop volume represent a most interesting set, made accessible as a whole for the first time, with sometimes eye-catching results. To give just an example, it appears that cesium-137 levels in mollusks are still more than one order of magnitude higher in the Black Sea and the Eastern Mediterranean than in the rest of the region, obviously reflecting the remaining impact, 15 years after the accident, of Chernobyl. However, because of the many differences between indicator species among regions, sampling dates, measurement parameters, and expression of results, spatial comparison of results is difficult and differences may possibly be attributed to differences in protocols rather than to real differences in radionuclide concentrations. This argues again in favor of the necessary harmonization and standardization of the monitoring procedures and methods at the regional level.

4. REACHING AGREEMENT ON MMW

The second half of the Workshop focused on essential scientific and technical issues in order to design a Mediterranean Mussel Watch through common strategies, procedures and methods. While some points of the program were not definitely established, requiring further testing and checking, the general framework of the monitoring network was largely settled. A comprehensive technical scheme was formulated, allowing for a rapid start. The general agreement reached among the Workshop participants is summarized in this section.

4.1 Objectives

Considering the growing public concern and the institutional enforcement over marine environmental quality, the main objective is to produce status reports and to identify spatial and temporal trends through long term monitoring at the regional scale. This will first require documentation of existing baseline levels of some key radionuclides in Mediterranean coastal waters, measured by the total body burden of filter feeder mollusks, preferably Mytilidae.

Another goal will be to fill the gap of knowledge on seasonal variations in contaminant concentrations due to yearly changes in inputs, environmental conditions, and to variations in bioaccumulation processes that are species-specific for the various indicators.

The ultimate aim will be to extend the program to other trace contaminants (metals and POPs) according to a common global approach.

4.2 Opportunities

There is a great deal of background knowledge and experience gained from other Mussel Watch programs throughout the world.

This project is the first attempt to establish and independently operate a permanent biomonitoring network dealing with radionuclides for the whole Mediterranean and Black seas. The originality of this project is primarily apparent in the effort to produce reliable information from the best available techniques across such a large and diversified geographical area. The CIESM sponsorship will represent a major opportunity, considering the scientific credibility of the organization and its is rich circum-Mediterranean network of marine scientists and research institutes. This will provide a framework for cooperation, shared expertise and technical know-how.

4.3 Monitoring strategy

4.3.1 Indicator species

The chosen bio-indicator species is the Mediterranean mussel *Mytilus galloprovincialis*, with a geographic distribution previously recorded on all coasts of the Basin (Fischer *et al*., 1987). However, participants to the workshop reported that the species has recently become rare in the southern and eastern parts of the Mediterranean and is nearly absent from the southern coast of Turkey to the southern coast of Tunisia. This change in distribution may result from habitat destruction linked with coastal development (Abdel-Moati, this volume) or from possible interference with other species, such as the migrant gastropod, *Strombus decorus persicus*, from the Red Sea (see Al-Masri, this volume).

Unambiguous interpretation of contaminant levels on a regional scale requires use of a single indicator species since large variations in contaminant concentration factors between species are well documented. An alternative solution for the affected areas (that is, those areas currently lacking *M. galloprovincialis*) is now available through the transplantation of mussels in cages (see Sauzade, this volume). Technical assistance can be provided from various participants to the MMW for the first trials.

Concurrently, the presence and abundance of natural populations of other species of Mytilidae can be investigated, in particular the genus *Perna* (African mussel), *Modiolus* (horse mussel) or *Brachidontes*.

4.3.2 Sampling sites

The MMW will start with a limited number of sampling sites in each area/country. Where national or international (MEDPOL) monitoring programs are already established, stations will be selected among the existing sampling sites, in particular where historical records are available, keeping in mind the overall objectives.

In case of a new implementation, the first phase will involve a coastal zoning exercise to identify the major coastal features (sandy margin, rocky shore, delta, large bay, etc.). Then, at least one relevant sampling site would be selected for each qualified sub-area, so as to provide optimal geographic distribution while keeping in mind the local feasibility of a long-term monitoring operation.

Proposals from participants collected during and right after the meeting appear in Fig. 2. Although still preliminary, this compilation already goes a long way to fulfill the objective of a comprehensive regional network.

4.3.3 Frequency of sampling

The need for accurate determination of baseline levels and future temporal trends requires the integration in the monitoring strategy of seasonal variations of environmental factors as well as inputs to the coastal zone and the biological/reproductive cycle of the mussel. Moreover, very low levels of some man-made radionuclides are expected to require frequent sampling to establish consistent "above detection limit" data sets.

It is further recommended that quarterly sampling be considered as optimal. If not possible initially, a minimum of two samples a year per site would be acceptable, provided that they are collected before and after the spawning period.

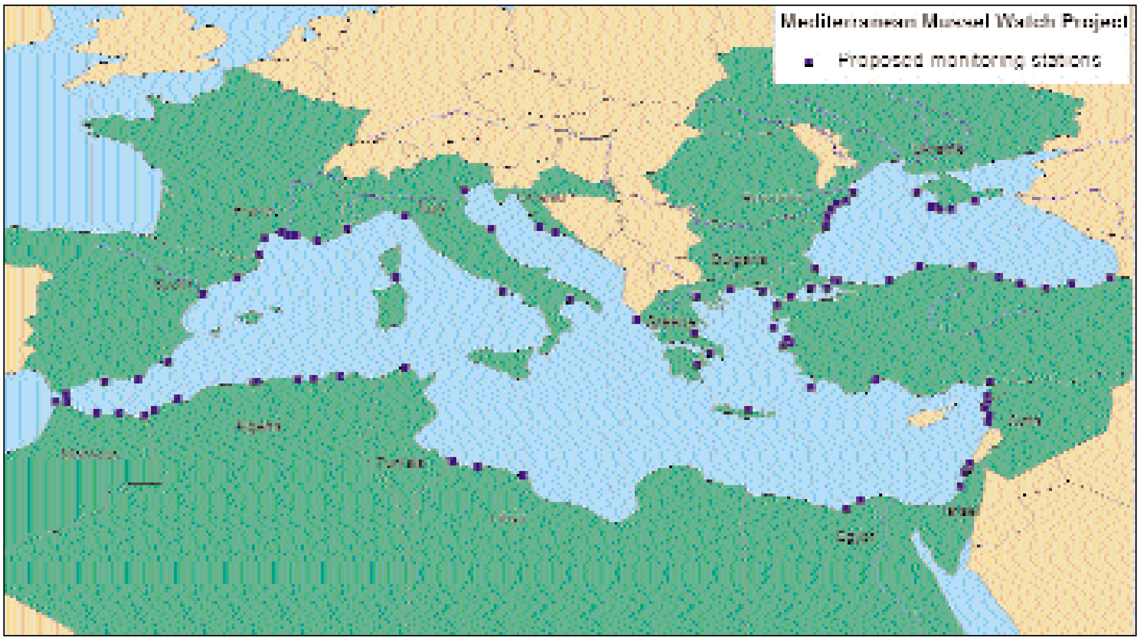


Fig. 2. First proposals of monitoring stations for the Mediterranean Mussel Watch (95 sampling sites).

4.3.4 Calibrated size/age individuals

The recommendation is to collect young adults ranging in size from 3 to 5 cm (total length). In any case, the individual length must be comprised between 70 and 90% of the maximum length of the local population.

4.4 Sampling and treatment of samples

The following recommended procedures are adapted for radionuclide measurements. They will be carefully revised for chemical pollutants for which the risk of sample contamination at every step of processing is quite high.

The sample amount must be adapted to measurement requirements, in particular to obtain enough solid material to completely fill the selected standard counting container. Typically 200 to 300 mussels (roughly equivalent to 3 to 5 kg) must be obtained for each sample to avoid analytical uncertainties. They must be rapidly cleaned with site seawater but no depuration is required. Samples, tightly packed in plastic bags, can be transported in refrigerated containers (8 to 15°C) for 48 hours. Ancillary measurements of site parameters include water temperature, salinity and, if possible, turbidity.

At the laboratory, mussels must be thoroughly cleaned with tap water, and the byssus and fouling organisms removed. The mussels can be either shucked raw with a stainless steel knife (for further freeze-drying) or opened in a microwave oven or pressure-cooker (for further heat drying). In both cases, it is recommended to collect the total intervalvar content and not only the soft parts, because any opening method can lead to a significant loss of internal fluids of delicate mussel tissues. Accordingly, the fresh weight is calculated as the initial sample net weight, minus the shells' dry weight. If participants use both methods, intercalibration within the group is required.

For heat -drying up to constant weight, the oven temperature must not exceed 100°C to avoid loss of some volatile elements like Po-210. Ashing of dried sample material is recommended prior to analysis to further concentrate the solid material. Ashing temperature must not exceed 500°C to avoid loss of Cesium. Ashed samples must be ground and carefully homogenized.

The condition index is an important ancillary biometrics parameter to estimate the physiological and reproductive stage of the sampled population. It is calculated for each pooled mussel sample, as the ratio of flesh dry weight to shell dry weight.

4.5 Trace level radionuclide measurements

The first common agreement is to perform direct gamma spectrometry as a routine technique. It allows the rapid detection of many artificial radionuclides, including Cs-137, Co-60 and Ru-106.

Considering the very low expected levels, hyper pure germanium detectors with a high resolution and high relative efficiency are recommended, but lower efficiency equipment can be compensated by increasing the sample mass and/or counting time. N-type detectors perform additional measurements of Pb-210 and Am-241. Gamma spectrometry also allows detection of natural radionuclides like K-40 as a standard parameter and some isotopes of the Th-232 and U-238 families.

Po-210 is a radionuclide of concern for many participants (as possibly significantly enhanced in the coastal environment by shipping or land-based activities) and will be measured by alpha spectrometry.

Pu isotopes measurements require time-consuming radiochemistry prior to analysis by alpha spectrometry and will only concern a limited number of samples.

Initial intercalibration of all participating analytical laboratories is a very important step in the MMW implementation phase, if only because of obvious differences in analytical equipment among scientific facilities. These differences will probably remain because of the high investment and operation costs associated with equipment upgrades.

4.6 Data management and reporting

Value-added and reliable information expected from the future network largely depends on effective data management.

After initial control and validation from laboratory expertise, all data collected from the participants will be directed to the coordinating unit and cross-checked for data quality and integrity.

Implementation in a relational database coupled with GIS will be made from the start of the program with special emphasis on description of raw data through the compiling of a common data dictionary.

User-friendly access to information for all participants and various stakeholders is a key feature of the project: the CIESM website could serve as a portal to on-line connection with all Mediterranean Mussel Watch resources.

5. NEXT STEPS

The workshop was successful in examining the implementation requirements for a permanent Mediterranean Mussel Watch dealing with trace contaminants, starting with radionuclides in the first phase.

Thanks to a motivated Task Force, the start-up phase of the project and its subsequent operation will only require a simple, flexible organization, relying largely on the core group established in the preparation of the workshop.

Participants will now report to their institutional authorities to set up the necessary arrangements, based on a memorandum of understanding which will detail the rules of good use of the produced data.

Further consideration is required on certain important issues like the final species selection for the whole region, technical feasibility of mollusk transplantation where necessary, the list of essential radionuclides to be measured at every site, or the organization of intercalibration. The first sampling campaign could be reasonably planned for the autumn, 2002.

In the meantime, a subgroup will start examining the conditions of extension, particularly in terms of monitoring strategy, of the monitoring network to chemical contaminants. MMW could be essentially operated on a shared cost basis, but the Task Force is aware of the need for financial support, in particular for participants not regularly involved in existing monitoring programs at the national or international level.

The Mediterranean Mussel Watch Egypt: case study

M.A.R. Abdel-Moati

Oceanography Department, Faculty of Science, Alexandria University, Alexandria, Egypt

Bioaccumulation in mussels reflects the changing levels of contaminants in the marine environment. Their accumulation depends on filtering activity, growth, biochemical composition, reproductive condition and metabolism which in turn are affected by environmental factors. Mussels are considered the appropriate bioindicator for pollutants derived from the local sources due to their sedentary, filter feeding habit, common abundance and ease of collection.

The coastal waters of Egypt are exposed to different types of pollutants discharging from landbased activities ranging from agricultural, sewage to industrial. Some areas receive a mixture of two or more types. Pollutants discharge is more concentrated at the area between longitudes 29°45' and 30°20' E (west and east of Alexandria city). Most studies on trace contaminants are concerned with metals; however, very few programs touched the concentrations of hydrocarbons and PCB's in the coastal marine ecosystem.

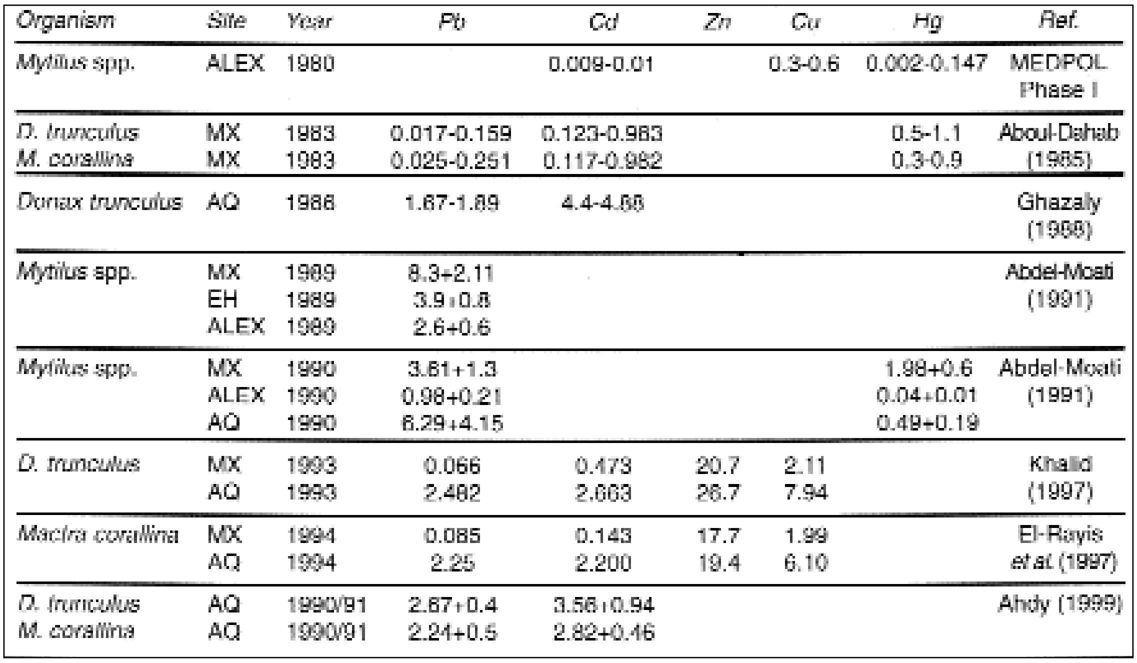
About 14 and 12 MT/Y of Pb are discharged to Abu Qir and Mex Bays, respectively. The latter received also, for a long period, huge amounts of Hg from a chlor-alkali plant. Concentration factors in bivalves varied between 1200 and 4000 (Abdel-Moati, 1990; 1995). Meanwhile, pesticides are highly concentrated off the River Nile and Nile delta lagoons.

Table 1 compiles the available published data on bivalves used as bioindicators for meta pollutions along Alexandria coast. Since the early eighties and within the frame work of MedPol Phase I, organisms have been used as bioindicators for monitoring pollution levels along the Egyptian coast. *Mytilus* and five fish species were monitored for Hg-t, Cu and Cd at five stations during 1980. *Donax trunculus* and *Mactra corallina* were used to monitor Pb and Cd (Aboul-Dahab, 1985). Abdel-Moati and Atta (1991) used Mytilus minimus to report Pb and Se enrichment along the coastal waters of Alexandria while the biochemical and physiological responses of *Mytilus* to Hg and Pb in the coastal waters of Alexandria were recorded by Abdel-Moati (1991).

During the nineties, *Donax trunculus* was widely used as a bioindicator for Zn, Cu, Pb and Cd levels (El-Rayis *et al*., 1997; Khalid, 1997 and Ahdy, 1999). It is widely distributed along the coast most of the year and edible to most marine food consumers.

Two scientific organizations, Alexandria University and NIOF are responsible for monitoring programs in both the Mediterranean and Red Seas. Currently, both are running national monitoring programs for identifying pollutants levels along the Egyptian coast. These programs are taking place after eight years of the Environment Law implementation to examine how most of polluting factories have treated and controlled their discharge. The program includes 32 locations for monitoring (once a year) sediments and bivalves for metals (Cd, Cu, Pb, Zn, Hg), hydrocar-

Table 1. Metal concentrations (μg/g) in bivalves from Alexandria coast.



Organism	*Site*	*Year*	*Pb*	*Cd*	*Zn*	*Cu*	*Hg*	*Ref.*
Mytilus spp.	ALEX	1980		0.009-0.01		0.3-0.6	0.002-0.147	MEDPOL Phase I
D. trunculus	MX	1983	0.017-0.159	0.123-0.983			0.5-1.1	Aboul-Dahab (1985)
M. corallina	MX	1983	0.025-0.251	0.117-0.982			0.3-0.9	
Donax trunculus	AQ	1988	1.67-1.89	4.4-4.88				Ghazaly (1988)
Mytilus spp.	MX	1989	8.3+2.11					Abdel-Moati (1991)
	EH	1989	3.9+0.8					
	ALEX	1989	2.6+0.6					
Mytilus spp.	MX	1990	3.61+1.3				1.98+0.6	Abdel-Moati (1991)
	ALEX	1990	0.98+0.21				0.04+0.01	
	AQ	1990	6.29+4.15				0.49+0.19	
D. trunculus	MX	1993	0.066	0.473	20.7	2.11		Khalid (1997)
	AQ	1993	2.482	2.663	26.7	7.94		
Mactra corallina	MX	1994	0.085	0.143	17.7	1.99		El-Rayis *et al.* (1997)
	AQ	1994	2.25	2.200	19.4	6.10		
D. trunculus	AQ	1990/91	2.67+0.4	3.56+0.94				Ahdy (1999)
M. corallina	AQ	1990/91	2.24+0.5	2.82+0.46				

bons (aliphatic and TPHs), pesticides (HCB, HCH's, DDT, DDE, lindane, aldrin, dieldrin) and PCB's (Congeners 28, 52, 101, 118, 138, 153 and 180). On the research level basis, radionuclides were observed in Abu Qir bay sediments (Nasr *et al*., 2001), mostly in areas dominated by black sand. However none of the previous or ongoing monitoring programs have touched radioactive nuclides measurements in marine organisms.

Long-term monitoring in Egypt is faced by lack of funding and is thus suffering discontinuity. Widening the coastal road by dumping huge rocky areas off Alexandria city lead to disappearance of large populations, specially *Mytilus* species, from some areas, and consequently temporarily excluding their use for biomonitoring. Most of ongoing bivalves monitoring programs depends on natural beds. Data on their biological cycles, specially the spawning season, is completely lacking.

A simple proposed monitoring program will aim to 1) define the present status and background levels of pollutant(s) in the coastal area, 2) assess the temporal changes in contaminant(s) levels, 3) determine the extent of bioaccumulation of target contaminant(s), 4) examine environment law implementation and 5) provide necessary technical and scientific inputs for preparation of outputs to decision makers. Such program is not restricted to provide temporal changes in contaminant(s) but could extend to use monitoring as a management tool. It comprises only three coastal stations : 1) Abu Qir bay (31°30'N and 30°20'E), 2) Mex bay (31°10'N and 29°50'E) east and west off Alexandria coast and 3) Al-Dabaa (30°00'N and 28°30'E) opposite to the nuclear power plant project area (120 km west of Alexandria with trace contaminants to be determined in mussels. The criteria for choosing sampling location is, in addition to the geographic coverage, the exposure of the selected areas to discharge from landbased activities, types of pollutants to be monitored, presence and absence of mussels and the ongoing national monitoring programs. Despite the increased public awareness, implementation of national regulations and wide spread of treatment plants, the two first locations are still receiving a mixture of contaminants. One reference station could also be monitored between Ras Al-Khema and Matrouh ((31°10'N and 27°58'E).

In absence of family Mytilidae representatives along the Egyptian coast, mussel transplantation is a proposed alternative in biomonitoring program. Transplantated organisms (recommended size 3-5 cm) could be sampled once a year during the pre-spawning period (expected to be between November and May). Organisms for transplantation could be imported from northern

Mediterranean countries. Contaminants may include ^{137}Cs, ^{40}K ^{238}U, and ^{232}Th measurements but could extend in the future to touch metals (Hg, Pb, Cd), TPH, PCB's, and PAHs . Radionuclides measurements (gamma spectometry) will take place at the Radioisotope Department of the Egyptian Atomic Energy Agency (EAEA). Biometrics include shell length, width, weight (wet/dry) and/or condition index. The lipid content of the mussel could also be determined as a biochemical indicator for stress. Measuring simple ancillary data such as salinity and temperature could reflect water quality conditions in the sampling sites. Sampling may take place after special events like maximum discharge periods, floods, Saharan dust blowing periods, etc.

A procedure handbook must be made available, including easy to operate and low cost techniques. Reference material should be made available for participants from IAEA through CIESM. Specific Regional intercalibration exercises should take place with other Mediterranean countries at least once a year. Data should be presented in an agreed upon format to facilitate comparison with other areas. Created data should be fed continuously to an easily accessible CIESM website. One annual workshop as well as two technical experts meetings should take place to discuss progress, overcome difficulties and exchange views and evaluate results.

Marine environment studies on the Syrian coast: biomonitors

Mohammed Said Al-Masri

Department of Protection and Safety, Atomic Energy Commission of Syria, Damascus, Syria

The lack of information about radioactivity along the Syrian Mediterranean Coast has urged the Atomic Energy Commission of Syria (AECS) to initiate marine environmental program in 1991. The main objective of this program was to determine radioactivity base line and to define the biota species (fish, algae, molluscs and others) distributed in the Syrian coast which can be used as biomonitors.

Samples of seawater, coastal sediments and marine biota have been collected during 1992 and 1993 from four main locations viz., Lattakia, Tartous, Banise and Jabla. These samples were analyzed for artificial radionuclides such as ^{137}Cs, ^{90}Sr, Pu isotopes and natural radionuclides such as ^{210}Po. Results have shown (Othman *et al.*, 1994, 1996) that Pu isotopes concentrations in all type of samples were in the same range of the Mediterranean region. ^{90}Sr concentrations in biota samples were relatively low and the highest activity was observed in Jania species.

Three types of marine algae distributed along the Syrian coast have been studied from a chemical and radioactivity point of view (Al-Masri *et al.*, 2001), viz. green, red and brown algae. Results have shown that ^{137}Cs concentrations in all the analyzed sample were low while the levels of naturally occurring radionuclides such as ^{210}Po, ^{210}Pb and radium isotopes were found to be high in red algae which indicates their selectivity to these isotopes. Other studies (Al-Masri *et al.*, 1999, 2002) reported high levels of ^{210}Po and ^{210}Pb in the brown alga Stypopadium sp (15.44 Bq kg-1 wet wt) collected near Tartous port. On the other hand, brown algae and especially Cystoseira has shown a clear selectivity for some trace elements such as As, Cr, Cd, Cu and Co, this selectivity may encourage the use of brown algae as biological indicator for trace elements pollution (Table 1). In addition, Algae collected and analyzed for ^{210}Po in 1993 along the Syrian coast (Othman *et al.*, 1994, 1996) showed concentrations between 0.52 to 8.5 Bq kg^{-1} wet wt. The highest concentration was found to be in Spridia sp (8.5 Bq kg^{-1} wet wt.).

Fresh fish samples were collected from the local markets in Syrian coastal cities and analyzed for radioactivity and trace metals. Sampling locations were verified by fishermen in the areas, only molluscs being analyzed for. Al-Masri *et al.*, 2000 showed that concentrations of ^{210}Po and ^{210}Pb in the edible tissues of about 36 species of marine fish varied between 0.27 and 27.48 Bq kg^{-1} fresh wt and 0.05 to 0.38 Bq kg^{-1} fresh wt for ^{210}Po and ^{210}Pb respectively. The highest levels were observed in *Euthynnus alletteratus* and *Sardinella* sp. In another study (Al-Masri *et al.*, 1999), cadmium, lead, copper and zinc concentrations were determined in the same samples and concentrations of Pb, Cd, Cu and Zn were found to be relatively low, ranging from 0.02 - 0.26, 0.004 - 0.127, 0.1 - 2.48 and 3.56 - 19.3 mg kg^{-1} of wet wt respectively; the highest level of lead being observed in Trachinotus sp species. In addition, the trace metals studied were also determined in bones and found to be correlated with their concentrations in fish muscle.

Table 1. Radionuclides distribution in some algae distributed along the Syrian coast.

Sample Type	Location	^{210}Pb BqKg^{-1}	^{210}Po BqKg^{-1}	^{226}Ra BqKg^{-1}	^{137}Cs BqKg^{-1}
Green Algae					
Zostera marina	Lattakia	13.86±1.18	13.42±0.01	5.40±0.8	<0.60
Ulva rigida	Tartous	3.49±1.68	13.93±0.95	<1.7	<0.68
Ulva fasciata	Banias	5.55±0.51	15.58±0.76	<1.14	<0.55
Ulva fasciata	Tartous	2.27±0.75	7.74±0.18	<2.25	<1.2
Zostera marina	Lattakia	5.23±0.23	9.67±0.60	<2	<1
Brown Algae					
Stypopodium zonale	Tartous	6.27±1.29	23.84±0.63	3.63±0.54	<0.75
Padina pavonica	Tartous	9.14±0.54	24.43±0.66	6.80±0.54	<0.91
Sargassum vulgar	Tartous	5.89±0.29	23.31±0.72	2±0.7	<0.72
Cystoseira barbata	Tartous	4.69±0.42	23.66±0.69	1.2±0.2	<0.47
Cystoseira sp.	Lattakia	15.85±0.71	26.40±1.10	<2.4	<1.10
Stypopodium zonale	Ras Ibn Hani	10.34±0.58	15.27±0.32	4.57±0.4	<1
Cystoseira ercegovicii	Ras Shamra	7.58±1.22	12.32±0.51	<2.8	<1
Padina pavonica	Tartous	5.04±1.72	9.95±0.35	6.36±0.5	0.55±0.1
Sargassum vulgar	Tartous	8.11±0.23	12.31±0.85	5.14±0.7	<1
Cystoseira sp.	Tartous	2.78±0.30	6.08±1.10	<1.5	<0.7
Red Algae					
Jania rubens	Ras Shamra	27.48±8.70	19.86±0.68	5±0.52	<0.60
Jania longifurca	OM Attouyour	26.01±2.60	27.43±0.58	3.8±0.33	<0.60
Jania rubens	Lattakia	7.77±0.90	19.46±2.45	<6	<1

Other species than algae and fish were also collected near Tartous phosphate port and analyzed (Al-Masri *et al*., 2002). Results have shown comparable values of ^{210}Po and ^{210}Pb for all marine organisms (algae, crab and fish). In this study, we concluded that the use of marine organisms as an indicator of phosphate impact on marine environment is not recommended since most of the marine organisms concentrate ^{210}Po and ^{210}Pb in their body.

Studies on radioactivity content in molluscs are insufficient, but molluscs beds distribution on the Syrian coast is well established (Al-Nimeh *et al*., 1997; Gosselck *et al*., 1986; Ibrahim, 2002).

Table 2. Most common Mollusca species identified along the Syrian coast.

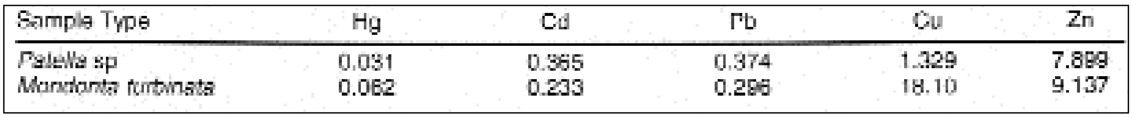
Mollusca
Patella rustica
Patella caerulea
Monodonta turbinata
Patella aspera
Strombus decorus persicus
Thais haematobium
Diodora graeca
Fissurella umbecula
Truncularioosis sp
Bivalvia
Lithophaga lithophaga
Pinctada radiata
Recellaria ubla
Callista chione
Mytilaster lineatus
Malleus regula
Mactra Stulorum
Echinoderma
Paracentrotus lividus
Holoturiam paticns
Asterina gibbosa
Astarpectin spinulosus

Common species identified in the littoral and sub-littoral zones are listed in Table 2. The most common species are Patella caerulea and Strombus decorus persicus. The suggested M. galloprovincialis suggested as biomonitors in the Mediterranean Mussel watch program is nearly absent (Ibrahim, 2002), and it is suggested that this species has been affected by the migration of the *Strombus decorus persicus* from the Red Sea to the region. Radioactivity, trace metals and other contaminants were determined in several species. Representative results are reported in Tables 3 and 4.

Table 3. Radionuclides distribution in some molluscs (sampling date: 10/1994) (Al-Nimeh et al., 1997).

Sample Type	^{210}Po BqKg^{-1}	^{40}K BqKg^{-1}	^{137}Cs BqKg^{-1}	239+240Pu BqKg^{-1}
Patella sp	37.67 ± 1.25	200 ± 12	2.68 ± 0.5	0.054 ± 0.01
Mondonta turbinata	67.24 ± 2.30	197 ± 11	-	0.125 ± 0.02

Table 4. Trace metals distribution in some molluscs (sampling date: 10/1994) (Al-Nimeh et al., 1997), mg kg-1 wet. w.



Sample Type	Hg	Cd	Pb	Cu	Zn
Patella sp	0.031	0.365	0.374	1.329	7.899
Mondonta turbinata	0.062	0.233	0.296	18.10	9.137

In conclusion, for future trend monitoring, the following biota species can be selected:

Fish: *Euthynnus alletteratus* and *Sardinella* sp for radioactivity;

Algae: *Jania rubens* for radioactivity;

Algae: *Cystoseira* for trace metals;

Molluscs: *Patella caerulea* for radioactivity and trace metals.

For future monitoring stations, the following sites were selected:

1. Tartous 2. Banise 3. Lattakia 4. Ras Shamra 5. Om Attoiyour.

However, a plan has to be established where more surveys are required for measurement of radioactivity and trace metals in mussel species distributed along the Syrian coast. This can be easily performed, where a specialist team is available for sampling (from AECS and HIMR) and well equipped laboratories for radioactivity and trace elements measurements are available in AECS (Gamma spectrometers, Alpha spectrometers, Liquid Scintillation Counter, INAA…).

Biomonitoring in the Mediterranean coast of Spain

M.P. Antón[1], C. Gascó[1], J.M. Benedicto[2]

[1] Departamento de Impacto Ambiental de la Energía, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas, CIEMAT, Madrid, Spain

[2] Instituo Español de Oceanografía, IEO, Centro Oceanográfico de Murcia, San Pedro del Pinatar, Murcia, Spain

Marine organisms have been used as bioindicators to monitor water pollution for more than 20 years. Different countries employ mussels for environmental monitoring within the so-called “Mussel Watch Programme”, such as France (Réseau National d´Observation de la Qualité du Milieu Marin, RNO), or at the international level, covering the coasts from South and Central America, the International Mussel Watch Programme, IMW. Mussels’ capacity to accumulate contaminants has favored their use as indicators of the contamination of the surrounding ecosystem, avoiding the analysis of other matrices which require more sophisticated and expensive techniques (water/sediments).

In Spain, for the last 10 years (1991-2001), biomonitoring of the Iberian Mediterranean coast has been carried out by the Group “Studies on Marine Contamination” ascribed to the Instituto Español de Oceanografía (IEO, Spanish Oceanographic Institute), within its Murcia Center, located in southeastern Spain. This group examines the quality of surface coastal waters along the Mediterranean shore, from Cadaqués (the most northern site) to Algeciras (the southernmost location) by performing a yearly monitoring of mussels (*Mytilus galloprovincialis*) in 40 different stations (see enclosed map). The contaminants analysed in a routine basis are heavy metals such as mercury, cadmium, lead, zinc and copper, and organic compounds like PCBs, DDTs, HCB, etc (Rodriguez *et al*., 1995; Benedicto J.M. *et al*., 1999).

Mussel sampling is performed following the guidelines established by the Joint Monitoring Program at the Oslo and Paris conventions (OSPARCOM) and the Mediterranean Pollution Monitoring Program (MED-POL). To minimize the variations due to biotic factors, 200 mussels are collected per station at the same season (from mid-May to mid-June) and in the same conditions year after year. In the majority of the stations, the size of the collected mussels is the most abundant, namely 3-4 cm. In those stations where the mussels population is plentiful, sizes 4-5 cm, 5-6 cm, 6-7 cm and 7-8 cm are taken to check the variation of the contaminants concentrations with the mussel sizes.

The sampling stations are located in a wide variety of ecosystems: marine fishing reserves (i.e. Islas Medas, Gerona), natural protected regions (i.e. La Herradura, Granada), non-protected areas of ecological interest (i.e. Punta del Carnero, Algeciras), fishing regions/marine farms (i.e. Ebro river mouth, Valencia mussel farms), industrial or heavily populated areas (i.e. Barcelona, Cartagena), etc.

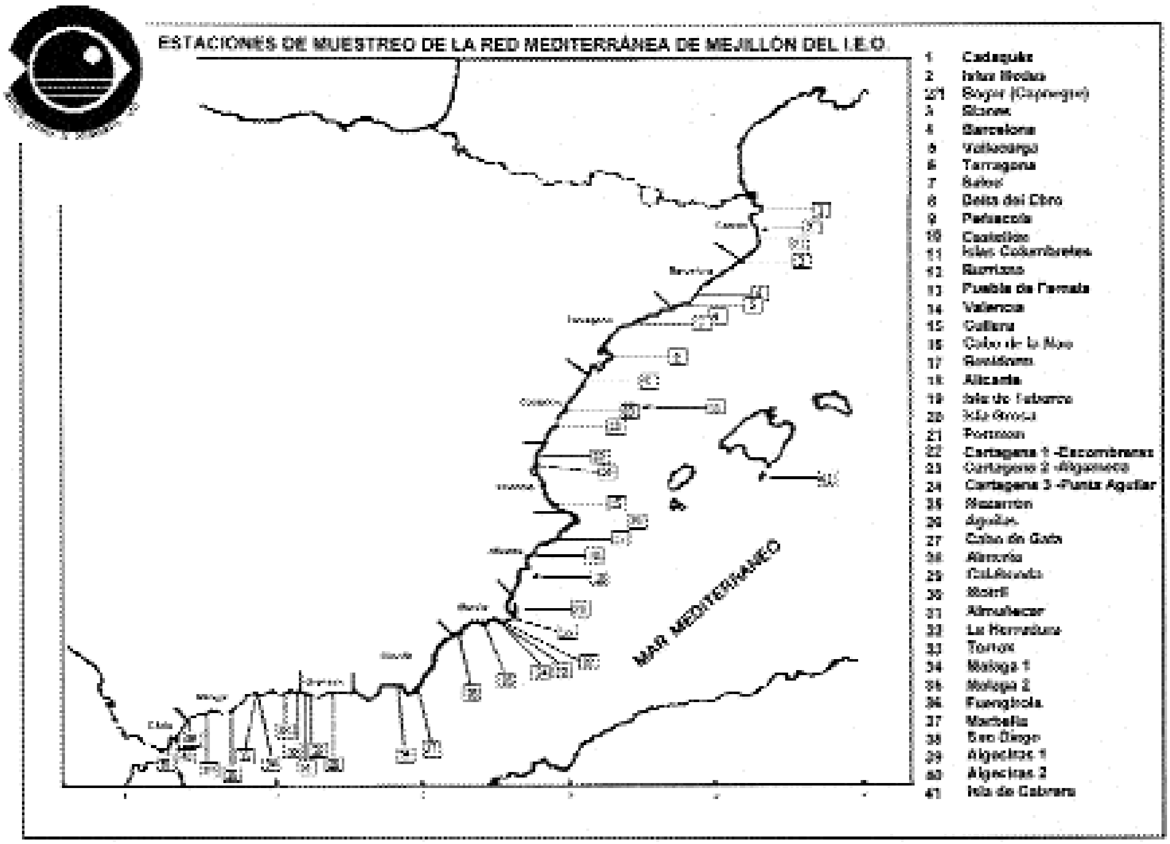


Fig. 1. Biomonitoring sampling network along the Mediterranean coast of Spain.

After mussel collection, samples are cleaned with seawater and appropriately packaged to fast-mail them to the laboratories in Murcia, making sure that the time span between sampling and their arrival is not more than 24 hours.

RADIOACTIVITY IN THE SPANISH MEDITERRANEAN ENVIRONMENT

The study of the temporal evolution concentration of selected radionuclides (^{137}Cs and ^{210}Po) in waters, sediments and biota from the Spanish Mediterranean coasts is very much limited by the non-existence of long term funding projects. However, some scattered data are available for ^{137}Cs from the western Mediterranean in surface waters and mussels (Molero, 1992) (Table 1).

Location	Water Bq/m³ (1s)				*Mytilus galloprovincialis* Bq/kg d.w.
	1988	1989	1990	1991	
Barcelona	5.2±0.1	5.0±0.2	5. 7±0.3	4.7±0.2	0.40±0.08
Vandellós	5.2±0.1	11.6±0.5	20.7±0.8	8.4±0.4	----
L'Ampolla	5.3±0.1	6.2±0.3	4.2±0.2	5.6±0.3	0.24+0.08
San Carlos	----	----	----	----	0. 82±0.09
Denia	4.6±0.2	4.5±0.2	5.3±0.3	4.3+0.2	----
Cabo de Palos	4.1±0.2	4.0±0.2	4.1±0.2	4.1+0.2	

There are also some results of ^{210}Po and ^{137}Cs in the Alboran sea obtained within the framework of CANIGO project (Gascó *et al*., 2002) (Table 2).

Water mass	^{137}Cs (Bq/m³, 1 SD)	^{210}Po (Bq/m³, 1SD)
Atlantic	2.52±0.28 (n=27)	1.53±0.34 (n=30)
Mediterranean	2.14±0.52 (n=21)	0.84±0.34 (n=22)

CONCLUSIONS

There is a well-defined monitoring network of mussels along the Spanish Mediterranean coast. Both sampling and determination of heavy metals and organic compounds in *Mytilus galloprovincialis* are performed by the IEO (C.O. Murcia) in a yearly basis.

The radioactivity levels measured in the Spanish coastal waters are homogenous, originating from fallout from atmospheric nuclear tests. To establish a radionuclides biomonitoring network, the sampling of 5-7 stations is contemplated, covering different ecosystems such as the Ebro river mouth, areas adjacent to industrial sites (Cartagena, Algeciras), etc. In this context, CIEMAT collaboration with IEO is essential, as well as other Spanish Institutions dealing with conventional contaminants.

Radioactivity measurements in the Environmental Radioactivity Measurement Laboratory (LMRE)

Evelyne Barker and Patrick Bouisset

lnstitut de Radioprotection et de Sûreté Nucléaire (IRSN)
Laboratoire de Mesure de la Radioactivité de l'Environnement (LMRE), Orsay, France

The environmental radioactivity measurement laboratory is in charge of the determination of trace of radionuclides in environmental samples. These radionuclides are gamma emitters by direct measurement, alpha emitters (^{239}Pu, $^{239+240}$Pu, ^{241}Am, ^{244}Cm), beta emitters (^{90}Sr, ^{3}H, ^{14}C), mass analysis by ICP-MS for long lived radionuclides (uranium, Pu and other alpha emitters, ...) and stable elements. Detection apparatus are located two storeys below ground level under a 3-m thick boron concrete slab. Ventilation with filtered out air allows stabilizing radon concentration.

Gamma spectrometry

Gamma spectrometry (Bouisset and Calmet, 1997; Bouisset *et al*., 1999) is performed with detectors of different measurement configurations. These configurations allow the coverage of investigated activity levels and are adapted to different environmental matrix. These configurations are based on hyper-pure germanium coaxial n-type detectors with a relative efficiency greater than 50% :

- conventional detectors (Ortec GMX) placed in a 5 cm thick standard lead shielding, internally lined with 0.7 cm electrolytic copper sheeting,
- low level detectors (high purity) with a streamline “J”, placed in a 15 cm lead castle (13 cm standard lead + 2 cm of ultra low-level activity lead), internally lined with 0.7 cm electrolytic copper sheeting,
- low-level detectors equipped with a veto-cosmic system or with a veto-Compton system,
- a very low-level detector located in an underground laboratory (Modane) shielded by a 15 cm lead castle (10 cm standard lead + 5 cm of ultra low-level activity lead).

Gamma measurements are generally performed with a counting time of 80 000 seconds. For low-level samples, counting time is 160000 to 240000 seconds. Density corrections are applied and matrix composition is taken into account below l00 keV (^{129}I, ^{210}Pb, ^{234}Th, ^{241}Am). With our high efficiency detectors, true coincidence summing corrections have to be done for some radionuclides (^{134}CS, ^{110}Agm, ^{60}Co, ^{214}Bi, ...). As an example, for ^{134}CS these corrections are around 30% for a 17 ml container (9 mm height) and around 15% for a 380 ml container (59 mm height).

Alpha spectrometry

Radionuclides (^{241}Am, ^{244}Cm, Pu isotopes) are extracted and purified by radiochemical procedures prior to alpha measurement (Goutelard *et al*., 1998; Agarande *et al*., 2001). Purified solutions are electroplated onto stainless steel discs and then measured by alpha spectrometry with a counting time ranging from 1 to 14 days. Alpha spectrometry measurements are performed with 48 passivated implanted planar silicon (PIPS) detectors.

Strontium-90

Strontium-90 detection follows the isolation of Sr from the rest of the matrix elements. After separation of ^{90}Sr from its daughter element, ^{90}Y, the latter is detected by proportional counting. Counting time is 72 hours.

Tritium

Tritium measurement (Fournier and Calmet, 1996, 1997) is performed on fraction water sample or a water fraction after a sample freeze-drying or a water fraction after combustion. The latter case is called organic bound tritium (OBT). Whatever the water preparation, a distillation is further performed before mixing the water fraction with a scintillant. Tritium is counted by liquid scintillation with low-level background Packard liquid scintillation counters. Counting time is 1000 minutes.

Carbon-14

Carbon 14 (Fournier and Calmet, 1996, 1997) is extracted by combustion under pressure (H par bomb) followed by an acetylene synthesis, and then mixed with benzene and scintillant before measurement. Counting time is 1000 minutes. Measurements are performed with low-level Quantulus liquid scintillation counters.

ICP-MS

Stable element and long-lived radionuclides are measured by ICP-MS (Agarande *et al.*, 2001). Purification is realised with ultra pure grade reagents. Measurements are performed with an HR ICP-MS axiom single collector (VG elemental), equipped with a double-focusing magnetic sector mass analyser of forward geometry. The mass spectrometer is installed in a class M 4.5 clean room.

Performances analysis

The sample size needed for measurements depends of the technique, which is performed. For ^{90}Sr determination, 20 g of ashed sample are sufficient. Alpha spectrometry measurements are performed with 20 g of ashed vegetable or animal sample and 100-200 g of dried soil or sediment. For tritium and carbon-14, sample mass is between 50 g-200 g depended of matrix. For ^{90}Sr alpha emitters and ^{3}H, Table 1 presents minimum detectable activities (MDA).

Table 1. Minimum detectable activity for measurement of some selected radionuclides.

	Minimum detectable activity (MDA)					
	a spectrometry [mBq.kg^{-1} dry weight]		Proportional counter [Bq.kg^{-1} dry weight]		Liquid scintillation [Bq.L^{-1}]	ICP-MS [mBq.kg^{-1} dry weight]
Matrix	Sediment (200 g)	*Mytilus edulis* (50 g)	Sediment (20 g)	*Mytilus edulis* (20 g)	All matrix	Sediment (100 g)
Counting time	14 days		72 hours		1 000 minutes	10 minutes
$^{239+240}$Pu	0.2	0.1				0.5 ^{239}Pu only)
^{238}Pu	0.6	0.6				
^{241}Am	0.2	0.1				20
^{244}Cm	0.2	0.2				
^{90}Sr			0.7	0.07		
^{3}H					1.2	

Table 2 presents detection limit for gamma spectometry. For carbon-14, because of the natural level, influence of a contribution can be studied only if measurements precision is important. For gamma spectometry, different containers with different sizes/volumes are proposed.

Table 2. Detection limit for gamma spectometry measurement of some selected radionuclides.

Posteriori detection limit for g spectrometry measurement (counting time : 80 000 s)				
	Mytilus sp. [Bq.kg^{-1} dry weight]			Sediment [Bq.kg^{-1} dry weight]
volume of container	17 ml (~20 g ashed)	60 ml (~ 70 g ashed)	380 ml (~ 370 g dry)	380 ml (~ 560 g)
^{210}Pb (46.5 keV)	11	6.3	-	-
^{241}Am (59.6 keV)	0.5	0.3	-	-
^{134}Cs (604.7 kev)	0.4	0.26	0.2	0.4
^{228}Th (621.8 kev)	4.3	2.3	2.4	4
^{137}Cs (661.7 kev)	0.6	0.38	0.24	0.5
^{60}Co (1332 keV)	1.1	0.3	0.25	0.4

Detection limit improvement

In the frame of IRSN mussel network, results are only performed by gamma spectometry.

Artificial radionuclide levels in the environment are low and decrease with time. As an example for 137 Cs (Fig. 1) in 2001 its level was around 0.1 to 0.7 Bq.kg-1 dry weight. In 2001, for all sampled Mediterranean French sites, we have found 56% results higher than our detection limit. In order to continue the time series, it is necessary to improve detection limit.

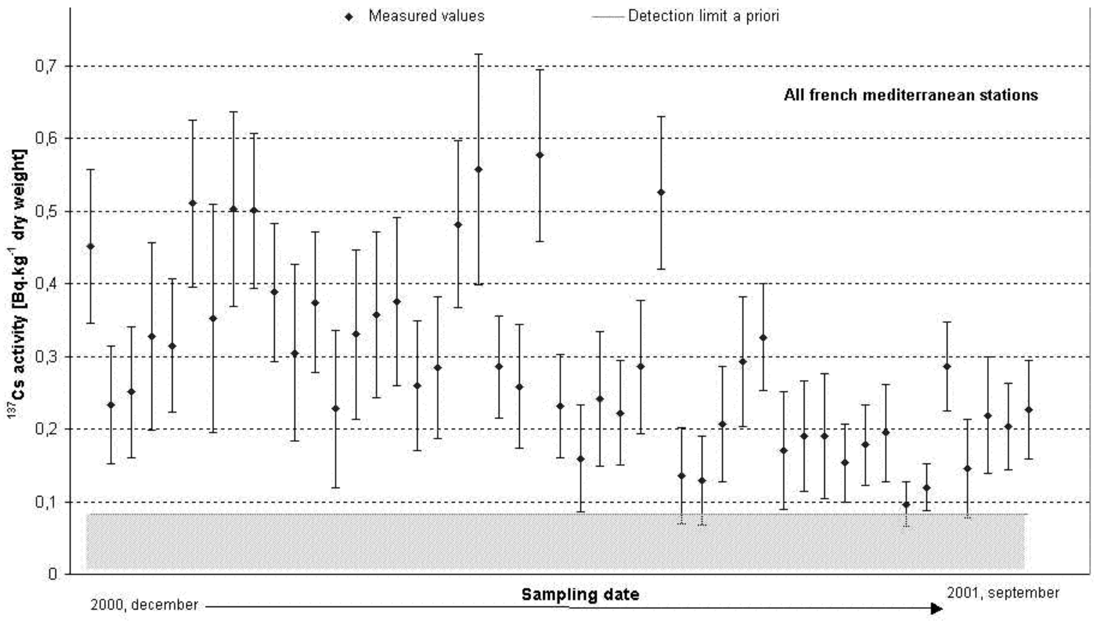


Fig. 1. ^{137}Cs activity in Mytilus galloprovincialis in French Mediterranean coast measured in 2001.

For natural radionuclides, such as 40K, there is no problem for detecting them, because even with their seasonal and biological variations, natural radionuclides levels are sufficient to be detected. Improvements can be achieved with

- counting time,
- sample mass,
- sample preparation,
- measurement equipment.

Counting time

Figure 2 presents the theoretical evolution of detection limit with counting time. With a routine counting time of 22 hours, doubling counting time does not decrease by factor 2 the detection limit but only by a factor of 1.4. In reality, detection limit is not decreased by the factor of 1.4, because the kind of detection limit approach presented in Figure 2 does not take into account

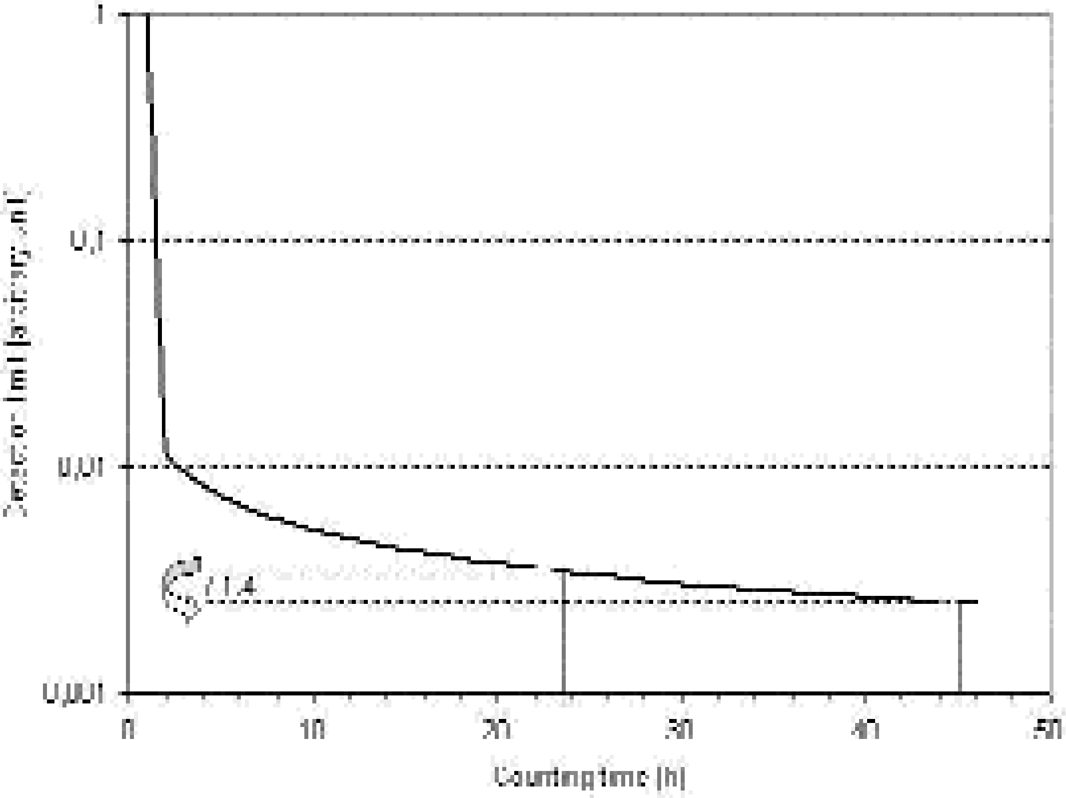


Fig. 2. Detection limit evolution with counting time in gamma spectometry.

the presence of other radionuclides in the sample. Multiplying counting time by factor 3 improves detection limit by a factor of 1.3 to 2 for radionuclides with energy around 600 ke V.

Sample mass

Gamma spectrometry can be performed with different container volumes. Theoretically, the more sample mass we have to measure, the better sensibility would be. But in fact, increasing container volume decreases detection efficiency. The final result is not a linear correlation with increased mass sample. Increasing sample mass in container increases self-absorption effects.

Sample preparation

The dried or ashed samples choice depends on sample matrix, container used and radionuclides searched. As an example, Figure 3 presents the detection limit evolution for 3 containers, for three radionuclides with different energies and for dried or ashed samples. Results are expressed in Bq.kg-1 dry weight. Self-absorption corrections are done (these corrections are more important for radionuclides of low energy (below l00 keV) such as 210Pb, 234Th, 24IAm; for radionuclides with energy higher than l00 keV a density correction is only necessary).

Figure 3 shows that for the same container a better detection limit for ashed samples is obtained than for the dried ones. But usually dried samples are conditioned in a higher volume container than ashed samples ones. As an example, for dried samples in 380 ml container and ashed samples in 60 ml container, and for Mytilus sp., detection limits are in the same order of magnitude. The container choice depends on the fresh mass collected and of course available in environment.

The better detection limit improvement will be observed for ashed samples in the greater container but self-absorption correction will be more important.

Measurement equipment

With a measurement performed with a low background gamma detector, some improvement can be observed (Table 3). Concerning background measurement, low background detector will give lower detection limit, which is not obvious when we look at the results of the Table. This is because with biological samples, presence of potassium 40 with high level creates a Compton

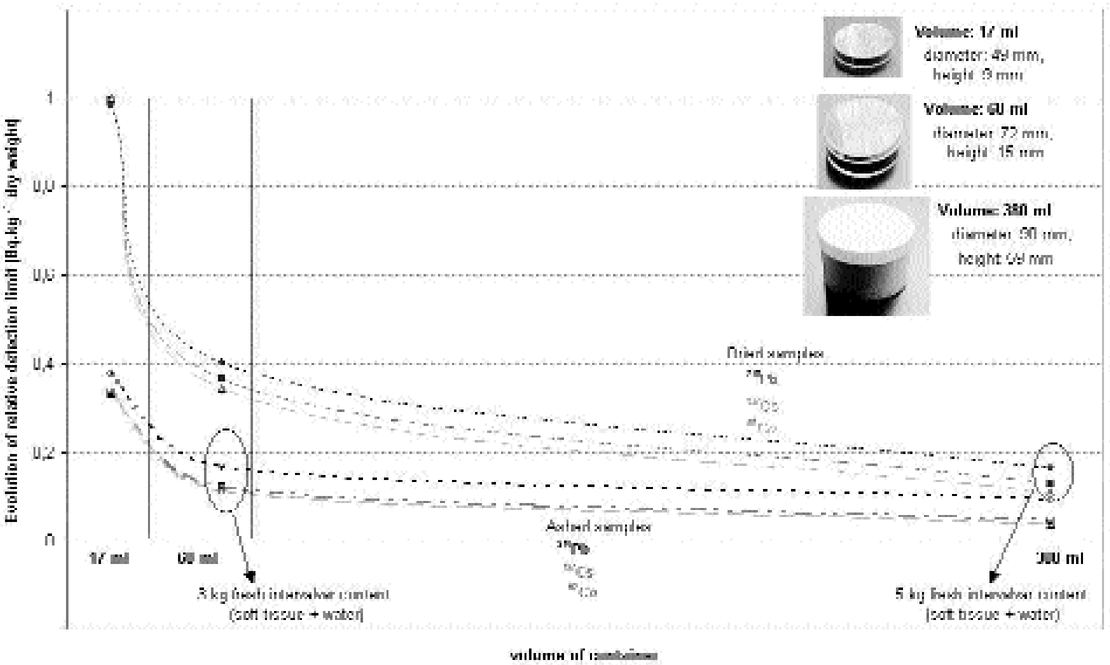


Fig. 3. Detection limit evolution for 3 containers, for 3 radionuclides, for dried and ashed samples in the case of Mytilus sp.

Table 3. Detection limit comparison for Mytilus galloprovincialis measured with different kind of gamma spectometry detectors.

Mytilus galloprovincialis			**Bq.kg^{-1} ashes samples**		
Vol. of container [ml]	Sample size [g]	Detector	^{210}Pb (46.5 keV)	^{137}Cs (661.7 keV)	^{60}Co (1332 keV)
60	81.72	Conventionnel	<30	<1.0	<1.2
60	67.31	Low background	<20	<0.65	<1
^{90}Sr			0.7	0.07	

continuum. Low background detector has a lower Compton continuum but sometimes it is not enough to measure radionuclides like caesium 137. To reduce this Compton continuum it is necessary to have an anti-Compton detector. With the anti-Compton system, ^{40}K Compton continuum line is reduced by a factor 5 (Fig. 4). It allows to reduce the detection limit by a factor 2.3 for ^{137}Cs. It means that this detector in 2001 activity should be given instead of detection limit.

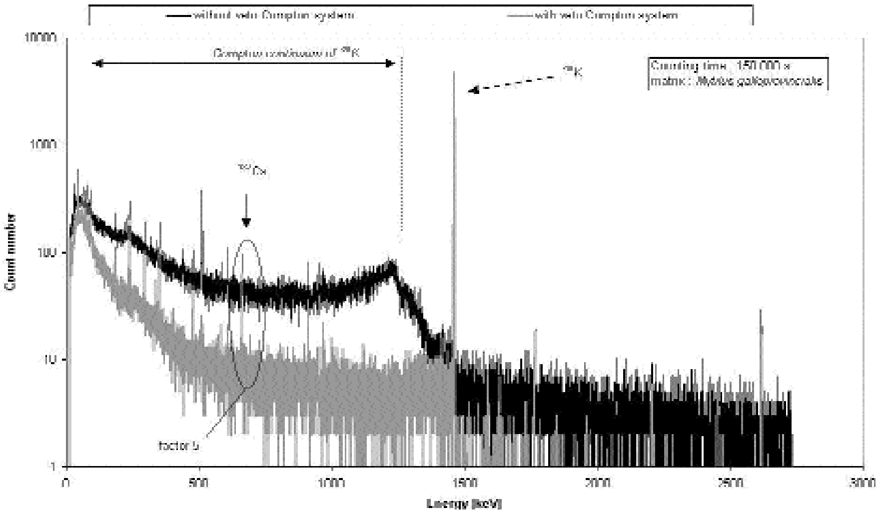


Fig. 4. Mytilus galloprovincialis spectra with and without anti-Compton system.

Monitoring of ^{137}Cs in mussel *Mytilus galloprovincialis* along the Romanian Black Sea coast

Alexandru S. Bologa

National Institute for Marine Research and Development "Grigore Antipa",
Constanta, Romania

The National Institute for Marine Research and Development "Grigore Antipa" (NIMRD), former Romanian Marine Research Institute (RMRI), is "*the technical operator of the national network of physical, chemical and biological monitoring of coastal marine waters and of the survey of coastal erosion*". Actually, RMRI/NIMRD has started the monitoring of the local marine environment within the National Integrated Monitoring System since 1975, considering contamination by excess nutrients (N, P, Si), heavy metals (Mn, Fe, Cu, Cd, Pb), artificial radionuclides (^{90}Sr, ^{137}Cs), total hydrocarbons, bacterial indicators, and parasite and saprophyte fungi, in emerged and submerged sediments, sea water and/or biota (Bologa, 1994, 2000; Bologa *et al*., 1999). NIMRD is continuing this monitoring to enable the appropriate management and protection of the marine environment in Romania.

The Global Environment Facility (GEF) through its Black Sea Environmental Programme (BSEP) specifies in its Strategic Action Plan for the Rehabilitation and Protection of the Black Sea (approved October, 1996), in chapter 54, Assessment and Monitoring of Pollutants : *"A Black Sea Monitoring System, based upon biological effects measurements and measurements of key contaminants, will be established in compliance with the Bucharest Convention (1992). It will consist of the integration of obligatory national monitoring programmes, to be included in the National Strategic Action Plans, and an independent quality assurance system. It is advised that the Istanbul Commission develop such quality assurance system through its Advisory Group on Pollution Monitoring and Assessment, by 1998"*. A pilot study for biological effects and contaminant residues was carried out within the International Mussel Watch in the Black Sea (Moore *et al*., 1999).

NATIONAL RESEARCH ACTIVITIES

Beginning in 1978, RMRI initiated the country's systematic study of marine radioactivity using a network of permanent stations along the whole Romanian littoral region, located between the Danube mouths and the southern extremity, and occasionally offshore up to 90 nautical miles (Bologa, 1994). The main purpose of this work was the completion of the data base on marine radioactivity levels. Data were used for generating partition coefficients (Kds) of radionuclides for marine sediments and biological concentration factors for key species. Assessments were also made of external and internal individual and collective doses from marine radioactivity due to immersion in sea water and/or seafood consumption (Bologa *et al*., 1988, 1991, 1995: Bologa and Patrascu, 1997; Osvath *et al*., 1990, 1992). After the Chernobyl accident (1986) special attention was paid to ^{134}Cs and ^{137}Cs in biota, including bivalve mollusks, i.e. the mussel *Mytilus galloprovincialis*, for which international organizations established maximum permissible limits as food products. In the Romanian Black Sea sector (Abaza, 1996-97; Petranu *et al*., 1999; Bologa, 20001a, b) , maximum values of ^{137}Cs in mussels, up to 3.3 Bq/kg (wet wt), were found in 1986 and in 1989 (see Fig. 1). In any case the highest ^{137}Cs concentration in mussels ranged below the maximum permissible level allowed for food by FAO in 1987 and the following years.

Fig. 1. Example of Cs137 trend activity in mussel (Mytilus galloprovincialis) from Constanta / Black Sea between 1986-1994.

INTERNATIONAL INVOLVEMENT

Regarding marine radioactivity/radioecology issues, RMRI/NIMRD has co-operated with various international bodies:

- International Atomic Energy Agency (IAEA):

- research contract N° 4805/ROM/RB/1987-92 : Monitoring of marine water, sediment and biota radioactivity in samples from the Romanian sector of the Black Sea by means of gamma spectrometry;

- co-ordinated research programme “Sources of radioactivity in the marine environment and their relative contribution to overall dose assessment from marine radioactivity (MARDOS) / 1989-92 (Aarkrog *et al*., 1997);

- research agreement N° K4.10.03/1989-92 :Dose assessment from marine radioactivity in the Romanian sector of the Black Sea;

- co-ordinated research programme : The use of nuclear tracers for the study of processes and pollution in the Black Sea;

- regional technical co-operation project RER/2/003 : Marine environmental assessment of the Black Sea region (with participation in both RADEUX research cruises in the eastern and western Black Sea in 1998 and 2000, respectively).

- International Commission for the Scientific Exploration of the Mediterranean Sea (CIESM):

- Global inventory of radioactivity in the Mediterranean Sea (GIRMED) / 1988.

- Other organizations/impact:

- Co-operative marine science programme for the Black Sea (CoMSBlack) / 1991-95 (with participation in the NW Black Sea research cruise aboard R/V *Professor Vodianitskyi*, IBSS/Ukraine, WHOI/USA, EPA/USA, August, 1992),

- Pilot study on cross-border environmental problems emanating from defence-related installation and activities (NATO/CCMS) / 1993-95,

- The radiological exposure of the European Community to radioactivity in the Mediterranean Sea (MARIN-MED) / 1994,

- Biochemical interaction between the Danube River and the north-western Black Sea (EC/EROS 21) / 1998-2000,
- Supportive of global programme of action for protection of the marine environment from land-based sources (GPA).

PRESENT POSSIBLE ENGAGEMENT IN THE "MEDITERRANEAN MUSSELWATCH" PROJECT

NIMRD is able to cooperate within the framework of the International Programme "Mediterranean Musselwatch" through the Nuclear unit devoted to marine radioactivity monitoring and radioecological research. Mussels can be sampled from various stations along the southern half of the Romanian Black Sea rocky shore (Constanta to Vama Veche) each season at agreed dates (e.g. quarterly) as noted in Fig. 2. Low background and high resolution gamma spectrometry equipment (GeHP detector, ORTEC - NORLAND 5500 MCD, PC AST-BRAVO 386) is available. ^{137}Cs data quality control was previously ensured by participation and validation in international intercomparison runs (SD-A-1, IAEA-300, IAEA-306, IAEA-307, IAEA-315, CoMSBlack, IBSS).

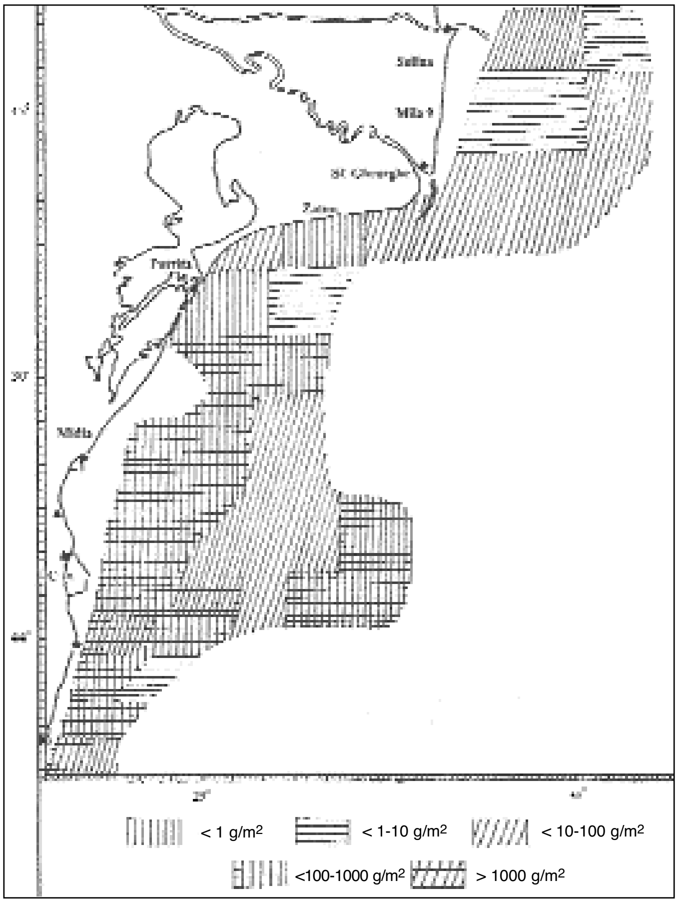


Fig. 2. Location of main mussel (Mytilus galloprovincialis) beds along the Romanian Black Sea coast.

Other marine chemical contaminants which could be included in the CIESM Mussel Watch from the Romanian Integrated Monitoring system, are heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Zn), total and polyaromatic cyclic hydrocarbons, detergents, and organoclorurate pesticides. From the monitored biological contaminants total and faecal coliforms and faecal streptococci could be considered. As to *Mytilus galloprovincialis* as bioindicator, cytological tests, histopathological analyses and data on early life stage injuries induced by contaminants (such as shell deformities) might also be added.

CONCLUSIONS

The marine radioactivity monitoring of the Romanian coastal waters during the last two decades allow the following main conclusions :

- reference values exist for ^{137}Cs concentration in the Black Sea mussel from 1985 until now ;
- maximum registered ^{137}Cs concentrations were always below “action levels” or highest admissible limits enforced by FAO;
- NIMRD may contribute to CIESM Mussel Watch.

Biomonitoring of the Moroccan coasts : outlook of the Mediterranean Mussel Watch in Morocco

Mohamed Cheggour[1], **Abdelghani Chafik**[2] **and Samir Benbrahim**[2]

[1] *Unité Ecologie, Dept. de Biologie, Ecole Normale Supérieure, Marrakech, Maroc*
[2] *Dept Qualité et Salubrité du Milieu Marin, Inst. Nat. de Recherche Halieutique (INRH), Casablanca, Maroc*

WHY BIOMONITORING THE COASTS ?

The Moroccan coastline, extending for more than 3500 km, is developing economically and socially on a rapid pace. Therefore, the coastal ecosystems sustain many pollution risks. Indeed, demographic pressure (more than 60% of the national urban population), urban (domestic wastewaters), industrial (more than 80% of the industrial activities), touristic (more than 53% of tourism), and agricultural development (intensive farming) are exposing coastal waters and hence marine organisms to increasing contaminant discharges that convey various chemicals into the sea. Furthermore, the Moroccan coast harbours several littoral systems, such as lagoons and estuaries which are ecologically and socio-economically interesting. Inclusively, their suitability (especially in lagoons) for aquaculture makes them important in terms of biological productivity (e.g., oysters and clams). Nevertheless, they may be also influenced by a number of pollution sources. Besides domestic wastes, they may especially face serious threats from agriculture, since the adjacent regions support extensive farming activity with corresponding use of pesticides and fertilizers.

The potential for damaging the fragile ecological balance of all these coastal ecosystems, as a result of unstable environment factors and enhanced anthropogenic pressure, justifies attempts of pollution monitoring.

NATIONAL BIOMONITORING PROGRAM

Based on the general observations above, and on its principal role in controlling pollution loads into the sea and preserving the marine resources, the INRH (Institut National de Recherche Halieutique) has carried out for many years an important program for long-term monitoring of the marine environment on the Atlantic and Mediterranean coasts of Morocco. The main axes of the program are: (1) studies of marine and littoral ecosystem functioning; (2) surveillance of marine environment quality and salubrity by a regular assessment of biological and chemical contaminants with their effects on marine fauna and flora. The phytoplankton and phytotoxins are subject to a specific surveillance.

The Moroccan Mussel Watch uses various species of molluscs bivalves (Fig. I), but mainly the Mediterranean mussel *Mytilus galloprovincialis* as a quantitative bioindicator of pollution (or sentinel species; Beeby, 2001), as in other worldwide monitoring programs (e.g., Cossa, 1989;

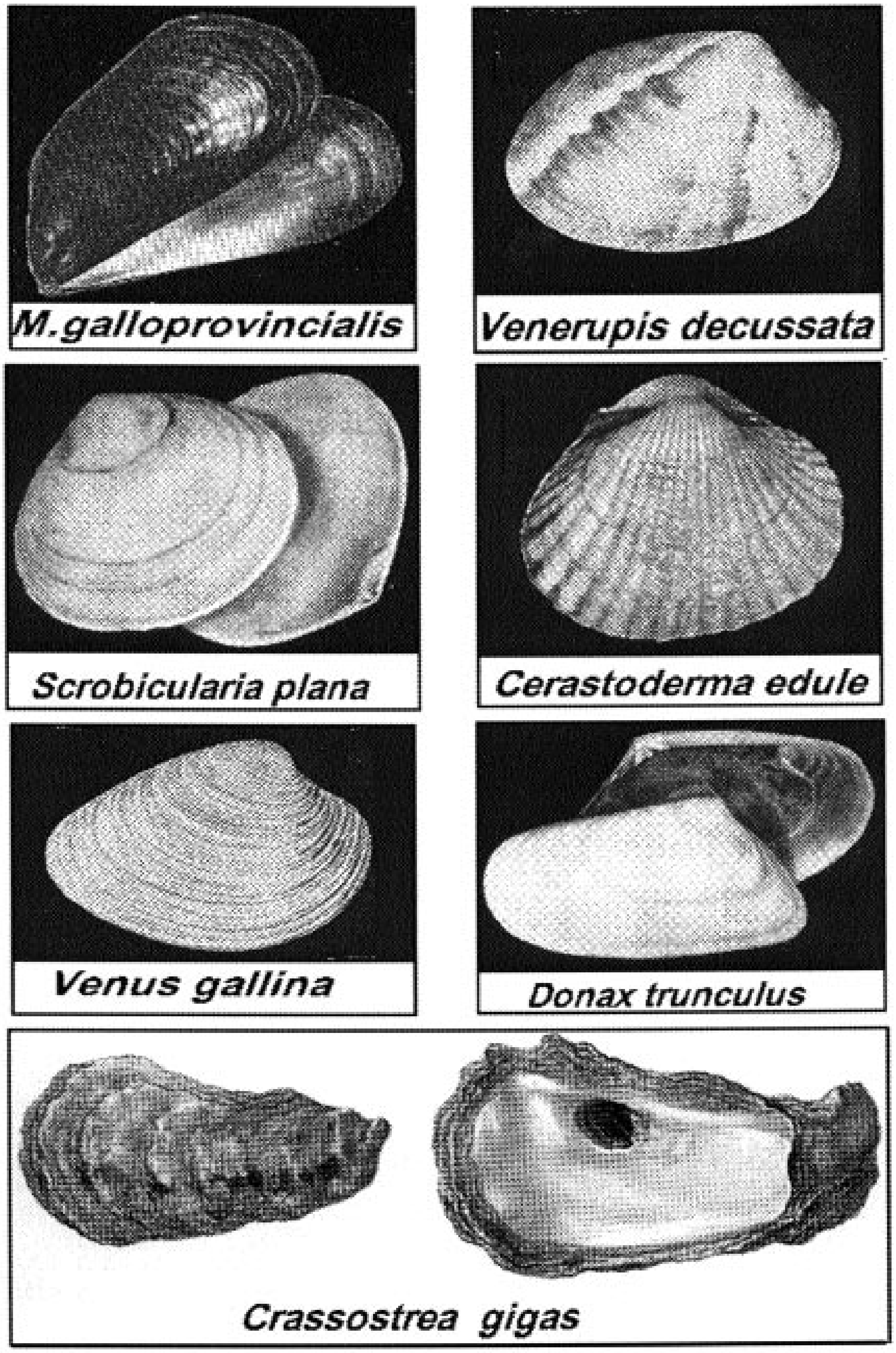


Fig. 1. Main studied molluscs.

Cantillo, 1998; Lares and Orians, 2001; Domouhtsidou and Dimitriadis, 2001). Moreover, various ecotoxicological studies have utilized mussels, particularly as they are commercially important and are components involved in the transfer of chemical contaminants along food chains including humans (Bryan, 1984; Claisse, 1989; Lauenstein and Dolvin, 1992).

The program strategy consists in seasonal sampling and analysis of waters, sediments and mussels (Fig. 2) in numerous locations along the coastline for many years leading to a Moroccan database. These locations are chosen to account for natural influences such as hydrographic inputs (upwelling, lagoons, estuaries) and anthropogenic ones such as urban, agricultural and industrial discharges. Furthermore, these locations harbor dense populations of mussels to ensure easier sampling.

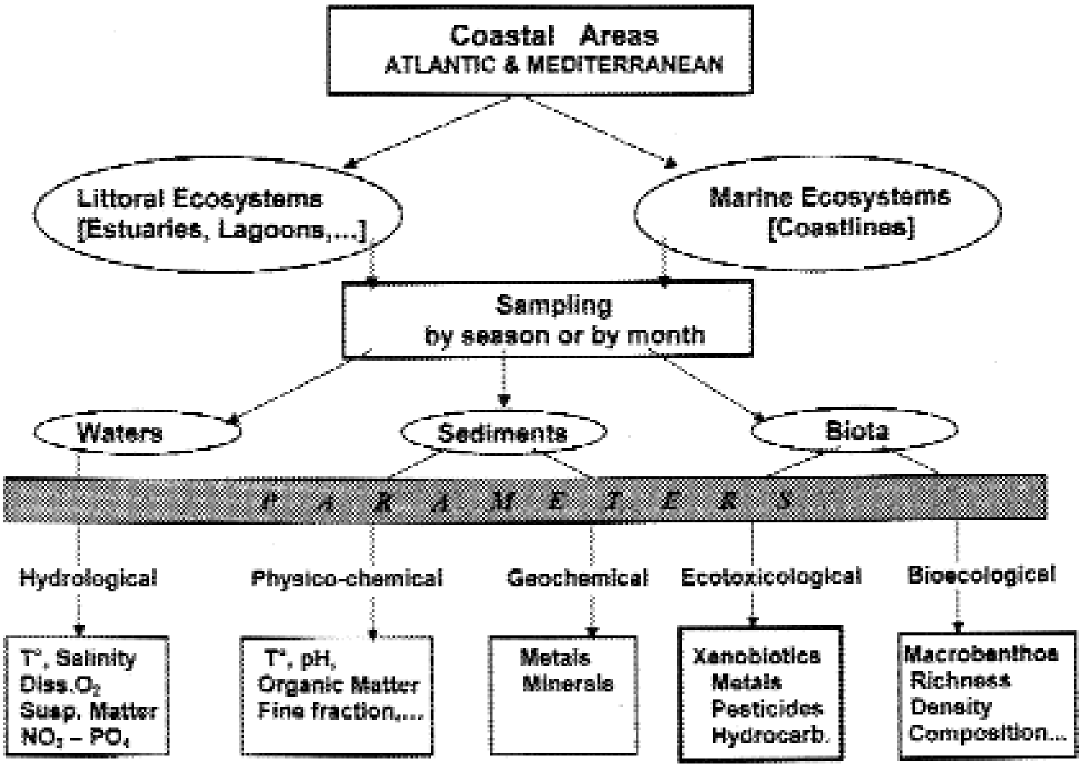


Fig. 2.

SPECIFIC STUDIES OF METALLIC POLLUTION

Environmental settings

The Mediterranean coast of Morocco

The Mediterranean coast of Morocco is irregularly extending for nearly 500 km, from the city of Tanger to Saïdia. Around 300 km of the coast is overhanging by the relief of the Rif's mountains, describing a large concave bow towards the sea (Fig.3).

On the west, from Tanger to Sebta, the coast is geomorphologically hilly with some capes and creeks. It becomes low and boggy between Sebta and Azla, containing some humid ecosystems such as marshes.

In the central region, from Oued Laou to Oued Nekkor mouth, the topography is elevated comparatively to the later zone. The dominant landscape on the littoral here consists of high hills and some abrupt cliffs. The narrow beaches are composed mainly by pebbles. Some alluvial plains are developing here with generally a little surface except those of Oued Laou and Oued Nekkor covering around 6000 hectares with some farming activities.

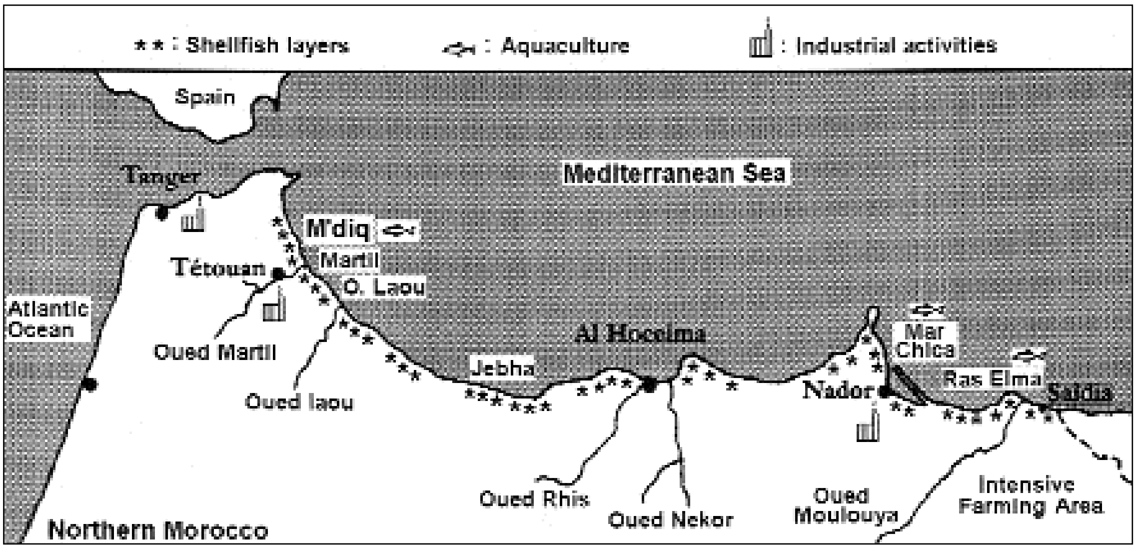


Fig. 3. Mediterranean coast of Morocco.

On the eastern part, the coast is heterogeneous, including some coastal and fertile plains, harbouring an intensive agriculture. Saïdia is the most large sandy beach (16 km) and probably one among the largest ones of the Mediterranean sea.

The Mediterranean coast of Morocco contains some interesting littoral ecosystems such as: estuaries (Oued Laou, Oued Nekkor and Oued Mouloya); lagoons (Mar Chica at Nador), bays (Tanger and Al Hoceima) and marshes (Fnideq, Martil).

The pollution sources are mainly urban and industrial activities in the western and the eastern parts of the Mediterranean coast, emanating from the cities of Tanger, Tetouan and Nador. In the later region, there is also a significant agricultural source of pollution. In the central sector, the inputs to the sea consist mainly in soil erosion and deforestation, added to urban wastes from Al Hoceima city.

The studied area, situated in the western part of the Mediterranean coast of Morocco, around Tetouan city, extends for almost 100 km. It is known for an important tourism, leading to high road's traffic around the watering places especially during summer. The fishing and aquaculture are well developed at M' diq station and Oued Laou. The industrial zone of Tetouan city could be considered as an important source of pollution into the sea. The most part of Tetouan industrial and urban wastewaters are discharged into Martil river (Oued Martil) which carries them until the sea via its mouth.

MATERIALS AND METHODS

Study stations

On the Atlantic coast, fourteen coastal stations were studied between the towns of Larache, a coastal stretch on the North Atlantic seaboard and Safi in the centre, that extends for nearly 400 km (Fig. 4). On the Mediterranean coastline, five locations are focused around Tetouan city in the western sector of the Moroccan Mediterranean sea (Fig. 3).

The stations are chosen in order to take into account the different coastal regions along the considered coastlines and the socio-economic activities likely to discharge pollutants into the sea. The existence of estuaries and lagoons was also taken into account.

Samples

Mussels *Mytilus galloprovincialis* were collected by hand at the mean low water of neap tides on rocky substratum on the Atlantic coastline. On the Mediterranean coastline, an other bivalve was studied, the cockle *Cerastoderma edule* collected on mud-sandy shore at the same conditions as mussels. Around fifty adult individuals (4-5 cm in size for the mussel, 2,5-3 cm for the cockle) were sorted on the field. They were then cleaned and stored in polyethylene flasks containing water from the sampling site and kept in a cooler (+ 4°C).

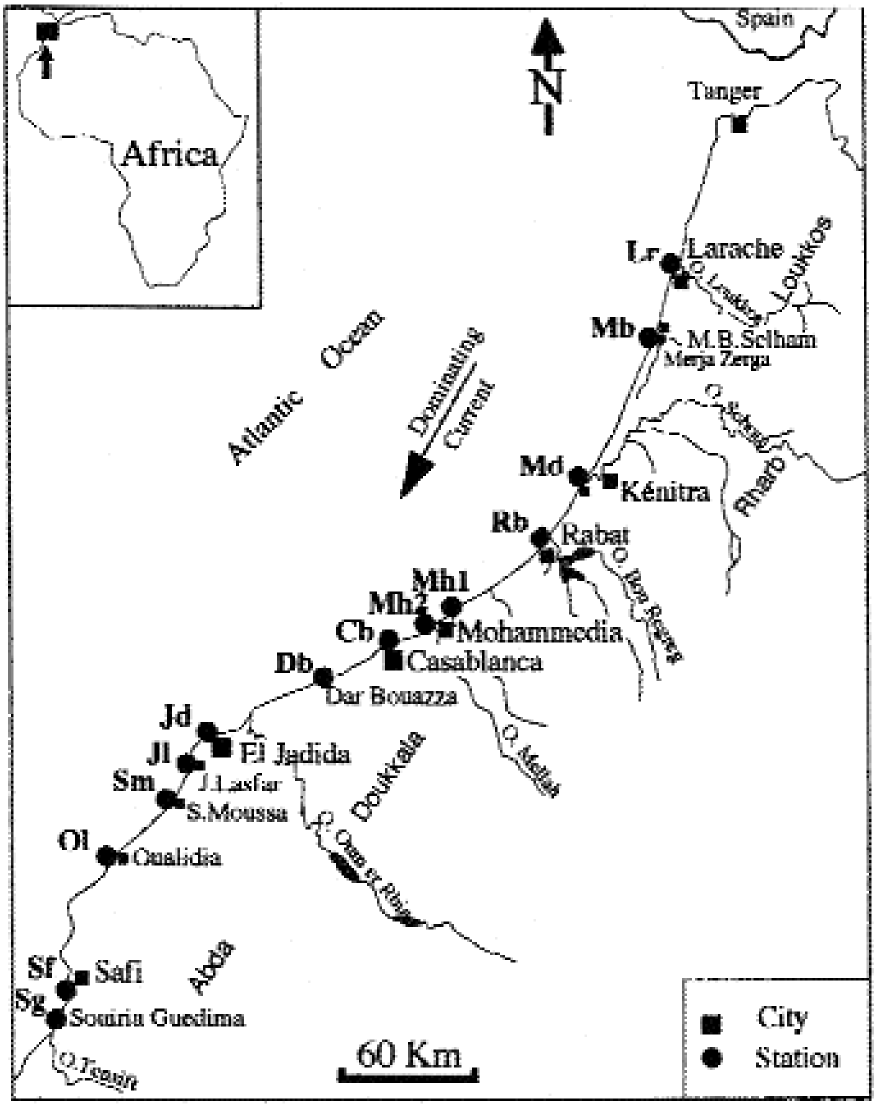


Fig. 4. Atlantic coast of Morocco

Analyses

After a period (up to 36 h) of purging in water from the sampling site, the soft parts of the bivalves were extracted from their shells, thoroughly cleaned and drained for 2 h. They were then minced fine using a grinder equipped with a stainless-steel blade and freeze-dried for conservation until analyses. A one gram sub-sample was homogenized in a porcelain mortar before the mineralisation step. Mineralisation was performed with 4 ml Suprapur nitric acid (Merck) in polyethylene tubes at room temperature for 12 hand then at 90°C for 3 h. After cooling, the digestat was brought to 50 ml by addition of bi-distilled water.

Metals (Cu, Cd, Zn, Mn in mussels and Cd, Pb, Hg in cockles) were measured by an atomic absorption spectrophotometer (Perkin Elmer 3100) equipped with a graphite furnace (HGA 600/700).

A special treatment was applied for Hg, using HNO3 (Merck quality), V_2O_5 (extra pure, Merck quality), $K_2Cr_2O_7$ (Merck quality), ultra pure and deionized water (Milli-Q) and FIMS 100 as analysis system equipped by an automatic sampler.

The calculation of the coefficients of variation for each metal analysis, based on reproducibility of a replicate analyses on one sample, show a range from 8% to 13%, i.e. a mean reproducibility of almost 90%. The accuracy of the method was tested using a Certified Reference Material obtained from the International Atomic Energy Agency (CRM N° MA-A-2/TM). The error brackets obtained for the various metals analyzed ranged from 7 to 9%. For Hg, the biological certified material comes also from IAEA (Reference: Standards Reference Material, S.R.M.) with a certified concentration in total Hg of 0.061 ± 0.004.

RESULTS AND DISCUSSION

Mussels on the Atlantic coast

Spatial variations in mean Cu concentrations were characterised by three clearly distinct peaks of variable intensity at Jorf Lasfar, Safi and Casablanca (which showed higher levels than other sites). Geographical variations in mean Cd concentrations were quite close to those for Cu (Fig. 5). The peaks at the Jorf Lasfar and Safi sites were markedly above those of the other stations. The nature of activities nearby (processing of phosphate ores) clearly indicates that inputs came almost exclusively from industrial operations. The lowest levels were recorded at Dar Bouazza. Mussels from Mohammedia-Casablanca showed the highest Zn concentrations, followed by those collected at Jorf Lasfar, Sidi Moussa and Safi. Similar levels were observed at Mehdiya and north Mohammedia, and lower levels at My Bou Selham and Dar Bouazza. Comparison of mean Mn concentrations showed fairly high levels at stations near estuaries and lagoons, notably Mehdiya, Mohammedia-town, Rabat, Moulay Bou Selham and Sidi Moussa (Fig. 4). These results tend to confirm the continental source of Mn.

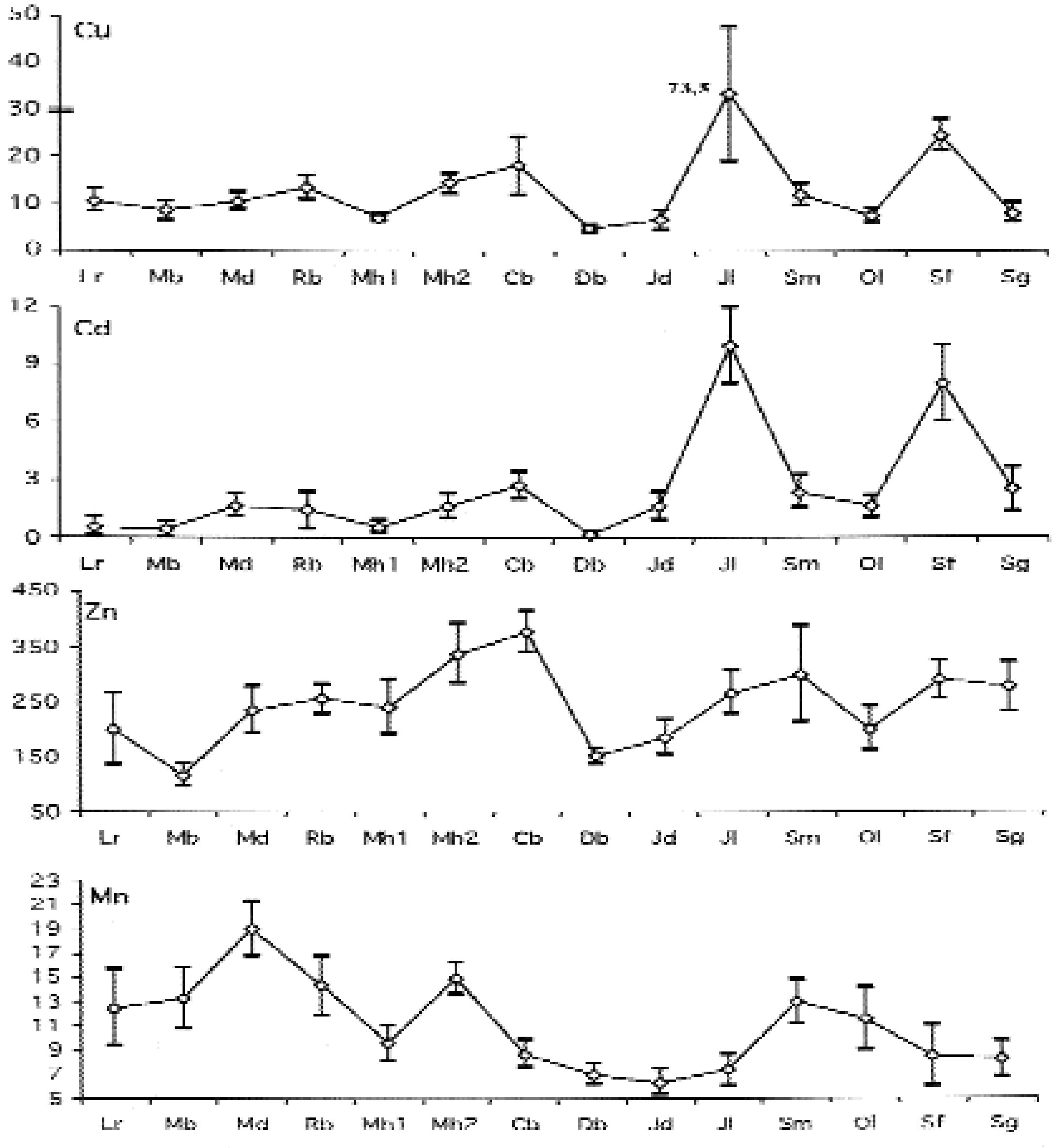


Fig. 5

Phosphogypsiferous discharges from local chemical complexes were responsible for much higher Cd and Cu concentrations in mussels at Jorf Lasfar and Safi than at other coastal stations (Chafik *et al.*, 1996; Cheggour *et al.*, 1999a). The relatively high Cu and Cd levels found at Mohammedia, Casablanca, Mehdiya and Eljadida are indicative of the role of urban and industrial activities in generating metal pollution. Cadmium concentrations recorded at "sites not primarily industrial" were generally lower, as at Oualidia and Sidi Moussa on the central Atlantic coast and to a lesser extent at Larache and My Bou Selham on the north coast. These concentrations could relate to the highly developed agricultural activities in these regions, which use large amounts of fertiliser and plant care products. It is also possible that the upwelling of waters rich in trace elements was a contributing factor (Bruland and Francks, 1983), notably at Oualidia and Sidi Moussa where this phenomenon is nearly constant.

The Zn concentrations recorded in mussels indicate the dominance of urban and industrial sources all along the Larache-Safi axis. Levels were particularly high in the Mohammedia-Casablanca region where waste water discharge outlets are large.

A continental origin was more apparent for Mn. The tissue concentrations of this metal in mussels were quite elevated in stations close to the mouths of estuaries and lagoons and remarkably low in typically urban areas distant from these points. These results are in close agreement with other Moroccan studies indicating that Mn is an excellent tracer of continental inputs into aquatic systems produced by the breaking up of rocks and soils of surrounding watersheds (Carruesco, 1977; Mayif, 1987; Texier *et al.*, 1994).

Generally speaking, the results suggest a dominant source of heavy metal contamination from urban and industrial activities (Fig. 5). The geographical distribution of metallic levels in mussels exhibit some hot spots especially for Cd as a result of exposure to industrial effluents (Cheggour *et al.*, 1999b). Localities relatively far from anthropogenic sources, especially the Atlantic lagoons, with a considerable potential for aquaculture, assessed both by mussels (Chafik *et al.*, 2001) and other molluscs as oysters (Cheggour *et al.*, 1999c) and cockles (Cheggour *et al.*, 2000; 2001) were, fortunately, little affected by chemical pollution.

Cockles on the Mediterranean coast

The results of the current specific study show clearly low concentrations of Cd and Hg in *Cerastoderma edule* tissues from all the studied stations, reflecting a good quality of the cockles and their environment (Fig. 6). This result is not surprising, since there is no industry in this region which may release these metals into the sea, comparatively to the Atlantic coast. The cockles could be contaminated from the Hg and Cd storage in sediments where they live and feed, in agreement with Cossa *et al.* (2001) in the Gulf of Cadiz (Spain).

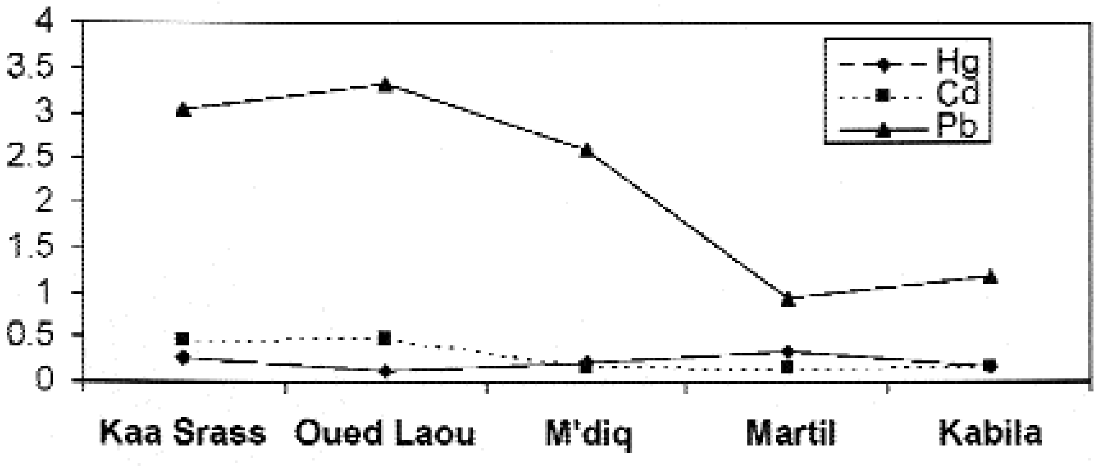


Fig. 6. Variation of interannual (1997-2000) for metals in the cockle Cerestoderma edule on the Mediterranean coast of Morocco.

The low measured levels in our study are likely mainly originating from urban waste waters discharged into the sea, added to “a natural baseline”. According to a mercury speciation study in the waters of the Alboran sea (Cossa *et al.*, 1994), the sub-thermocline constitutes an important reservoir of dimethylmercury (DMHg) available for accumulation by deep-Iiving organisms, such as fish. Passive diffusion of dissolved gaseous DMHg through the gills may be a significant pathway for its bioaccumulation. Nevertheless, the authors accurate that the DMHg accumulation by the coastal organisms is lower than those of off-shore waters, since this metal is absent or at very low concentrations in coastal regions due to its volatility. On the French coast, Claisse *et al.* (2001) reported that molluscs exhibited quite high total mercury concentrations in some sites, but relatively low proportions of methyl-Hg, suggesting an increased bioavailability of non-methylated forms of the element in the coastal areas, under the influence of terrigenous inputs. This observation raises the issue of the chemical speciation of mercury in coastal environments, atopic poorly understood.

Despite the magnitude of urban (and industrial) liquid-solid discharges and continental inputs, which would seem to account for most of the Cd and Hg concentrations found in cockles tissues, atmospheric emissions cannot be overlooked. Indeed, atmospheric deposition is an important source of trace metals such as Hg and Cd to aquatic environments and is a potential contributor to enhanced concentrations of these metals found in aquatic organisms (Mason *et al.*, 2000). Nriagu and Pacyna (1988) estimated that atmospheric emissions of Cd from anthropic activities represent several thousand tons (between 3 and 12) per year, mainly from industrial sources. For the Western Mediterranean, Migon *et al.* (1991) estimated annual atmospheric fluxes (in thousands of tons) at 0.14 to 0.17 for Cd. For North America, Allen and Halley (1980) noted that atmospheric inputs represent a large proportion of the aquatic load for Cd (nearly 42%). On the other hand, it has been estimated that the anthropogenic load to the atmosphere has increased total emissions by a factor of 5 for Cd, and 3 for Hg (Nriagu, 1989; Mason *et al.*, 1994).

The concentrations of Pb found in *C. edule* tissues are significant and indicate a noticeable contamination level of the bivalve, especially in Kaa Srass, Oued Laou and M’diq locations. This result could be related to an important traffic on the coastal roads, especially during the summer season, when the tourism activities are clearly enhanced. The run-off of these roads especially during the rain falls season, is an important source of Pb, since this element is a fundamental compound in the motor’s fuel. Furthermore, the fishing effort (mainly at M’diq) could be an other reason for the Pb increase in the environment.

Although the current study only covered a small area comparatively to the Atlantic one, the results show a good quality and salubrity of the coastline, especially for Cd and Hg which are high toxic trace metals. It will be useful to extend the study to the other sectors of the Mediterranean coast of Morocco.

THE MEDITERRANEAN MUSSEL WATCH IN MOROCCO

The results of the specific studies presented above are just an example of the biomonitoring activities carried out by the INRH, on the Moroccan coasts. In particular, on the Mediterranean coastline, the INRH has two marine stations at Nador and M’diq, where the same strategy (as for the Atlantic) is followed in monitoring the marine environment. The analyses are done in the central laboratory at Casablanca. To improve the Mediterranean sea monitoring, it will be useful to add more sites in the Mussel Watch network. Some potential locations would be chosen along the area between Tanger and Saïdia since they contain mussels in dense populations.

In terms of pollutant inputs to the sea, three sectors could be distinguished on the Moroccan Mediterranean coastline (Fig. 3): (1) western coast with a dominance of urban and industrial sources of pollution discharges especially from the agglomerations of Tanger and Tétouan and their industrial areas, which develop rapidly; (2) central coast where the main inputs to the sea are terrestrial, emanating from soil erosion and deforestation. Nevertheless, AI Hoceima, which is the main city in this sector, accounts for an important urban source of pollution, since its waste-waters are discharging directly into the sea; (3) eastern coast: as for the western part, there is here a dominance of urban, and industrial sources of pollution load, especially from Nador city.

Furthermore, the intensive farming activities developed in this area may also discharge a considerable amount of chemicals into the sea, since they use high quantities in pesticides and fertilizers. Beside these sources, a marine origin of the pollution load must be considered in the total budget of the contamination, due mainly to two factors: the marine traffic especially closer to Gibraltar Strait and the harbor activities, especially at Tanger, M'diq, AI Hoceima and Nador.

We suggest to take, at least, one station in every sector described above to represent different morphological areas and also various inputs of contaminants into the sea, especially urban and industrial wastes. Many parameters would be measured such as the hydrological ones to assess water quality.

For contaminants, beside radionuclides to which the mussel watch network is first dedicated, other xenobiotics could be further integrated as a second step (metals, pesticides, hydrocarbons,...). Some additional parameters could be taken as complements to the principal aim of the network, such as the enzymatic biomarkers (biochemistry), the biological characteristics of mussel populations (densities, geographical distribution) and the bio-ecology of intertidal communities including mussels around pollution sources. On the other hand, some exceptional events have to be steadily surveyed: (1) marine traffic to prevent accidents and potential black tides; (2) toxic phytoplankton development to avoid human intoxications by consumption of toxic mussels and (3) atmospheric inputs since it is an important source of contaminants.

Radioactivity biomonitoring in the Italian marine environment

R. Delfanti, F.G. Pannacciulli and C. Papucci

ENEA, Marine Environment Research Centre, La Spezia, Italy

Since 1976 mussels have been largely used for environmental monitoring (Goldberg *et al.*, 1978). In Italy biomonitoring of mussels is performed through two main national networks: The Italian National Network for the survey of marine radioactivity and The Italian Programme for Marine Coastal Monitoring. The first was initially coordinated by the Italian National Agency for New Technologies, Energy and Environment (ENEA) and is presently run by the Italian National Agency for Environmental Protection (ANPA); the second one is coordinated by the Ministry of the Environment. Below is an outline of the main features of the two networks which includes parameters in use, sampling sites and methodologies.

THE ITALIAN NATIONAL NETWORK FOR THE SURVEY OF MARINE RADIOACTIVITY

The Italian National Network for the Survey of Marine Radioactivity is operating since 1957 (run by ENEA until 1995 and later by ANPA; ENEA, 1958 and ANPA, 1998) and requires the measurement of gamma-emitting radionuclides in seawater, sediments, algae or aquatic plants, fish and mussels at 5 main stations along the Italian coasts (Fig. 1):

- La Spezia (Ligurian Sea),
- Naples (Tyrrhenian Sea),
- Taranto (Ionian Sea),
- Venice (Adriatic Sea),
- La Maddalena (Sardinia, Tyrrhenian Sea).

Samples are collected twice a year, in summer and winter. Regular measurements of gamma-emitting radionuclides in mussels began in the eighties and the results are regularly published since 1986 (ENEA, 1989). The aim of the network is to survey the pattern of environmental and dietary contamination in order to assess the radiation doses which the Italian population may receive.

Mussels (*Mytilus galloprovincialis*) were collected, when possible, from natural mussel beds but, as very often they were not available, we convened to take samples from mussel farms located in the study area, before treatment for commercial use. The soft parts were separated and samples dried at 105°C to reach constant weight, homogenised, transferred to calibrated containers for gamma spectrometry with high purity germanium detectors. The system was calibrated using a certified standard liquid source and the accuracy of the results regularly checked by analysing standard reference materials.

Before the Chernobyl accident, in the period 1980-85, ^{137}Cs was the only gamma-emitting radionuclide detectable in mussels along the Italian coasts. Its concentration in the soft parts

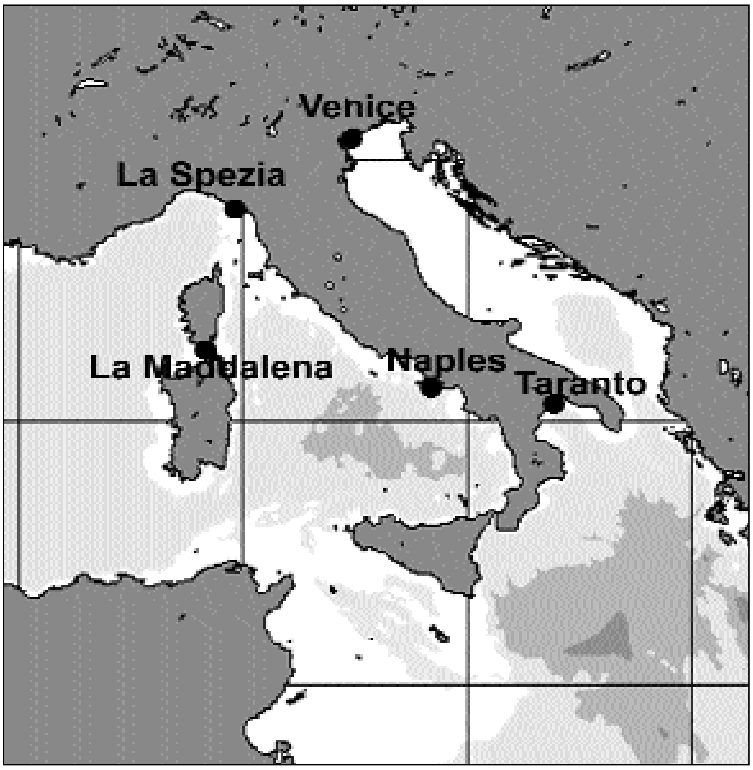


Fig. 1. Location of the sampling stations of the Italian National Network for the Survey of Marine Radioactivity.

ranged between 0.14 and 0.30 Bq/kg (wet weight of soft parts), the concentration factor was in the range 25-45 and there were no significant geographical differences, reflecting the homogeneous levels of the radionuclide in seawater.

The Chernobyl accident, and the patchiness of its fallout deposition in Italy, completely changed the scenario in the following years. The fallout deposition was highest in the Northern basins, and particularly in the Northern Adriatic Sea, where ^{137}Cs levels in seawater were one month later still 20 times higher than before the accident and, due to water circulation and input from rivers, remained higher than in the other basins until 1990.

From 1986 to 1990, ^{137}Cs concentration in mussels has always been higher in the Adriatic than in the Ligurian Sea. The sampling frequency was increased at some stations after the accident and it was possible to follow the loss of ^{137}Cs from mussels after the pulsed input in May 1986. Fig. 2 shows the time trend of ^{137}Cs concentration in seawater and mussel soft parts in Venice from 1986 to 1990. After 1990, the levels in seawater and mussel soft parts have slowly decreased and are now about 3 Bq m^{-3} and 0.15 Bq kg^{-1} w.w. respectively. The surveillance network proved then to be adequate to describe the new situation of radionuclide distribution in Italy derived from sudden accidental releases.

THE ITALIAN PROGRAMME FOR MARINE COASTAL MONITORING

Since 1996 the Italian Ministry of the Environment is running a national network aimed to collect data, produced at a regional level, for the survey of the coastal environment (Ministry of the Environment and ICRAM, 2000). In 2001 the programme has been implemented in terms of spatial scales, matrices and number of parameters analysed. Mussels are used as bioindicators for:

- organic compounds: chlorinated hydrocarbons, TBT, polyciclic aromatic hydrocarbons;
- trace metals: Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, V and Zn.

At the same sites where mussels are collected, physico-chemical and trophic characteristics are also evaluated (temperature, salinity, dissolved oxygen, chlorophyll-a, nutrients, phyto- and zooplankton biomass).

Sampling is carried out twice a year in correspondence of the time when gonads reach their largest and smallest size. The spatial scale of this programme (73 sites in total, all along the

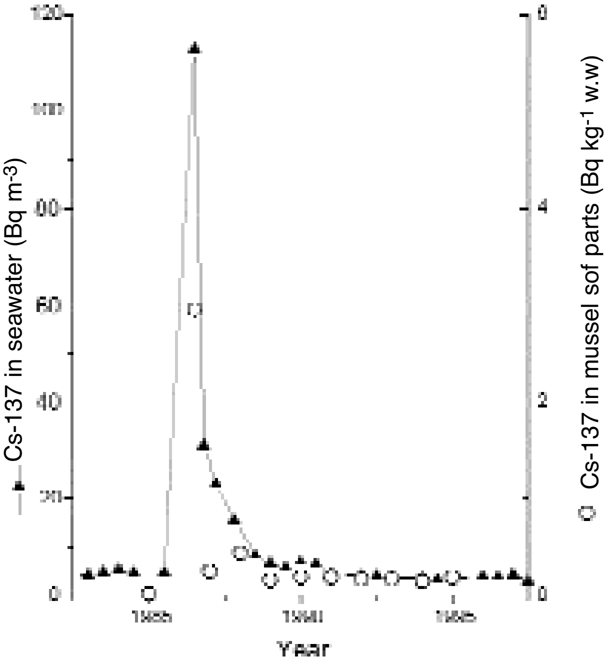


Fig. 2. ^{137}Cs concentration in mussel soft parts and in seawater. Venice, 1986-1990.

Italian coast) is much finer than the radioactivity monitoring network: in each region sampling is carried out at 3-4 impacted sites versus one control area (generally a protected area). The protocol recommends the use of natural mussels when available, or from transplanted and caged specimens. All collected data will be stored in the central information system of the Ministry of the Environment for processing and analysis.

CONCLUSIONS

A well structured network of mussel sampling sites including natural populations, caged and farmed mussels exists along the Italian coasts. As in Italy the radioactivity levels in seawater are homogeneous and there are no important point sources, we propose to participate in the Mediterranean Mussel Watch Programme with a limited number of monitoring sites for routine sampling (La Spezia, Naples, Taranto and Venice) can be guaranteed by ENEA through collaboration with other institutions. Specimens will be collected from mussel farms before stabulation. Sampling will be carried out twice a year, in May-June and September-October (respectively one month after and one month before spawning). Initially the focus will be on gamma-spectometry, determining levels of ^{137}Cs, later the analyses will be extended also to ^{210}Po. Close collaboration will be maintained with the Italian Ministry of the Environment, in relation to its programme for Marine Coastal Monitoring. This link will allow a quick expansion, if and when necessary, of the basic structure of the radioactivity network. In addition, it will provide for the future the opportunity to extend the present Mediterranean Mussel Watch Programme also to conventional pollutants.

The Sevastopol bay radio-chemoecological monitoring programme using the mollusc *Mytilus galloprovincialis*

Egorov V.N., Polikarpov G.G., Kulebakina L.G., Kostova S.K., Zherko N.V., Mirzoyeva N.Vu., Stokozov N.A., Lazorenko G.E. Malakhova L.V.

Institute of Biology of the Southern Seas, National Academy Science of the Ukraine, Sevastopol

Due to its geographical position, the Black Sea was strongly polluted with artificial radioactivity: by direct fallout from atmospheric nuclear weapons tests and from the Chernobyl NPP accident. In the early 1990s the decrease of industrial production was registered as a result of the USSR disintegration. In this context, long-term monitoring has been carried out in the Sevastopol bays (Egorov *et al*., 2000; Zherko *et al*., 2001; Kostava *et al*., 2001) using concentrations of ^{90}Sr, ^{137}Cs, ^{210}Po, PCB and Hg as an indicator of the ecological state of the mussel *Mytilus galloprovincialis*.

FOCUS OF INVESTIGATIONS

- determination of contents of the artificial radioactive nuclides (^{90}Sr and ^{137}CS), and natural ones (^{210}Po and ^{210}Pb) as well as chemical contaminants (mercury, PCB) in water and the mussel *M. galloprovincialis*;
- trends of radioactive and chemical contamination the components of the Sevastopol bays ecosystem, using *M. galloprovincialis* as the bioindicator.

MATERIALS AND METHODS

The study was carried out in the Sevastopol bays. The sampling location is illustrated by Fig. 1. Average errors of ^{90}Sr, ^{137}Cs, ^{210}Po, Hg and PCB measurements in components of the Sevastopol bays ecosystem are equal to 7-18%. The intercomparison of the radionuclides measurements was fulfilled with the IAEA, WHOI and EPA (USA), RISOE National Laboratory (Denmark), as well as such of PCB measurements - with IAEA. The standard solutions are used for Hg calibration measurements. LLD for Hg determination is not more than 1 ng per sample.

RESULTS AND DISCUSSIONS

Concentrations of ^{90}Sr and ^{137}Cs as recorded since 1986 in the water and *M. galloprovincialis* of the Sevastopol bays are shown in Figs. 2 and 3. They demonstrate that in the water of the Sevastopol bays a rapid decrease of ^{90}Sr and ^{137}Cs concentrations in the water was observed during the first year after the Chernobyl NPP accident. For the next observation period ^{90}Sr concentrations in the water decreased exponentially with the time constant 8.7 years *versus* 10.5 years for ^{137}Cs. Mussels as bioindicators reflected these processes in environment. The approximation of ^{90}Sr and ^{137}Cs concentrations change in mussels by exponential functions allows to estimate the tendencies of the marked radionuclides concentrations changes in water with the errors of 6,9% and 14,3 % accordingly.

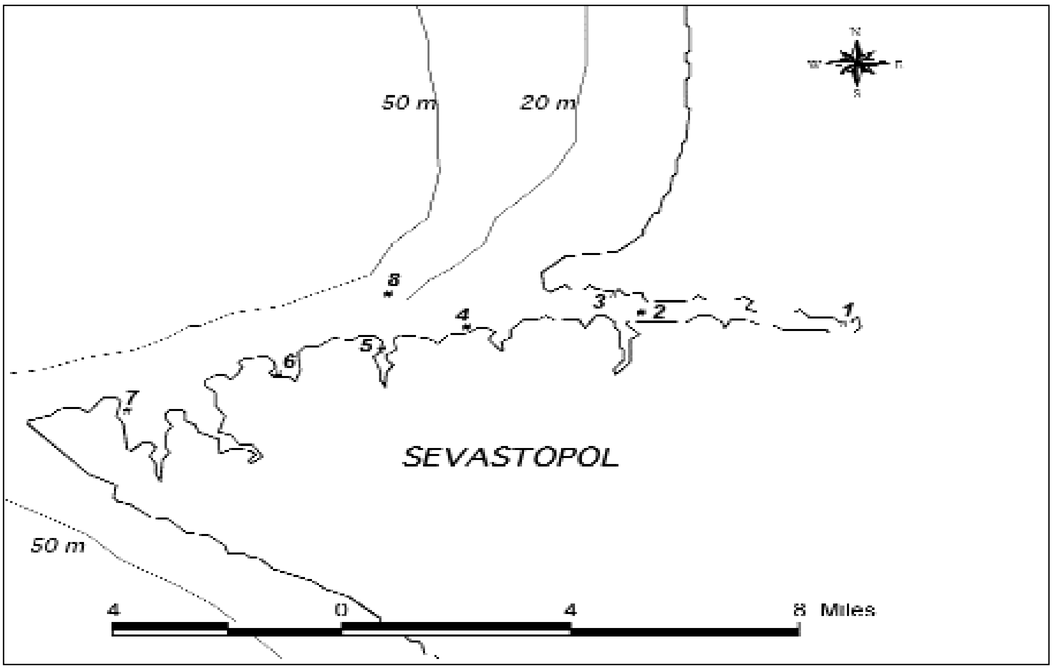


Fig. 1. list of stations
St. 1 - The Black River Mouth. 44°36.45N; 33°36.08E, depth = 3-6 m
St. 2 - Southern Bay (Pavlovskiy Cape). 44°37.06N; 33°32.13E depth = 12-15 m
St. 3 - Northern Bay (Corrosionnaya st.). 44°37.63N; 33°31.90E, depth = 10 m
St. 4 - Martynov Bay. 44°36.97N; 33°30.30 E, depth = 5 m
St. 5 - Streletskay Bay. 44°36.55N; 33°28.21 E, depth = 1-4 m
St. 6 - Omega Bay. 44°35.95N;33°26.65E, depth = 1-3 m
St. 7 - Kazachja Bay 44°35.26N; 33°24.31 E, depth = 10 m
St. 8 - Fishery Station 44°36.90N; 33°28.60 E, depth = 20-50 m

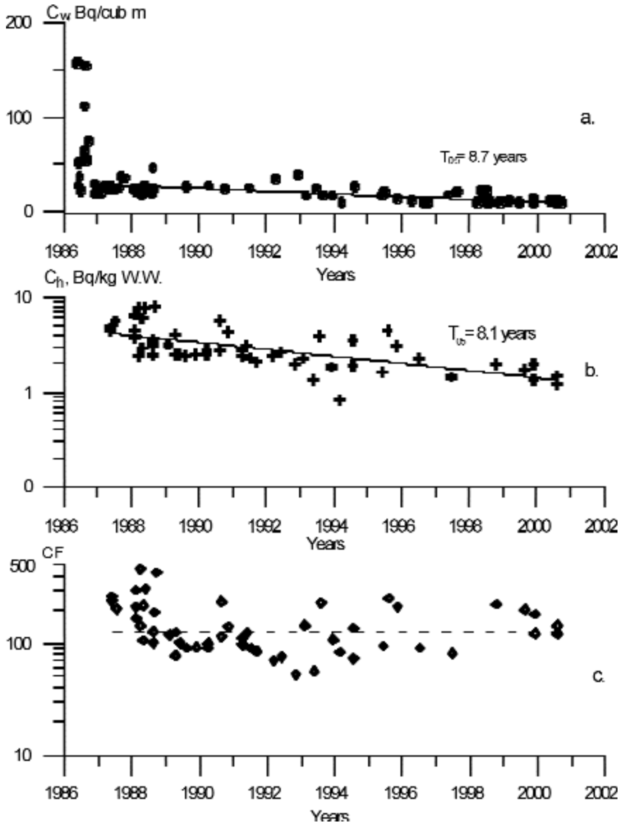


Fig. 2. ^{90}Sr concentration in **(a)** water and **(b)** molluscs Mytilus galloprovincialis (shell 3-5 cm) of the Sevastopol bays; **(c)** Concentration factor (CF=C_h/C_w).

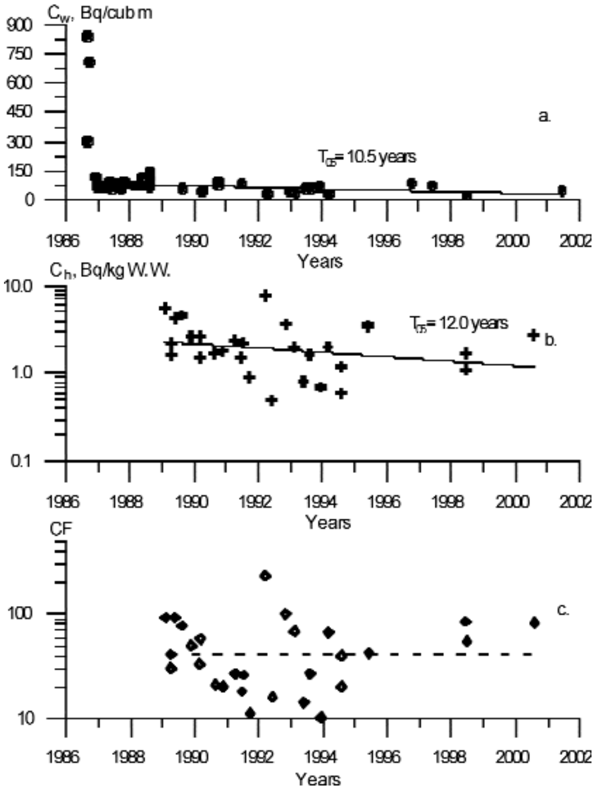


Fig. 3. ^{137}Cs concentration in **(a)** water and **(b)** Mytilus galloprovincialis (soft tissue) of the Sevastopol bays;
(c) Concentration factor (CF = C_h/C_w).

Calculations of ^{90}Sr and ^{137}CS concentration factors (CF) of *M. galloprovincialis* have shown that these data are highly variable, likely reflecting the dynamic state (Fisher *et al.*, 1996) of the system "radionuclide in the aquatic environment - mussels".

The distributions of ^{210}Po concentrations in soft tissues of mussels (of different size) is shown in Fig. 4, indicating an inverse relation with the animal weight.

Changing PCB levels in water, mussels and bottom sediments are shown in Fig. 5.

Changing Hg concentration in water and in *M. galloprovincialis* is shown in Fig. 6.

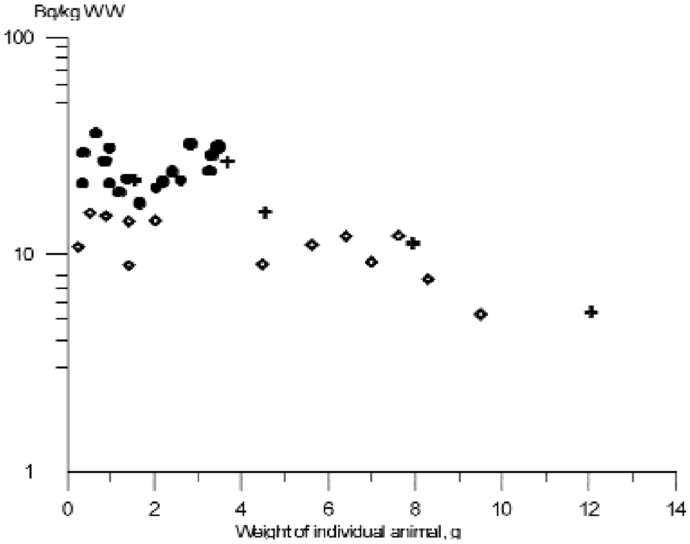


Fig. 4. Dependency of ^{210}Po concentration in soft tissues of Mytilus galloprovincialis (wet weight of individual) :
• Kazachya bay;
+ Belbek river mouth;
◊ St. 3 of the Sevastopol bay.

The overall relation between Hg concentrations in the water of the Sevastopol bays and in *M. galloprovincialis* is illustrated in. Fig. 7. These data show that CFs of Hg decrease for *M. galloprovincialis* upon increase of Hg concentration in water. The dependence between Hg concentration in water (Cw) and in mussels (Ch) *M. galloprovincialis* is described by the Langmuir equation :

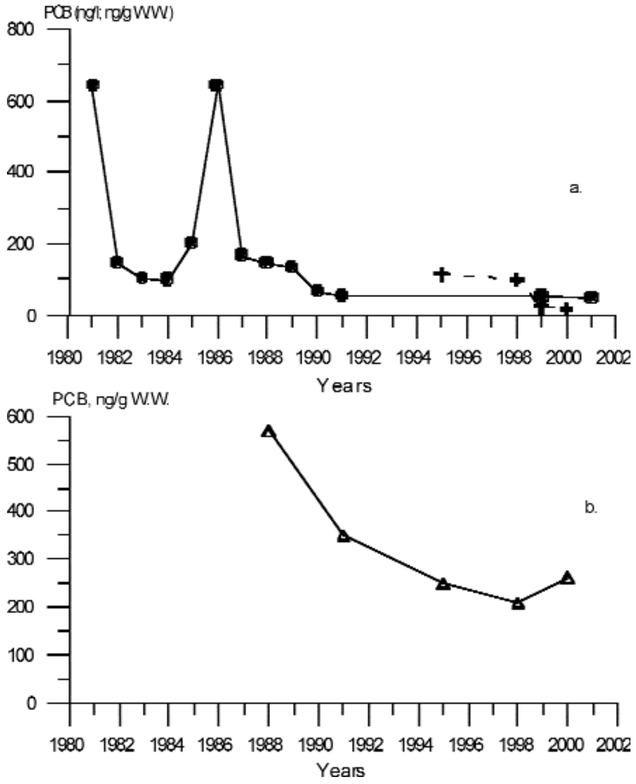


Fig. 5. Change PCB concentration in water (+), in soft tissues of Mytilus galloprovincialis (•) and (Δ) in bottom sediments (layer 0-5 cm) of the Sevastopol bays.

$$C_h = \frac{k\, C_{max}\, C_w}{1 + k\, C_w},$$

where: k = 0.0432 ±0.0115 (1/I) and C_{max} = 46.7 ± 8.9 (ng/g W.W.).

CONCLUSION

Within the framework of radio-chemoecological monitoring, the use of *M. galloprovincialis* as the bioindicator has allowed (with a sufficient degree of adequacy) to research the time trends of radioactive and chemical pollutants in the components of the Sevastopol bays ecosystem. Precision increase according to the increase of the ratio between the scales of time of examination of tendencies and scales of time of sorption proceeding and metabolic processes in mussels in relation to the studied pollutants.

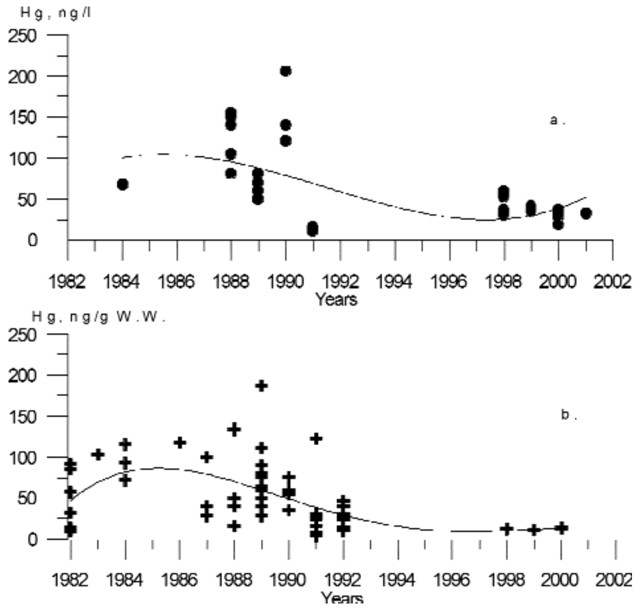


Fig. 6 Change Hg concentration in water (•), in soft tissues of Mytilus galloprovincialis (+) in the Sevastopol bays.

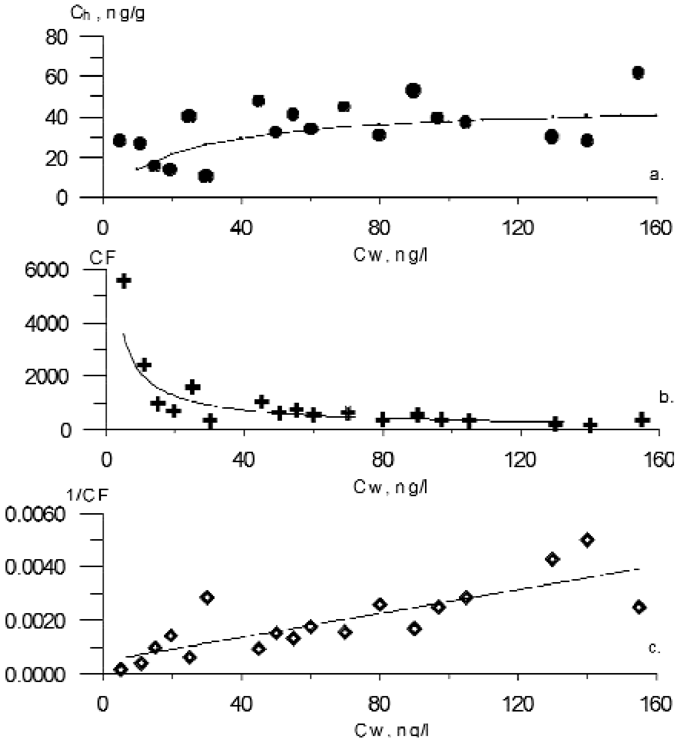


Fig. 7. Dynamic of the system “Hg in the Sevastopol bays water – Mytilus galloprovincialis molluscs “:
a – average Hg concentration in molluscs (soft tissue) vs Hg concentration in water (C_w);
b – Hg Concentration factor (CF = C_h/C_w) in molluscs vs C_w;
c – Langmuir equation (Nesmeyanov, 1978) in the scale modified Laynuiver -Bark equation (Patton, 1965).

Considerations for a mussel-based monitoring program for radioactive contaminants in the Mediterranean Sea

Nicholas S. Fisher

Marine Sciences Research Center, State University of New York, Stony Brook, New York, USA

The idea of using marine mussels to serve as sentinels of coastal contamination was proposed and first tested in the 1970's (Goldberg *et al*., 1976, 1983) and has since been widely accepted throughout the world. It is currently in practice in different sized national programs in many regions. Numerous reviews have identified the factors which make mussels useful as bioindicators of coastal contamination (e.g., Phillips, 1980). Among the most attractive aspects of using these animals as bioindicators of contaminants are the following attributes: mussels are ubiquitous, sessile, easy to collect, not easily poisoned by high contaminant concentrations, and any contaminant within the tissues of the animals is, by definition, a contaminant that is in a form that was available for bioaccumulation ("bioavailable"). Clearly, knowing the bioavailable fraction is critical, since the principal concern regarding contaminants is tied to their potential effects on living organisms (including man), but only bioaccumulated contaminants have the potential to impact living organisms. Further, we now know that many contaminants, most particularly metals and metalloids, can speciate in different forms in natural waters and that not all forms are available for biological uptake (Campbell 1995). Thus, the uptake of contaminants in mussels gives us an idea of the concentration of bioavailable contaminants in a region. Moreover, contaminants in mussel tissues, including shells, have been shown to reflect ambient dissolved contaminant concentrations (Bjerrgaard *et al*., 1985; Wang *et al*., 1996), and the mussel serves to integrate its past contaminant exposure over time; thus, analyzing mussels does not just provide an instant "snapshot" of the immediate conditions but rather a picture of contaminant levels over a period of weeks to months. Because contaminant concentrations in mussel tissues seem to generally reflect ambient contaminant concentrations, the underlying assumption that these organisms actually reflect environmental concentrations appears to be valid.

However, it is also important to recognize that mussels, being animals, eat, and can in fact accumulate contaminants from their food (usually phytoplankton, but other suspended particles are also ingested) in addition to accumulating contaminants from the dissolved phase. This can greatly complicate the overall picture because different contaminants can be assimilated out of ingested food with greatly different efficiencies and the relative contribution of contaminant uptake from food varies enormously among contaminants and can vary as well with the quantity and quality of the suspended particulate load (Wang and Fisher, 1999a,b). By using the modeling approach described below, the relative proportion of a mussel's total body burden for any given metal that is attributable to dietary uptake has been shown to vary from as little as about 20% for Cd to as much as 99% for Se.

Various studies (Fisher *et al*., 1996; Wang *et al*., 1996) have demonstrated that contaminant accumulation in marine animals can be described mathematically as:

$$dC_m/dt = (k_u \bullet C_w) + (AE \bullet IR \bullet C_f) - (k_e + g) \bullet Cm \qquad \text{Eq. 1}$$

where C_m = metal concentration in mussel soft tissues,
t = exposure time,
k_u = metal uptake rate constant from the dissolved phase (note, k_u = metal absorption efficiency from the dissolved phase times the filtration rate of the mussel),
C_w = metal concentration in the dissolved phase,
AE = assimilation efficiency of ingested metal,
IR = ingestion rate of food,
C_f = metal concentration in suspended particles,
k_e = efflux rate constant,
g = growth rate (taken to be negligible for adult mussels).

Eq 1 solves to:

$$C_m = \frac{(k_u \bullet C_w) + (AE \bullet IR \bullet C_f)\ [1 - \exp^{-(ke + g)\ t}]}{(k_e + g)} \qquad \text{Eq. 2}$$

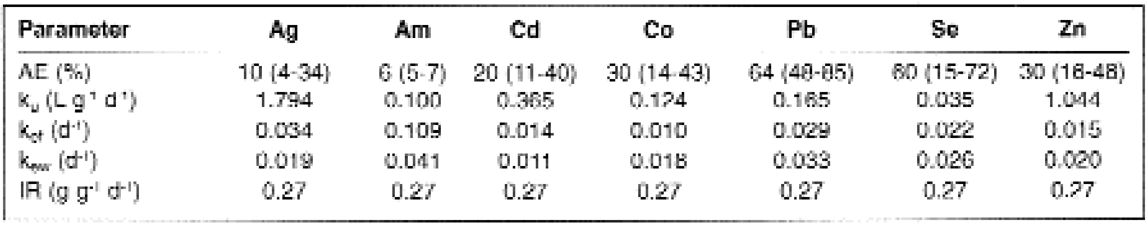
Under steady-state conditions, which can take 1-6 months to achieve (Roesijadi *et al*., 1984),

$$C_{mss} = \frac{(K_u \bullet C_w)}{(k_{ew} + g)} + \frac{(AE \bullet IR \bullet C_f)}{(k_{ef} + g)} \qquad \text{Eq. 3}$$

where C_{mss} = steady state metal concentration in mussel soft parts,
k_{ew} and k_{ef} = efflux rate constants following metal uptake from the dissolved phase or food, respectively.

Thus, the concentrations of any contaminant in mussel tissues are dependent on the contaminant concentrations in food (C_f) and in the dissolved phase (C_w), the efficiency with which ingested contaminant is assimilated (AE), and the efflux rates of the contaminant out of the mussel (k_{ew}, k_{ef}). The AE and the efflux rate constants are parameters that are influenced by the physiological state of the mussel which in turn is influenced by numerous environmental conditions (temperature, salinity, DOC, season, food supply, etc.). These kinetic parameters have been measured in numerous laboratory experiments in recent years in which radioisotopes have been used to quantify uptake and depuration rates (Wang and Fisher, 1997). Table 1 presents a compilation of assimilation efficiencies and efflux rate constants of select metals from different food particles in the mussels Mytilus edulis, the most commonly used mussel species used in North America and other regions, and *Mytilus galloprovincialis*. Note that efflux rates do not differ enormously among the various metals nor do kef and kew values differ greatly from each other for any given metal. However, assimilation efficiencies of ingested metals vary greatly among metals and even appreciably for any single metal with the food source and diverse environmental factors (Wang and Fisher, 1997). Uptake rate constants of metals from the dissolved phase (k_u) also vary appreciably among the metals, with values for Ag and Pb being highest and values for Se (examined in the form selenite) and Am being the lowest (Table 1). Although fewer metal bioaccumulation studies have been conducted with the Mediterranean mussel, *M. galloprovincialis*, evidence sug-

Table 1. Kinetic parameters describing metal accumulation in Mytilus edulis (Ag, Cd, Co, Se, Zn, from Wang and Fisher, 1999a) and in M. galloprovincialis (Am, Pb, from Fusher et al., 1996). Values are



Parameter	Ag	Am	Cd	Co	Pb	Se	Zn
AE (%)	10 (4-34)	6 (5-7)	20 (11-40)	30 (14-43)	64 (48-85)	60 (15-72)	30 (18-48)
k_u (L g^{-1} d^{-1})	1.794	0.100	0.365	0.124	0.165	0.035	1.044
k_{ef} (d^{-1})	0.034	0.109	0.014	0.010	0.029	0.022	0.015
k_{ew} (d^{-1})	0.019	0.041	0.011	0.018	0.033	0.026	0.020
IR (g g^{-1} d^{-1})	0.27	0.27	0.27	0.27	0.27	0.27	0.27

gests that this species behaves comparably to *M. edulis* with respect to metal bioaccumulation patterns (Fisher *et al*., 1996).

Equation 3 has been used to make predictions of metal concentrations in marine (*Mytilus edulis*) and freshwater (*Dreissena polymorpha*) mussels on site-specific bases, where the values of C_w and C_f were independently measured, AE, k_u, k_{ew}, and k_{ef} were experimentally determined, and values for IR and g were taken from the literature. Model predictions of metal concentrations in the mussel tissues have come strikingly close to independent measurements of these concentrations in the mussels (Wang *et al*., 1996; Roditi *et al*., 2000), suggesting that we can account for the major processes governing metal concentrations in mussels and that the laboratory-derived kinetic parameters are applicable to natural systems.

Because biological factors can greatly influence the extent to which metals concentrate in mussel tissues, existing monitoring programs make sure that these are considered in their sampling protocols (NOAA, 1998). For example, mussels that are spawning can mobilize certain metals more than others and a misleading picture of the bioavailable concentrations of those metals can result if spawning mussels are sampled. Therefore, monitoring programs such as the National Status and Trends Program in the US avoid analyzing mussels in this condition.

In envisioning a mussel-based monitoring program for evaluating radioactive contamination in the Mediterranean Sea, it is important to realize that most of the long-lived radioisotopes emanating from the nuclear fuel cycle are, in fact, metals, and their behaviors are essentially the same as their stable isotope counterparts. In fact, consideration should be given to focusing on a variety of potential contaminants, including such metals as Hg, Pb, Cd, Ag, and other toxic metals in addition to the long-lived radionuclides. Presumably, persistent organic pollutants would also be important to assess in the Mediterranean, but their analysis requires much different procedures from the metals/radionuclides and may need to be considered in future efforts. A number of studies have experimentally examined the factors which influence the bioconcentration of important components of radioactive wastes (e.g., isotopes of Pu, Am, Tc, and others) in marine mussels (Guary and Fowler, 1981; Dahlgaard, 1986; Bjerregaard *et al*., 1985; Fisher and Teyssié, 1986; Fisher *et al*., 1996). Clearly, the same processes governing metal uptake and retention in these animals are applicable to the long-lived radioactive wastes. Radionuclide concentrations in mussel tissues have been regularly monitored in the US for decades (Valette-Silver and Lauenstein, 1995; NOAA, 1998). Consequently, the mussel-based monitoring program for radioactive wastes in the Mediterranean should build on already established sampling, analytical, and statistical protocols developed for metals (NOAA, 1998). Ideally ancillary measurements should be considered as well as measuring radionuclide concentrations in mussel tissues. These would include radionuclide concentrations in the dissolved phase, in suspended particulate matter $> 1\ \mu$m, and in sediments in shallow water columns. Such measurements would help in interpretation of the mussel data.

A first screening of mussels in regions around the Mediterranean will provide a snapshot of the current state of radioactive contamination in different regions, but without subsequent measurements in future years will, of course, not provide any useful information on temporal trends in contamination in these waters. Thus, it is important that a long-term (on the order of 20-30 years perhaps ?) monitoring program be undertaken if any temporal variability is to be assessed. It is recommended that samples be taken every 2-3 years from the same locations. It is also imperative that all samples be treated and analyzed identically so that differences in contaminant levels among sampling locations can be directly compared. Clearly, all participating analytical laboratories need to be involved periodically in an intercalibration exercise, ideally supervised by the IAEA Laboratory in Monaco. Similarly, there should be a brief training exercise for all participating labs to ensure that mussels are collected from the sea following the same protocols.

In the National Status and Trends Program, typically 300 g (dry wt) of soft mussel tissues (roughly equal to 200 mussels) are obtained for each sampling site (less than this amount can lead to high analytical uncertainties). Mussels are packed in plastic containers, frozen with dry ice until shucked, after which soft tissues are freeze-dried and weighed. Ashed samples are analyzed for the different radioisotopes of interest using α, β and γ spectrometry (Valette-Silver and

Lauenstein, 1995). As in other geochemical studies, analyzing the data for isotopic ratios in the mussel tissues can provide information useful for tracing the origin of the isotopes in the environment, although experience with the US work has shown that errors associated with these ratios can be quite high, particularly in cases when there are insufficient sample sizes or low radioactivity of some isotopes.

Environmental radioactivity monitoring in Greece: present knowledge and planned study

H. Florou, Ch. Chalalou, Ch. Lykomitrou and M. Ladopoulou

NCSR "Demokritos"/INT-RP, Environmental Radioactivity Laboratory, Athens, Greece

1 – INTRODUCTION

1.1. Monitoring networks - surveys

A routine environmental monitoring programme is run by the Environmental Radioactivity Laboratory since 1960, aimed at the radiological protection of the public and contributing to the radiological risk assessment in the case of accidental releases. Since then, several modifications/ improvements have been made, dictated by the updated demands on the environmental protection in conjunction to the innovations of the use of nuclear energy and techniques for social benefit.

Therefore, the programme is being carried out as a three branch environmental monitoring control, as regards to the situation considered: a) the routine monitoring as a part of the REM (Routine Environmental Monitoring) programme of the EU, covering the national demands in collaboration with the GAEG (Greek Atomic Energy Commission), b) the emergency plan schedule, which is a part of the national emergency situation programme and is based on the routine branch arranged-expanded according to the existing information about the potential primary regional pollution, and c) the case controls, which concern radiation measurements and monitoring upon radiological protection demands on request of anyone, anywhere, anytime. An example of case c is the monitoring of the marine environment in the host areas of the nuclear vessels visiting the Greek ports.

The methods and techniques used have been tested and calibrated through international intercalibration programmes e.g. the ALMERA Proficiency Test of the IAEA (Reporting date 25 April 2001).

The schedule of the environmental radioactivity programme under routine and case monitoring is shown in Fig.1.

1.2. Marine radioactivity

In terms of marine radioecology, several studies have been carried out mainly through international scientific frames such as CRC programmes and GIRMED of IAEA, RTD programmes and MARINAMED of EU (Aarkrog *et al.*, 1994), oriented research studies in national level. Besides, a large bulk of data have been derived from projects as third party services for radiation protection purposes. Thus, a background knowledge has been established on the dispersion and behaviour of radionuclides in the marine environment, and a "radiation archive" is now available concerning the inventory of artificial and natural radionuclides (selected) in the abiotic components and

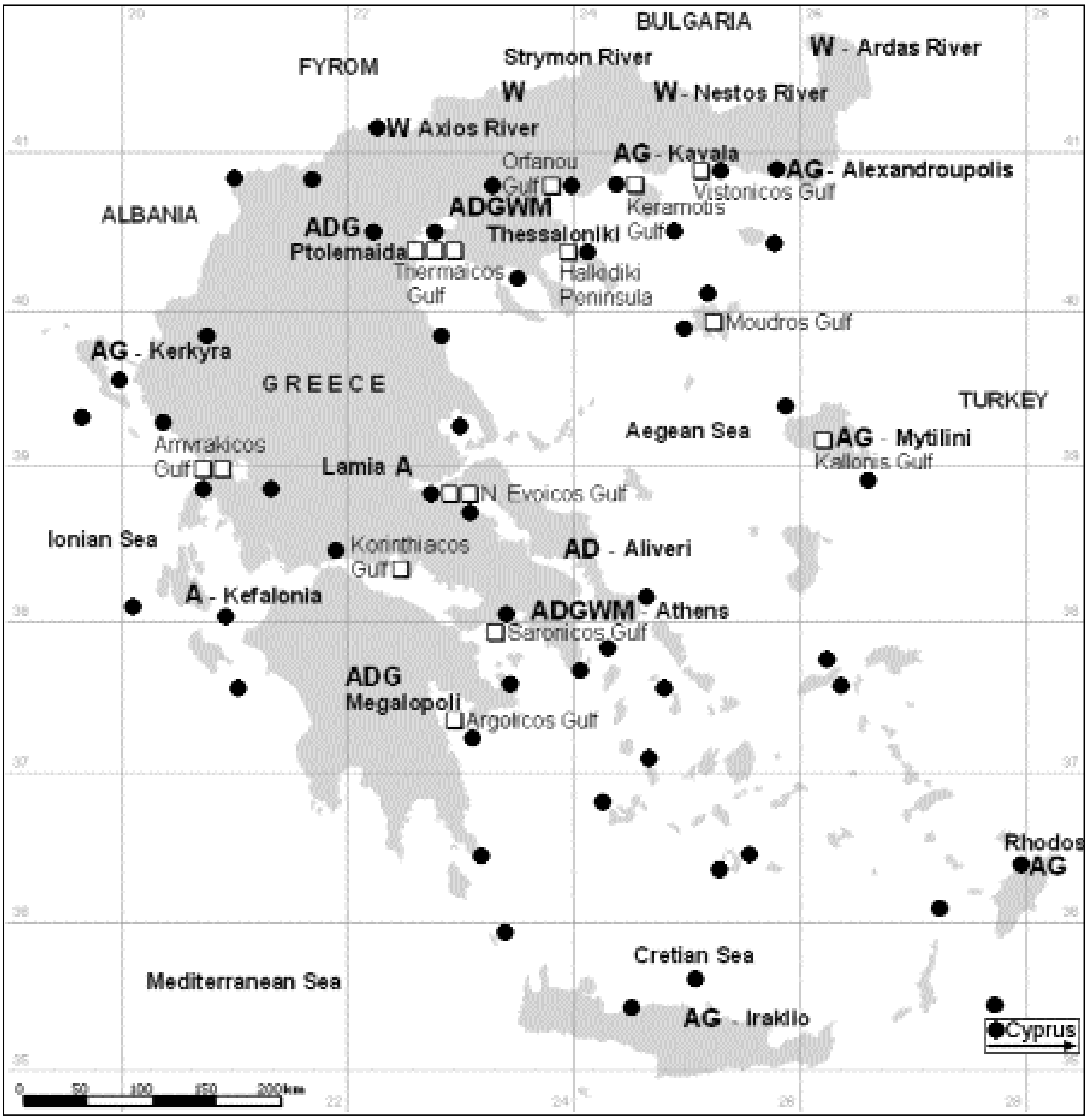


Figure 1. Map of the sampling and measuring locations of the ERL in the frame of the REM programme of the EU indicating also the areas of case studies on aquatic radioactivity and the wide areas of mollusk aquacultures in Greece.

A - Air sampling
D - radioactive deposition sampling
G –gamma intensity measurement
W - surface and/or tapwater sampling
M - milk and mixed diet sampling
● - marine radioactivity case studies
☐ - areas where units of mollusc aquacultures are located

organisms of the Eastern Mediterranean. Published and unpublished data of ERL/INT-RP/NCMR"D" (Florou and Kritidis, 1994; Florou *et al*., 2000) are available to study the environmental monitoring using biomonitors as "organism–sentinels" for radioactive contamination.

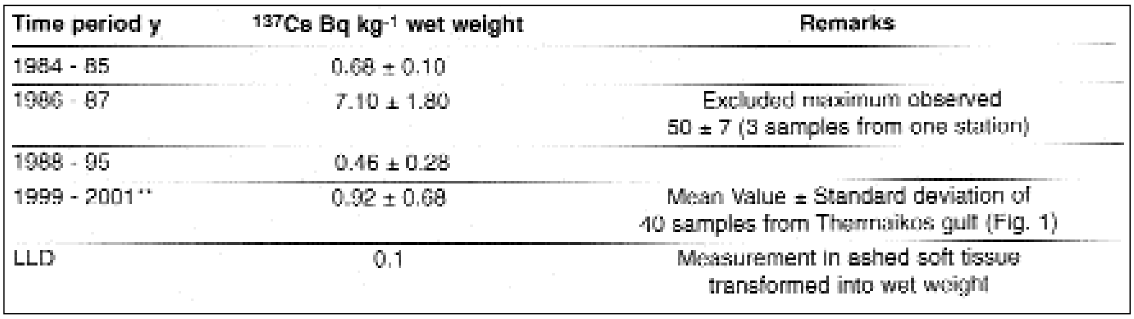
2. BRIEF STATE OF ART OF BIOMARKERS - *MYTILUS GALLOPROVINCIALIS* AS A BIOINDICATOR

Marine pollution levels tend to increase worldwide and, require control strategies and routine monitoring. Radioactive pollution results in long term effects on ecosystems but even their impact has no visible influence, in comparison to other pollutants. Evaluation of marine radioactivity levels is based not only on direct measurements of the abiotic components but also on measurements of the abundance and availability of radionuclides in selected marine organisms. The bioaccumulation of isotopic contaminants by tissues and organs by marine organisms has been studied in an extent scale and led to the adoption of the bio-indicator concept for the environmental quality assessment (Catsiki *et al*., 2001). In addition, by using biomonitors a radiological risk could be assessed early and support the countermeasures for public protection warning (IAEA, 1992).

Mussels are recognised worldwide as pollution bioindicator organisms (mussel-watch) because they accumulate pollutants in their tissues at elevated levels in relation to pollutant biological availability in the marine environment. Besides, they are recognized to show an early response to the radioactive contaminants added to ecosystem. It is notable that concentrations up to 50 ± 7 Bq kg^{-1} (wet weight) of ^{137}Cs were detected in *Mytilus galloprovincialis* sampled in Evoikos Gulf in the central Aegean Sea (Table 1, Fig. 1) a few days after the Chernobyl nuclear accident, whereas the respective mean value concerning the Aegean and Ionian Seas was 7.10 ± 1.80 (maximum value excluded from the mean), which was more than one order of magnitude higher compared the pre-accident levels. The levels of ^{137}Cs in *Mytilus galloprovincialis* were back to the pre-accident observed in the Eastern Mediterranean since 1988 (Florou, 1996). The summarized evolution of ^{137}Cs concentrations is shown in Table 1, whereas the distribution of ^{137}Cs in time series measurements from Thermaikos Gulf is illustrated in Fig. 2.

As it is shown in Fig.1, *Mytilus galloprovincialis* (and other Bivalves) are widely cultured in Greece. More than 32 000 tons were produced during 2000, while a comparable tonnage was

Table 1. ^{137}Cs activity concentrations (Bq kg^{-1} wet weight) in Mytilus galloprovincialis in the Eastern Mediterranean (Aegean and Ionian seas).



Time period y	^{137}Cs Bq kg^{-1} wet weight	Remarks
1984 - 85	0.68 ± 0.10	
1986 - 87	7.10 ± 1.80	Excluded maximum observed 50 ± 7 (3 samples from one station)
1988 - 95	0.46 ± 0.28	
1999 - 2001**	0.92 ± 0.68	Mean Value ± Standard deviation of 40 samples from Thermaikos gulf (Fig. 1)
LLD	0.1	Measurement in ashed soft tissue transformed into wet weight

*The respective values from Ionian Sea vary in the same range (sparsed samples).
** The limited data of 2002 are in the same range as for 2001.

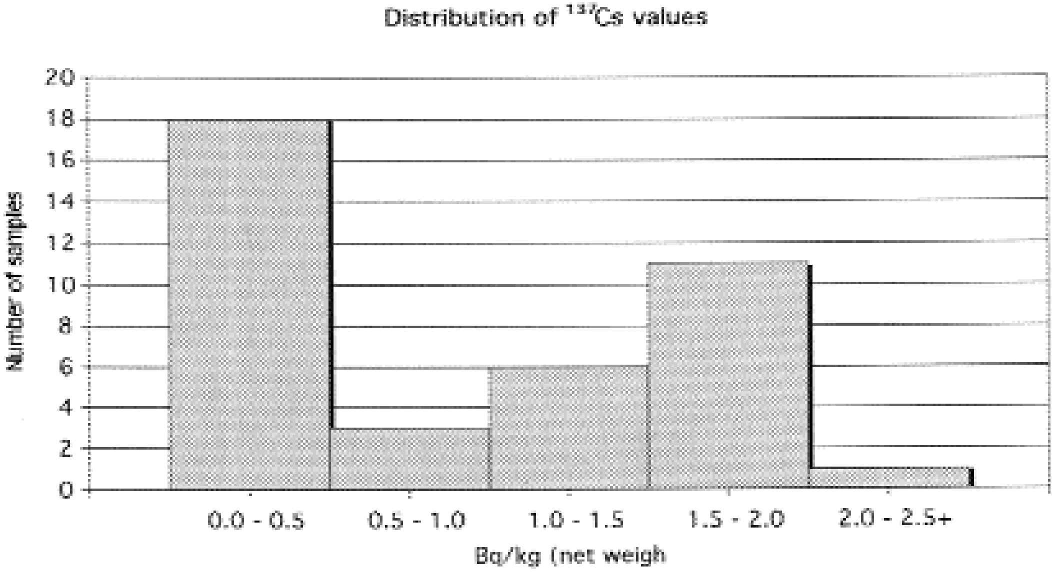


Fig. 2. Distribution of ^{137}Cs (Bq kg-1 wet weight) in Mytilus galloprovincialis from Thermaikos gulf, North Aegean Sea during the period 1999-2001.

imported from other EU and third countries. All fishing goods are being transported in the EU through the market routes (Ministry of Agriculture).

3. PROPOSITIONS

3. 1 Monitoring - methods

The guidelines of the co-ordinated monitoring network to be carried out in the Mediterranean and the Black Sea are encoded as:

- selection of radionuclides (^{137}Cs, Pu isotopes, ^{210}Pb/^{210}Po, ^{238}U, other artificial and/or natural);
- intercalibration exercise;
- sampling (networks, protocols, time series sampling, supporting parameters, biometrics, tissue
- critical organ selection, quantities of samples);
- homogenization of treatment procedures;
- measurements parameters (e.g., LLD, efficiency, SD);
- harmonization of data;
- statistical formulae selection;
- data presentation.

3.2. Research

The cytogenetic effects of ionizing radiation on natural populations of aquatic ecosystems in comparison to conventional pollutants can be determined based on the methodology of the distribution pattern of the chromosomal aberrations in cells, as well as the frequency of the type of the recorded damage. Chromosome aberrations in germ cells can be used as a gauge for the genetic damage induced by radioactive and chemical pollution (Blaylock and Trabalka, 1988; Tsitsugina, 1998). Mussel gonads constitute a relevant system for studying the genetic hazard of environmental mutagens. Thus, a co-ordinated research project based on key radiocontaminant effects on mussels in comparison to conventional pollutants under the leadership of IAEA' CRC is proposed jointly with the monitoring planned to achieve a sufficient ecological coverage for the Mediterranean.

Acknowledgement. This paper could not be materialized without IAEA's programming and funding. Appreciation is owed.

A Mediterranean Mussel Watch for radionuclides: what is the best information that can be gained?

Scott W. Fowler

International Atomic Energy Agency (IAEA), Marine Environment Laboratory (MEL), Monaco

Bivalve molluscs and particularly mussels of the genus *Mytilus* have been used worldwide to document or "monitor" the existing levels and spatial and temporal trends in concentrations of a wide variety of marine contaminants. Many of these bioindicator, or "sentinel", monitoring exercises using mussels have often met with mixed success and resulted in merely identifying hot spots of contamination that were either already known or suspected beforehand (Goldberg *et al*., 1978, 1983; Farrington *et al*., 1983). Much of the ambiguity that may have arisen was partially caused by a poor understanding of the behaviour of the contaminant in the mussels, and as a result proper interpretation of the environmental data was difficult to achieve. One of the problems is that mussels often reflect only the bioavailable fraction of a contaminant, and in this respect, do not give a true picture of the existing ambient levels in the surrounding waters. Another is that for various physiological reasons mussels are poor sentinels for environmental contamination of specific heavy metals. Radionuclides like the long-lived transuranic elements are no exception because they too behave as heavy metals, although there is far less information on their physiological behaviour in mussels than exists for the conventional heavy metal contaminants. For this reason, it is wise to review some of the main environmental and physiological factors which may play a role in the bioaccumulation and retention of radionuclides that are measured in Mussel Watch activities.

A second important point to make is that in order to know what kind of useful information will be gained from this exercise, the project must have a well-defined goal, i.e., what is the specific purpose of gathering these data? A rather large perspective is needed, for ultimately one must understand the complex relationship between contaminant loading in the ambient environment, the mussel's response to it, and the actual entity or target being protected (White, 1984). This strategy has been underscored by an earlier Mussel Watch workshop (NAS, 1980) which stated that implicit in proposing a biomonitoring programme is having a conceptual model of the physical, biological and social systems affected. Furthermore, it would be impossible to construct any rational monitoring system without such a concept, and its development should be a primary goal of the proposed Mediterranean programme. Such a conceptual, holistic model might take the form of that shown in Fig. 1.

As radionuclide monitoring will be the first stage of this effort, what can be said about the application of this model to establishing criteria for monitoring radionuclides in mussels from around the Mediterranean? First of all, the radionuclide loads for the key nuclides (e.g., ^{137}Cs and $^{239+240}$Pu) are fairly well-known for coastal waters in many areas of the western Mediterranean. Most of the radionuclides entering the Mediterranean originate from atmospheric fallout with rel-

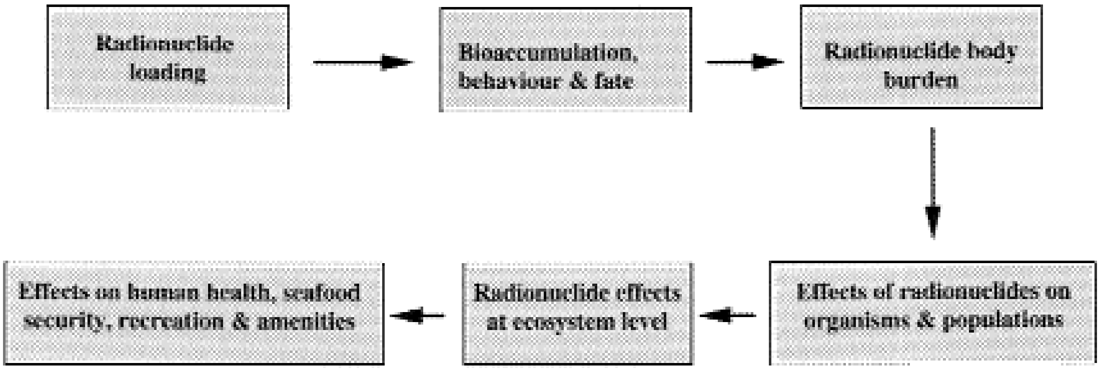


Fig. 1. Six box conceptual model for setting goals and priorities for monitoring radionuclides using sentinel organisms like marine mussels.

atively very small amounts coming down rivers like the Rhône, Ebro and Po. Hence, outside the proximity of those minor, localized source terms, most artificial radioactivity accumulated by Mediterranean mussels will be fallout-derived including minor amounts of ^{137}Cs from the Chernobyl accident which now are barely measurable in marine environmental samples. With respect to the second box in Figure 1, information on bioaccumulation, elimination and fate of artificial radionuclides in Mediterranean mussels (either *M. galloprovincialis* or *M. edulis*) is somewhat limited and restricted to only a handful of published papers (e.g., Keckes *et al*., 1967; Fowler *et al*., 1975, 1981; Guary and Fowler, 1977, 1978, 1981; Whitehead *et al*., 1988; Nolan and Dahlgaard, 1991; Fisher *et al*., 1996). Nevertheless, transfer rate information in this literature is important to take on board when establishing sampling strategies for the various radionuclides of interest, and for interpreting field data obtained from mussels living under the different environmental conditions found around the Mediterranean basin.

Obtaining data on existing radionuclide levels or "body burdens"in mussels (third box) has been the central focus of previous Mussel Watch efforts (Goldberg *et al*., 1978, 1983; Farrington *et al*., 1983; Vallette-Silver and Lauenstein, 1995), and it will likely remain so in the proposed project for the Mediterranean. However, in the case of radionuclides, focusing attention on the remaining boxes and protecting those entities as a primary goal of this project is open to debate. Most Mussel Watch programmes rightly have made pleas to research the relationships between contaminant body burdens, reproduction and survival (NAS, 1980), and some have included examining sub-lethal effects such as scope for growth measurements in mussels (Martin and Severeid, 1984). However, the focus there has been on toxic contaminants known to cause effects at the organism level such as POPs and certain toxic heavy metals. The case for artificial radionuclides is different in that wherever examined in the marine environment, no effects of ionizing radiation or radionuclide toxicity at extant ambient concentrations, or impacts of radionuclides, have been found in organisms or ecosystems (IAEA, 1976, 1988). Thus, based on both historical and scientific perspective, GESAMP (2001) have recently concluded that the "impacts" of authorized releases of radionuclides "*on human health and the environment at global and regional levels are generally of minor significance*". This is why according to GPA/LBA criteria for food security, public health and safety, marine resources and ecosystem health, the impact of radionuclides vis à vis all other types of contaminants is considered low or negligible (*ibid*.). Given these conclusions drawn from a broad spectrum of scientific study, it seems clear that the only realistic goal for a Mediterranean Mussel Watch focused on radionuclides will be to obtain radionuclide body burden levels on a spatial and perhaps temporal scale for use as baseline information in the event of any major accidents or deliberate releases in the future.

It also should be kept in mind that far more information is available on radionuclide levels in Mediterranean water and sediments than for concentrations in mussels. Hence, to date, most estimates of spatial and temporal trends of long-lived fallout radionuclides in the Mediterranean have come from broadbased, decadal monitoring and research studies of changes of concentrations and resultant inventories in the water from coastal and open sea areas (e.g., see Fowler *et al*., 2000). Such studies clearly show that fallout-derived radionuclide levels are decreasing in the Mediterranean as elsewhere. In the case of mussels, and likely for other sentinel organisms as well, comparable systematic biomonitoring data for radionuclides do not exist for the Mediterranean outside of "snapshot" measurements made in certain locations. For example, $^{239+240}$Pu analysis of mussels from Marseilles made some 30 years ago showed a concentration of approximately 40 mBq/kg dry in soft parts (Murray and Fukai, 1978). This concentration lies about midway in the range of values (1.2-88.4 mBq/kg dry) reported for $^{239+240}$Pu mussels from the US coast collected in 1990 (Vallette-Silver and Lauenstein, 1995). As fallout levels in the Mediterranean have fallen considerably since 1973, it is likely that present day plutonium levels in mussels will be somewhat lower. The same Mediterranean study showed that mussel shell contained roughly three times higher concentrations than soft parts. The increased concentrations of $^{239+240}$Pu in a shell sample coupled with the knowledge that residual plutonium is strongly retained in shell with little loss over time (Fowler *et al.*, 1975), suggests that mussel shell may be a better integrator of past and present plutonium levels in the water than soft parts of the animal.

As mentioned above, in any monitoring programme involving the use of mussels as sentinels, it is important to keep in mind certain factors which can affect the levels of the radionuclides measured. As often stated, one chief disadvantage of using mussels is the rapidity with which they purge themselves of contaminants. However, for certain radionuclides this may not be the case as attested to by biological half lives for long-lived radionuclides like plutonium ranging from six months to two years (Fowler *et al.*, 1975; Guary and Fowler, 1981). Nevertheless, a priori knowledge of elimination half-lives is important when establishing the sampling frequencies for a given radionuclide. Such transfer rates are often governed by temperature, salinity, the physical-chemical species of the radionuclide, and the amount of time and the manner in which the mussels have been exposed to the radionuclide. Thus, given the wide variety of environmental conditions under which mussels live in the Mediterranean coastal environment, some knowledge of the behaviour of the radionuclide in mussels will greatly assist in the interpretation of any observed variations in radionuclide body burden over time and space. Where radiotracer technology and appropriate experimental facilities are not available, carefully designed mussel transplant experiments at specific locations may furnish similar rate data. In any event, implementing a Mussel Watch exercise in which only information on body burden levels is obtained would be extremely short-sighted, and wherever possible ancillary data on radionuclide concentrations in the surrounding waters and sediments should be acquired, particularly in areas where measurements have not been made previously.

Another obstacle to obtaining basin-wide data for the Mediterranean is the fact that mussels do not exist or are poorly represented along many coasts on the eastern and southern shores. This limitation may be partially overcome by the use of non-endemic mussels transplanted to those locations (provided they can survive there), or by selecting a closely-related bivalve species although both these alternatives also carry some disadvantages.

It is hopefully evident from the facts presented above that an initial Mediterranean Mussel Watch for radionuclides can only have a limited goal, i.e. to obtain a basin-wide snapshot of current levels of long-lived radionuclides in mussels where they exist. Further temporal measurements may have some value only if sampling frequencies are properly determined, and this will have to be based on the targeted radionuclides. Such an effort should also depend on a thorough cost-benefit analysis with the real benefits of further basin-wide artificial radionuclide monitoring being carefully weighed.

While contaminant radionuclide monitoring is the primary goal of the initial stages of the proposed project, some additional effort should be made to establish levels of the natural radionuclide ^{210}Po in the mussel samples. This is important for two reasons. First, ^{210}Po is considered to be the most important contribution to the radiation dose received by humans via seafood consumption (Aarkrog *et al.*, 1997) and information on extant levels in seafood from many areas of the Mediterranean is lacking. Second, elevated levels of ^{210}Po can enter the coastal waters through industrial, mining and agrochemical activities. Thus, enhanced ^{210}Po concentrations in mussels can provide a strong signal for various types of land-based activities which contaminate coastal areas. Therefore, with respect to technological enhancement of natural radionuclides, ^{210}Po could be considered as a marine contaminant.

In conclusion, we should keep in mind the limited goals and scope of a Mediterranean Mussel Watch for radionuclides and use it to assemble a qualified group of analysts and environmental scientists who can devise a truly integrated monitoring network which uses a sentinel organism to gain more insight into existing levels of key radionuclides around the Mediterranean basin. When this initial task is accomplished, it would then be most profitable for the Mussel Watch project to turn its attention to an examination of the more high priority pollutants such as POPs, various heavy metals, and anti-foulants.

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Present use of mollusks as bioindicator in monitoring Israel's Mediterranean coastal waters

Barak Herut

Israel Oceanographic and Limnological Research, National Institute of Oceanography, Haifa, Israel

INTRODUCTION

The rapid increase in population density and growth rate along the Israeli Mediterranean coastal plain in the past half-century and its consequent urbanization generated land reclamation schemes and possible short or long-term impact on the marine systems. The main environmental problems along the coast are: a) coastal erosion or negative sand budget, b) pollution from land-based sources and c) biodiversity variations and habitat damage.

Herut and Galil (2000) summarized the environmental status of these problems. Since the early 1980's the overall pollution load into the coastal zone has declined due to the combination of the following factors: a) the increase of public awareness and governmental actions, b) the national commitment to implement international conventions on marine pollution prevention, and c) the progress and expansion of the National Sewage Project and sewage treatment. At present only two cities (Akko and Naharya) discharge raw sewage (after primary filtration) into the sea. Protective measures and monitoring activities are implemented by several legislative/administrative systems, evacuating and abating land-base pollution sources. Albeit, significant quantities of heavy metals and nutrients are introduced into Israel's Mediterranean coastal waters both from point sources (marine outfalls and the coastal rivers) and from diffuse sources (runoff waters and atmospheric deposition). As a result, a number of local pollution problems exist along the coastline. The main problems are in Haifa Bay, in ports and marinas, in the vicinity of the sewage sludge outfall of the Tel-Aviv Region sewage treatment plant (TAS) (located ca. 5 km offshore) and near the outlets of some coastal rivers (Fig. 1).

The aim of this brief summary is to present information on the environmental quality of Israel's Mediterranean coastal waters, emphasizing the present use of mollusks as bioindicators in monitoring. These records are based on the National Marine Environmental Monitoring Program (NMEMP) and related activities carried out by the National Institute of Oceanography of Israel Oceanographic and Limnological Research (IOLR).

MONITORING ACTIVITIES

The NMEMP includes four components (Herut *et al*., 2000b):

- monitoring of heavy metals in the coastal waters (carried out since 1978);
- monitoring of the introduction of nutrients and particulate metals into the coastal waters through coastal rivers (since 1990);

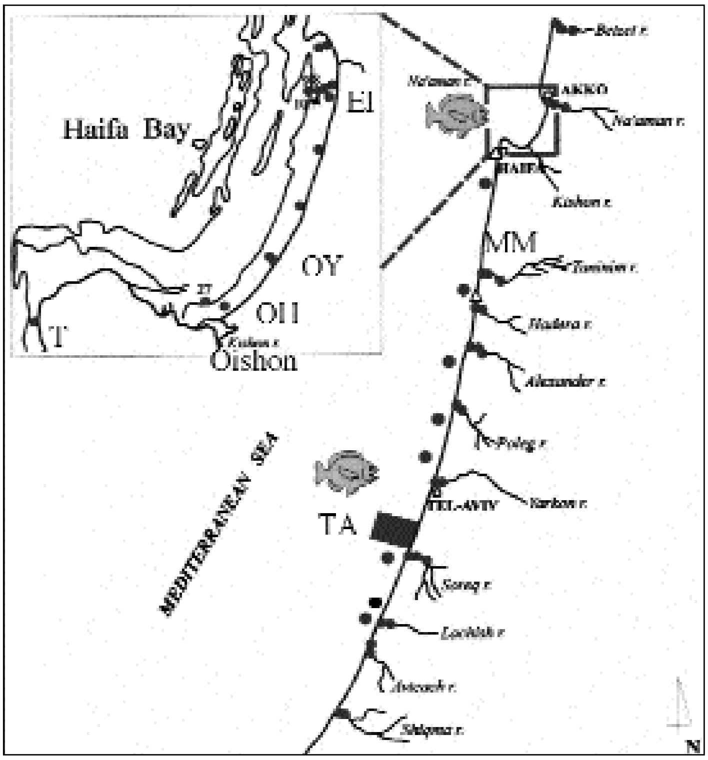

Fig. 1 Location of monitoring stations along the Mediterranean coast of Israel and at the lower reaches of the coastal rivers. The fish symbols represents areas where fish were collected. Triangles represent stations of atmospheric monitoring. The black square south of Tel-Aviv represents the monitoring area of sewage sludge disposal at sea (TAS).

- monitoring of the atmospheric fluxes of nutrients and heavy metals into the coastal waters (since 1996);
- monitoring of nutrient levels and algal populations in the shallow area of the coastal waters (started in 2000).

The following Table summarizes the status and trends of some environmental indicators. Fig. 1 presents a general map with location of the monitoring sites.

PRESENT USE OF MOLLUSKS AS BIOINDICATORS IN THE NMEMP

Two bivalve species (*Mactra corallina, Donax* sp.) and two gastropod species (*Patella* sp. and *Cellana rota*) are sampled at polluted and relatively clean sites. Their soft tissue is analyzed for Hg, Cd, Zn, Cu, Mn and Fe concentrations. In the past, representative samples were screened for organic contaminants (DDE, PCBs and PAHs), which exhibited very low concentrations at all sites (Herut *et al*., 1999).

Hg and Zn are enriched in two bivalves (*M. corallina* and *Donax* sp.) from Haifa Bay, both species have an ongoing a long-term decrease in Hg (Fig. 2). Contamination of Hg in Haifa Bay is from two sources : a chlor-alkali plant in the north (EI, Fig. 1) and the Qishon River estuary at the southern part of the bay. The latter is the main source of anthropogenic Zn and Cd. In general, *Patella* sp. specimens from Haifa Bay exhibited higher levels of Cd as compared with other sites along the coast (Fig. 3), attributed to the enrichment of Cd in suspended particulate matter. During the past 3 years, a decreasing trend in Cd concentrations was detected which we attributed to the decrease in its load into the Qishon River and to dredging activity at its estuary.

During the past two decades Hg levels in sediment, in benthic organisms and in fish in the northern part of the bay have decreased drastically (Fig. 4). It is probable that with regard to Hg pollution, the marine system of northern Haifa Bay has reached, or is approaching, a state of equilibrium whereby the annual input of anthropogenic Hg equals the amount of Hg removed from the area towards the open sea.

Outside Haifa Bay, the concentrations of Hg, Cd and other metals in the sediments near the TAS outfall reflect a high degree of pollution. However, no significant enrichment of these metals was found in benthic organisms from this area.

Table 1.

Environmental Indicator	Status in 2000	Trends in Last Decade
Heavy metals in sediments	**Haifa Bay:** Mercury, lead and zinc enrichment relative to other areas. **Coastal rivers (outlet areas):** Moderate pollution in the Qishon; enrichment in Soreq, Naaman, Alexander, Betzet. **Along the coast:** Several local pollution problems. High degree of mercury and moderate degree of cadmium pollution in TAS area.	Mercury pollution decreased. Pollution in the Qishon estuary decreased; no clear trend in other rivers. Pollution increased in TAS area after winter 1992. No significant trend in last 5 years.
Heavy metals in SPM	**Haifa Bay:** Higher concentrations than in sediments. High mercury conc. in northern part of the Bay. Copper and lead enrichment. **Coastal rivers:** High mercury concentrations in most rivers. Chromium, copper and cadmium enrichment in Hadera River. **Along the coast:** Enrichment opposite river outlets.	No change in mercury levels. Cadmium levels in the southern part of the Bay decreased as of July 2000. No significant trend No significant trend.
Heavy metals in fish	**Haifa Bay:** Mercury enrichment relative to other areas. Fish fit for consumption with respect to national safety limit. **Along the coast:** Fish fit for consumption.	Mercury concentrations stabilized at a lower level than in previous decade. No change.
Heavy metals in benthic organisms	**Haifa Bay and Akko**: Mercury enrichment relative to other areas. Cadmium and zinc enrichment in Qishon estuary. **Along the coast (selected sites):** Natural levels	Mercury concentrations decreased. Cadmium decreased in Qishon estuary. No change
Heavy metals in dust	Concentrations similar to Europe and higher than in open sea areas.	Lead concentrations decreased.
Sediment pollution in ports and marinas	High degree of mercury pollution and moderate cadmium pollution in Haifa and Qishon ports. Moderate cadmium pollution in Ashdod Port. Relatively high concentrations of pesticide residues and PAH's in Hadera Port and Tel-Aviv Marina.	Similar and higher levels of pollution found previously in Haifa and Qishon ports. Other sites not checked in details previously
Nutrients and acidity in rain water	Nitrogen and phosphorus fluxes into the coastal waters smaller than in Europe but higher than in open sea areas.	Percentage of acid rains decreased. Nitrogen flux increased
Nutrients in coastal rivers (outlet areas)	Moderate to high levels of pollution in most rivers.	Some decrease in nutrient concentrations.
Nutrient load from point sources	**Nitrogen**: coastal rivers > TAS outfall > others **Phosphorus**: TAS outfall > coastal rivers > others	Reported decrease in quantities of nutrients discharged into the rivers.
Nutrients in coastal waters	**Haifa Bay:** N and P enrichment. **Along the coast:** In winter, enrichment mainly near Yarkon, Soreq, and Taninim outlets, TAS outfall and Hertzelia sewage treatment plant outfall; in summer, enrichment mainly near Taninim and Poleg outlets and above outfalls.	No significant change. Not checked in detail previously.
Microalgae in coastal waters	**Haifa Bay:** High concentrations in southern part. **Along the coast:** Relatively high concentration at depths < 10 m. Genera that might include toxic species found along entire coastline.	No change. Not checked in detail previously. Toxic species found once in Haifa Bay.

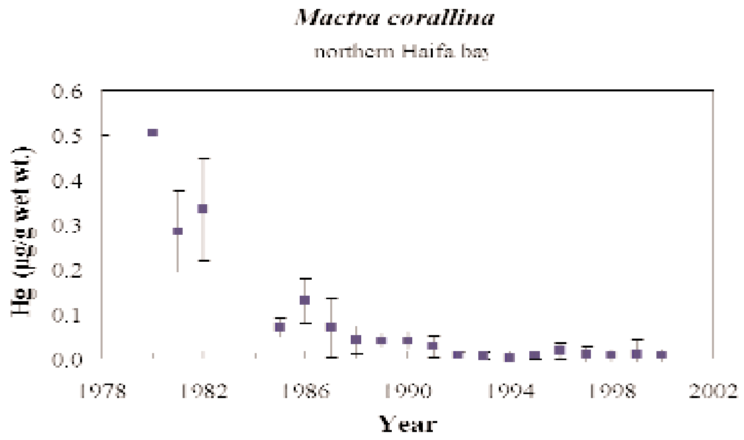

Fig. 2. Decrease of mercury concentrations (µg g-1 wet wt.) in the bivalve Mactra corallina (1978-2001) from northern Haifa Bay.

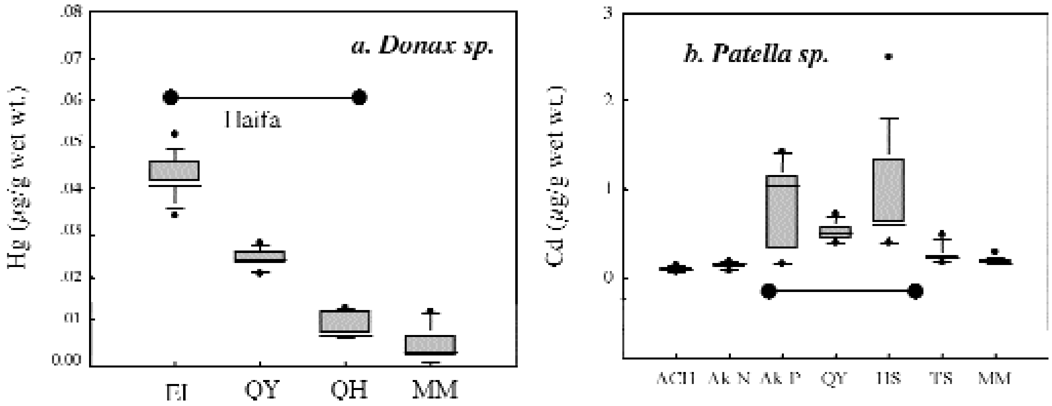

Fig. 3. Box plots of Hg and Cd concentrations in the gastropods Donax sp. (a) and Patella sp. (b) collected at different sites during 2000. The bottom and the top edge of each box are located at the sample 25 and 75 percentiles. The center horizontal line is drawn at the sample median (**MM**=Maagan Michael, **TS**=Tel shiqmona, **HS**=Hof Shemen, **QY**=Qiryat Yam, **QH**=Qiryat Haim, **AK-P**=Akko port, **AK-N**=Akko north, **ACH**=Achziv, **E**I=Electrochemical industries; See Fig. 1).

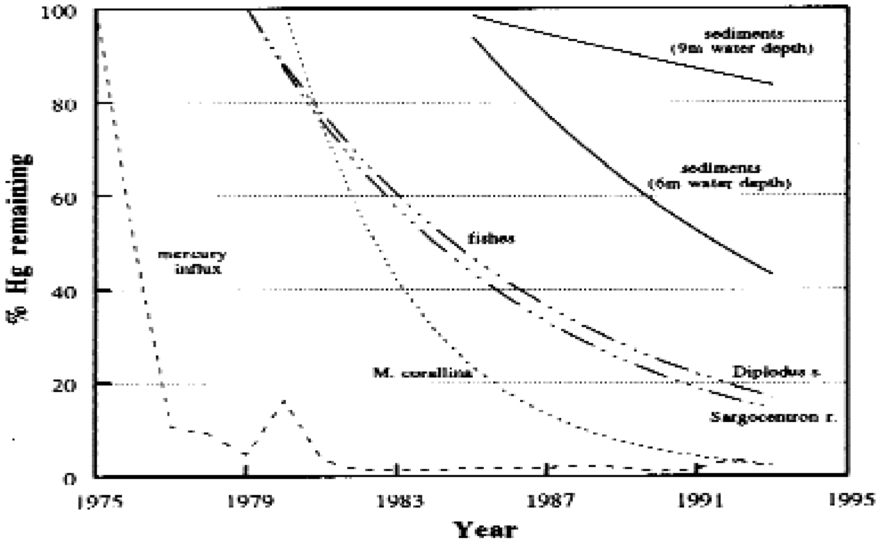

Fig. 4. The relationships between the reduction of mercury influx into northern Haifa Bay, the reduction in the amount of mercury in the top 50 cm of the sediments and the reduction of mercury concentrations in the biota (bivalves and fish) of Haifa Bay (Herut et al., 1996).

POSSIBLE PARTICIPATION IN A MEDITERRANEAN MUSSEL WATCH FOR RADIONUCLIDES

Until now, radionuclide concentrations in mussel tissues have not been monitored in the coastal waters of Israel. It is proposed to include a few representative stations (2-3) along the Mediterranean coast of Israel within the framework of the proposed Mediterranean Mussel Watch for radionuclides. The specific location will be determined based on the presence of an existing alternative species, *Brachiodontes pharonis* or the need of transplantation of either *M. galloprovincialis* or *M. edulis* from the closest areas. The former is regarded as a Red Sea immigrant settled on rocks in the tidal zone. Dr Gustavo Haquin will perform gamma spectometry analysis at Soreq NRC. Sampling frequency and methodology will follow the procedures established in the framework of the proposed Mediterranean network.

Investigations related to the monitoring of bivalves and other marine organisms on the Eastern Adriatic Coast (Croatia)

Goran Kniewald and Delko Barisic

Center for Marine and Environmental Research, Rudjer Boskovic Institute, Zagreb, Croatia

The use of sentinel organisms as an indicator of the level of contamination of the sea with a variety of pollutants has been effectively applied in northern Europe and the USA since the late 1960s, and has since been adopted by an ever increasing number of countries. Such monitoring programmes have two general aims:

- to provide information on contemporary problems such as the extent of contamination of coastal ecosystems and food resources and
- to advance the scientific knowledge and understanding of coastal systems and their dynamics by providing intercomparable regional and global data on the abundance of anthropogenic contaminants.

Croatia has been involved in monitoring programmes within the Mediterranean Action Plan MED-POL frameworks I, II and III. A national monitoring programme currently forms part of the scientific project “Systematic research of the Adriatic Sea as a base for sustainable development of the Republic of Croatia” (Project Adriatic) which was launched in 1998.

Since the introduction of the ”International Mussel Watch” in the late seventies (Goldberg, 1975, 1980) a substantial amount of data has been collected within research programmes related to the monitoring of contaminants in the mussel *Mytilus galloprovincialis* from Croatian waters. First published data on toxic metal levels in bivalves from selected sites on the eastern Adriatic sea coast appeared in 1980 (Martincic *et al*., 1980; 1984). Considerable attention was given to the distribution of trace elements between bivalves, sediment and ambient waters (Martincic *et al*., 1987), and to heavy metal pollution assessment of the marine environment by determination of metal binding proteins in bivalves (Pavicic *et al*., 1987, 1993; Raspor *et al*., 1987; Raspor and Pavicic 1991). The contents of organo-metallic species of toxic elements (Mikac *et al*., 1985, 1996; Tusek-Znidaric *et al*., 1985) as well as the distribution of trace elements in transplanted mussels were also extensively studied (Martincic *et al*., 1992; Odzak *et al*., 2000).

Some research has also been directed towards the development of methods for the quantification and characterization of metalothioneins induced in marine bivalves (Raspor and Pavicic, 1996, 1997; Erk and Raspor, 1998, 1999, 2000; Raspor *et al*., 2001). Assessments of metal exposure of marine mussels has been done by means of biomarkers (Raspor *et al*., 1998, 1999; Erk *et al*., 2001).

Specific diseases of marine bivalves, including legal aspects thereof, have been investigated over many years and were recently published in a comprehensive study (Topic Popovic and Tesekeredzic, 1999).

Fig. 1. Stations for radionuclide monitoring on the coast of Croatia.
station 1 - Sibenik.
station 2 - Kastela bay.

On the Croatian coast of the Adriatic sea, marine aquaculture has been gaining in importance, particularly over the last 25 years. At present, there are 92 registered aquaculture businesses including ca. 60 bivalve mariculture locations. In addition, there exist a number of smaller family-owned incentives, with direct sale of aquaculture products at local markets. In general, the yearly production of aquacultured bivalves in Croatia is modest, presently amounting to approximately 1000 tons/year (Overview, 2002). Most of the production pertains to the mussel (*Mytilus galloprovincialis*), while the production of oysters *(Ostrea edulis*) only amounts to some 40-50 tons.

Studies of radionuclides in marine bivalves from Croatian waters have been comparatively few, and little data has been published in scientific literature. Some recent investigations, dealing with the distribution of radionuclides between mussels and associated sediments showed the following activities for some nuclides in mussel tissue (wet weight) collected from various sites on the Adriatic coast: ^{40}K = 94-105 Bq/kg, ^{232}Th = 0.9-2.3 Bq/kg, ^{137}Cs = bdl - 1.2 Bq/kg, ^{238}U = 3-20 Bq/kg (Barisic, 2002).

Following the above, the proposed scope for monitoring strategies within the framework of a Mediterranean Mussel Watch programme should encompass:

- radionuclides (with emphasis on artificial and natural radionuclides);
- toxic and trace metals (with emphasis on speciation);
- persistent organic pollutants, including halogenated hydrocarbons and other compounds derived from fossil fuel production and burning;
- health of the mussels.

For specific purposes of radionuclide monitoring in the mussel *Mytilus galloprovincialis* on the Croatian coast, two stations will be established with respect to the identification of possibly elevated radionuclide activities due to specific activities in these areas : station 1, in the harbour of the city of Sibenik within the estuary of the Krka river ; station 2, in the Kastela bay, south-east of the city of Split.

Surveillance du milieu marin le long des côtes tunisiennes

Nadia Mzoughi

Institut National des Sciences et Techniques Marines (INSTM) La Goulette, Tunis, Tunisie

INTRODUCTION

Dans les écosystèmes aquatiques naturels, les contaminants chimiques peuvent avoir des concentrations supérieures aux charges naturelles. Ce problème est devenu de plus en plus préoccupant. Pour gérer rationnellement et maîtriser la pollution, il faut arriver à étudier tout ce qui concerne les apports (charges), la distribution et le sort des contaminants qui se déversent dans les écosystèmes aquatiques, il faut en particulier en étudier les quantités et les caractéristiques qualitatives, ainsi que leurs effets sur les organismes vivants.

Le laboratoire du milieu marin à l'Institut National des Sciences et Technologies de la Mer en Tunisie (INSTM) s'intéresse principalement à l'analyse des micropolluants dans l'environnement marin et à leurs effets sur les organismes marins. Nous citons en particulier les programmes suivants :

- Le programme de surveillance continue de la pollution en Méditerranée (MED-POL); il a débuté en 1978, il englobe tous les pays méditerranéens et intéresse plusieurs sites d'échantillonnage; les échantillons analysés sont des sédiments et des organismes marins.
- Le réseau de surveillance national du milieu marin; il englobe plusieurs paramètres : eau de baignade, bactériologie, métaux lourds, hydrocarbures et pesticides; plusieurs institutions y participent.
- Le réseau de surveillance du phytoplancton toxique dans les zones conchylicoles; il implique la recherche du phytoplancton toxique, les biotoxines, les bactéries et les micropolluants dans les bivalves collectés dans les zones de production des coquillages.
- Le programme d'étude de la dynamique côtière ; il s'agit d'étudier les déplacements des masses d'eau le long de la côte tunisienne et dans les lagunes.
- Le réseau de biosurveillance du littoral : il s'intéresse à l'analyse des biomarqueurs de la pollution (métallothionéine, EROD, altérations lysosomales, altérations de l'ADN, catalase, acétyl cholinesterase…) dans les moules et les palourdes prélevées dans plusieurs sites le long de la côte tunisienne.

Plusieurs travaux de recherche utilisant les deux espèces bio-indicatrices, *Tapes decussatus* et *Mytilus galloprovincialis* ont été publiés dans ce thème (Mzoughi *et al*., 2001a, b ; Chouba *et al*., 1998a, b).

MATÉRIEL ET MÉTHODES

L'analyse des différents micropolluants se fait comme suit :

- échantillonnage (UNEP/IEA/IOC, 1994) : généralement l'échantillonnage est semestriel (été et hiver) des mesures de Température, Salinité, pH, Oxygène dissous, Nitrate, Phosphate,… se font *in situ* et au laboratoire pour suivre en même temps la qualité physico-chimique de l'eau;

- lyophilisation des échantillons de sédiments et des organismes marins ;
- minéralisation pour les dosages des métaux lourds avec une attaque acide (UNEP/IAEA/IOC, 1995; UNEP/IAEA, 1985; UNEP/FAO/IAEA/IOC, 1984) et extraction avec des solvants organiques à l'aide d'un extracteur de Soxhlet pour l'analyse des hydrocarbures totaux et les pesticides (UNEP/IAEA/IOC, 1986, 1992 ; UNEP/FAO/IAEA/IOC, 1996).

Les métaux toxiques sont le mercure, le plomb et le cadmium. Le mercure est déterminé par spectrométrie d'absorption atomique couplée à un système de vapeur froide; le cadmium et le plomb sont déterminés par spectrométrie d'absorption atomique avec four à graphite.

Les pesticides organochlorés (PCBs, DDT et ses métabolites, HCB, HCH, aldrine, endrine, dialdrine, lindane) sont déterminés par chromatographie en phase gazeuse avec détecteur à capture d'électron et les hydrocarbures totaux sont déterminés par spectroscopie de fluorescence (UVF).

Pour chaque type d'analyse, nous utilisons des échantillons de références standards fournis par l'agence internationale de l'Energie Atomique (IAEA) qui nous permettent de valider la méthode utilisée et de contrôler nos analyses.

L'analyse des différents biomarqueurs de la pollution se fait comme suit (UNEP, 1999) :

- technique de L'EROD : elle est basé sur la détermination de l'activité catalytique de l'éthoxyrésoruffine O decethylase, fondée sur l'incubation du substrat (éthoxyrésoruffine) avec la préparation enzymatique et un cofacteur (NADPH) dans un tampon adapté (CYP1A1); l'analyse est réalisée par spectrofluorimétrie;
- mesure de la catalase : l'activité de l'enzyme n'est pas reliée quantitativement à la concentration des contaminants métalliques mais elle nous renseigne sur la présence et l'effet des contaminants présents dans le milieu;
- dosage des méthallothionéines : la quantification des teneurs en ces protéines est faite par tests Elisa par utilisation d'anticorps spécifiques;
- altération de la membrane lysosomale : consiste à mettre en évidence la stabilité lysosomale membranaire *in vivo*. Ceci est réalisé par test de rétention du rouge neutre. En effet, toute altération tissulaire résultera en une diminution du temps de rétention du rouge neutre dans les lysosomes;
- altération de l'ADN mitochondrial : effectuée sur ADN mono caténaire après élution, on mesure l'augmentation de fluorescence due à la liaison du fluochrome à l'ADN par spectrofluorimétrie.

RÉSULTATS

Le tableau 1 montre quelques résultats relatifs à l'analyse des micropolluants (métaux lourds et hydrocarbures totaux) et des biomarqueurs dans les deux espèces *Tapes decussatus* et *Mytilus galloprovincialis* durant l'année 2001.

Tableau 1. Métaux lourds, hydrocarbures totaux et biomarqueurs (métallothionéine, EROD et catalase) dans les deux espèces T. decussatus et M. galloprovincialis durant l'année 2001 (programme MEDPOL).

contaminants	***Tapes decussatus*** (Station Barraka au sud de la Tunisie)	***Mytilus galloprovincialis*** (Menzel Jemil au nord de laTunisie)
Mercure (µg/g)	0.137	0.476
Plomb (µg/g)	0.584	2.41
Cadmium (µg/g)	0.421	0.9
HT (µg/g eq chrysène)	-	6.84
EROD (pmol)	$2\ 10^{-12}$	$2\ 10^{-12}$
Catalase (pmol)	-	146.66
Metallothioneine (pmol)	-	0.55

PROPOSITIONS

L'évaluation périodique de l'état de pollution de la mer Méditerranée peut nous fournir des renseignements sur la nature et la quantité des polluants directement libérés dans l'environnement. Il est nécessaire aussi de surveiller les zones situées à proximité du littoral, qui sont sous l'influence directe de polluants émis par des sources identifiables primaires (émissaires, rejets et sites côtiers d'immersion) ou secondaires (cours d'eau); la surveillance continue des zones du large (zones de référence) fournissant des renseignements sur les tendances générales du niveau de la pollution en Méditerranée.

En Tunisie, il existe deux grandes stations de mytiliculture qui sont situées dans la lagune de Bizerte, au nord de la Tunisie. Ces moules sont destinés à l'exportation. Le suivi de ces deux stations d'élevage de moule est important.

Dans le cadre du programme "Mussel Watch Méditerranéen" nous proposons de travailler dans les deux sites d'élevage de moules situés dans la lagune de Bizerte au nord de la Tunisie (Menzel Jemil et FMB). Etant donné l'absence de moules dans l'est et le sud tunisien, on se propose de mener ces projets de suivi de la pollution (surveillance) et d'analyse des tendances (biosurveillance) sur les palourdes. Les paramètres à mesurer de manière trimestrielle concernent l'évolution des concentrations du plomb, cadmium et mercure ainsi que la détermination des teneurs en pesticides et en hydrocarbures totaux et bien évidemment un suivi mensuel des paramètres hydrobiologiques (T, S, O^2…..). D'autre part, on procèdera à l'analyse des biomarqueurs de la pollution causée par ces micropolluants. Nous axerons nos suivis principalement sur l'analyse des metallothioneines, de l'Erod et de l'altération lysosomale.

Monitoring of radionuclides in Algerian coastal waters

Abdelkader Noureddine

Commissariat à l'Energie Atomique (COMENA), Centre de Recherche Nucléaire d'Alger (CRNA.) Laboratoire d'Etudes d'Impact Radiologiques, Algiers, Algeria

ABSTRACT

Samples of surface (0-15 cm) marine sediments of different grain sizes, namely sand, muddy and fine sand, and surface and deep seawater were collected in different locations along the Algerian coast during the period of 1993 to 2001 using Van Veen type grab, box corer for sediment and bottles for water columns. Sediments samples were analysed directly by gamma spectrometry for ^{40}K, uranium and thorium series radionuclides and ^{137}Cs. Some of them have undergone radiochemical separations for the extraction of ^{90}Sr, Pu isotopes, ^{210}Pb and ^{210}Po.

Seawater samples were first preconcentrated on board of the *M.S. Benyahia* vessel of Institut des Sciences de la Mer et de l'Aménagement du Littoral (ISMAL) using AMP and then analysed at the laboratory by direct counting gamma spectrometry.

The analysis revealed measurable quantities of artificial radioisotopes, $^{239+240}$Pu (0.3- 0.6), ^{137}Cs (0.6-9), and ^{90}Sr (1.4-7.4) Bq Kg^{-1} dry mass and showed natural radioactivity ranging from (12-128), (7-28), (5 -35), (5-42), (11-48), (127-632), (60-131), (52-118) Bq Kg^{-1} dry mass, for ^{226}Ra, ^{214}Pb, ^{214}Bi, ^{228}Ac, ^{212}Pb, ^{40}K, ^{210}Pb and ^{210}Po, respectively.

Concerning the biological indicators such as mussels and seaweeds, we did not have yet the opportunity to analyse the eventual radioactive concentration.

INTRODUCTION

Different radioactive sources may contribute to the introduction of radioactivity into the marine environment. In addition to the artificial radioactivity, natural radionuclides can occur, due to weathering and recycling of terrestrial minerals and rocks, in the sea floor and in sea water to give rise to ^{40}k, ^{87}Rb, uranium and thorium series (IAEA, 1988).

Consequently, several works were launched from 1993 to 1996 by collecting very simply, due to the lack of equipment at that time, surface sediments from some bays along the Algerian coast, in order to determine the concentration of gamma-emitting radionuclides.

Thereafter, following the acquisition of counting equipment by our laboratory and the introduction of a TC project ALG/2/006 through the IAEA (1996-1998) and the collaboration with ISMAL, we managed in undertaking an oceanographic sampling campaign along the Algerian coast during October-November 1999. to collect surface sediment, core sediment and surface and deep water (down to 500m) samples (Nourredine *et al*., 1997).

These works were strengthened by a second oceanographic campaign through the participation of Algeria to the regional project RAF/7/004, where mainly three water column (down to 2000m) and two core sediment samples were collected along the Algerian littoral. This campaign was organized by the COMENA in collaboration with ISMAL and IAEA and was undertaken during August 2001 on board of the *M.S. Benyahia* scientific vessel (Nourredine *et al*., 2001).

At present, it would be a very good opportunity to complete such studies by including another marine bioindicator species through participation to the "Mussel Watch Project".

The data that could be necessary for the achievement of the above mentioned aspect consist on the industrial releases carried out along the Algerian coast. One finds , in the west, zinc industry in Ghazaouet, a cement plant in Beni-saf, the petroleum port in Arzew and the releases of the El-harrach and Hamiz rivers; in the east, the petroleum ports in Bejaia and Skikda, leather treatment in Jijel and the contribution of the Soummam river near Bejaia.

Regarding the occurrence of mussels namely *Perna Perna* and *Mytilus galloprovincialis*, these can be found in the west (Beni-saf and the port of Oran), in the north (Port of Algiers and isle of Sandja near Cap-Matifou), in the east (port of Bejaia, a small island at about 45 km north of the port of Annaba, Skikda and the Lake Mellah in El-Kala) as shown in Figure 1.

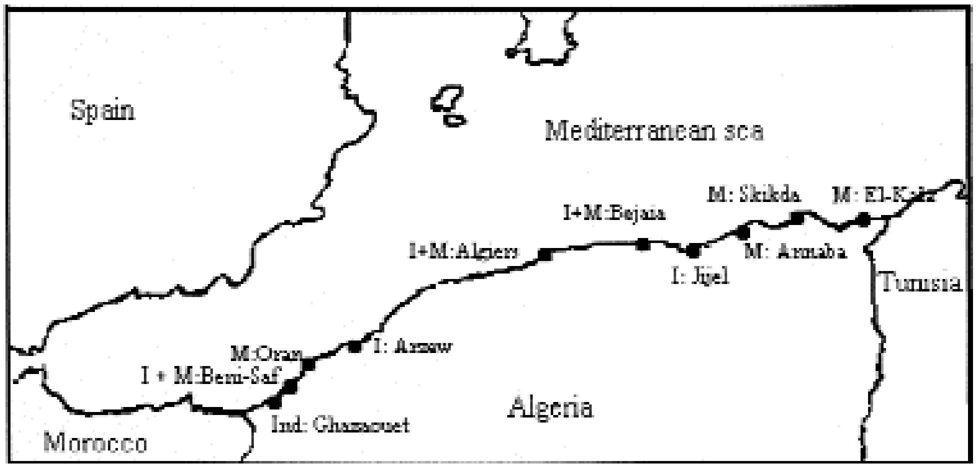

Figure 1.
Localisation of industrial and rivers releases: I. Ghazaouet (zinc); Beni-Saf (cement plant); Arzew (petroleum port); Algiers (river releases); Bejaia (petroleum port and Soummam river releases); Jijel (leather treatment).
Occurrences of mussels : M
Beni-Saf, Port of Oran, nearby the bay of Algiers, Port of Bejaia; Nearby the port of Annaba, Lake Mellah : El-Kala.

EXPERIMENTAL METHODS

Sampling

Surface sediment samples were collected at different locations by means of a Van veen type grab from undisturbed areas at a depth ranging between 20 to 100m. Core sediment samples, were sampled using two box corers, Kahl Sico for a depth of 350m and box corer 750 (IAEA) for a depth of about 1000m. Seawater samples were collected by means of Niskin bottles of 50l volume (COMENA) and a stainless steel sampler of 270l volume (IAEA). In addition, seawater was passed on board through cartridge filter to retain Cs isotopes.

The samples were stored in plastic bottles, labeled, and brought to the laboratory to be oven dried at 100°C, crushed and homogenised prior to either direct counting by gamma spectrometry, or radiochemical separations for beta-counting or alpha-spectrometry.

Radioactivity Measurement

The samples were analysed to detect any radioactive contamination and eventually its origin. Another objective was to determine the uptake of radioactivity by marine surface sediments and seawater and at the same time to study the radioactive distribution in the marine environment. Using a combination of direct gamma spectrometry, radiochemical separations, alpha-spectrometry and beta-counting, activity levels of the most significant naturally-occurring (^{210}Po, ^{210}Pb, ^{226}Ra, etc.) and artificial radioisotopes (^{137}Cs, ^{90}Sr, and Pu isotopes) were determined. The gamma-emitting radionuclides were determined using GeLi and HpGe at two different geometries, namely, 500 cm^3 Marinelli and 250 cm^3 plastic cylindrical form beakers. For the alpha and beta emitters, after dissolution of the samples, radiochemical separations were undergone. The source of plutonium isotopes was prepared by coprecipitation method on a 0.1μm pore size membrane filter (Larosa *et al.*, 1992). Regarding the ^{90}Sr, the chemical recovery was determined by gravimetry and the final source was measured by liquid-scintillation counting (LSC) (Vajda *et al.*, 1992)

RESULTS AND DISCUSSION

The analysis revealed measurable quantities of artificial radioisotopes, $^{239+240}$Pu (0.3-0.6), ^{137}Cs (0.6-9), and ^{90}Sr (1.4-7.4) Bq Kg^{-1} dry mass and showed natural radioactivity levels ranging, in Bq Kg^{-1} dry mass : 12-128 (^{226}Ra), 7-28 (^{214}Pb), 5-35 (^{214}Bi), 5-42 (^{228}Ac), 11-48 (^{212}Pb), 127-632 (^{40}K), 60-131 (^{210}Pb), 52-118 (^{210}Po). The values of the average concentration of radioelements are given in Table 1. The origin of the radioactive contamination, and the uptake of radioactivity by some marine sediments, are discussed and compared with other published data. ^{137}Cs, ^{90}Sr and $^{239+240}$Pu, in the sediments are clearly observed, and concentration ratios of ^{137}Cs to $^{239+240}$Pu, and 238Pu to $^{239+240}$Pu, which are around 17.5 and 18 respectively, are used to determine eventually their origin. Regarding the uptake of radioactivity, the level is seen to be correlated with the variations of the fine grain-size sediments (Ivanovich and Harmon, 1982; Delle Sitte *et al.*, 1984).

Table 1. Range values of activities (Bq kg^{-1} dry mass) of some alpha, beta and gamma radionuclides in marine surface sediment from different bays along the Algerian coast.

Sampl.Locat.	Nature of sed.	^{226}Ra	^{214}Pb	^{214}Bi	^{210}Po	^{210}Pb	^{228}Ac	^{212}Pb	^{40}K	^{137}Cs	^{90}Sr	^{239}Pu	^{238}Pu
Bay of Ghara	muddy	74±23	10±2	7±2 ???	52±1	80±2	20±7	23±4	446±85	7±2	1.5±0.4	0.3±0.02	0.02±0.01
West		128±35	22±4	28±8	118±3	131±3	35±8	26±4	518±98	8.5±2	7.4±1.4	0.6±0.02	0.05±0.02
Bay of Ghaza	muddy	27±3	8±2	5±2	–	–	12±1	11±1	101±216	0.7±0.1	—	—	
West		133±36	22±4	28±5			35±8	35±6	32±114	9±2	—	—	—
Bay of Algiers	Differ. nature	25±3	9±1	8±1			7±1	15±2	127±24	0.6±0.3	—	—	—
		66±8	20±2	18±2			29±3	41±4	447±49	7.0±1.0	—	—	—
Bay of Zem.	Differ. nature	12±2	7±1	6±1			5±1	13±1	159±15	0.6±0.1	—	—	—
East		63±7	28±3	29±3	—	—	30±4	48±4	309±35	1.5±0.2	—	—	—
Bay of Bejaia.	Differ. nature	63±5	24±2	16±2	—	—	18±2	41±3	252±22	8±2	—	—	—
East		112±30	28±5	35±8	—	—	42±10	48±4	542±100	12±1	—	—	—

Concerning the surface seawater, the concentrations range from 2.3 to 3.2 Bq.m^{-3}, and the average value is around 2.6 Bq.m^{-3} (Gheddou *et al.*, 1998). After comparison with the literature (MARINA MED Project, 1994) it was found that our average concentration value is close to that of Mediterranean sea in coastal regions.

Other samples of surface and core sediment collected during November 1999 and August 2001 are being analysed by direct counting and radiochemical separations and the results are to be shortly obtained (Nourredine *et al.*, 2001).

Spatial and temporal trends in the distribution of contaminants and their biological effects in the NW Mediterranean

Cinta Porte and Joan Albaigés

Environmental Chemistry Dept. IIQAB-CSIC, Barcelona, Spain

A variety of studies have been conducted along the Spanish coast (NW Mediterranean, North Atlantic), to assess spatial and temporal aspects of the distribution of organic and organometallic contaminants. Different organisms (molluscs, crustaceans, and fish) have been used to this end, and the distribution of PAHs, PCBs, DDTs, organotin compounds, organochlorinated and organophosphorus pesticides, among other pollutants, was considered. A summary of our main experiences within the "Mussel watch" concept is indicated below.

- Selection of suitable sentinel species. Generally, mussels and benthic fish (*Mullus barbatus*) are selected as suitable indicator species of coastal pollution, as they give complementary information on chemicals bioavailable in the water column and sediments, respectively (Porte et Albaigés, 1993). Bioaccumulation patterns of the different chemicals vary substantially among species. Habitat, season, and food web play a key role on the bioaccumulation process. Even within bivalve species, differences up to 10-fold in terms of bioaccumulation of some POPs (e.g. PCBs) have been observed, and related to different habitat and lipid content of the species studied (mussels, oysters, clams, western oysters) (Solé *et al*., 1994a).
- Influence of the biological cycle of the organism. The biological cycle of the organisms strongly affects body burdens of liphophilic persistent chemicals; this has been particularly investigated in cultured mussels from the Ebro Delta, and related to cycles of food availability and reproduction (Solé *et al*., 1995). Important differences in terms of organochlorinated compounds accumulated in muscle of *Mullus barbatus* have been recorded before (high levels) and after (low levels) the reproduction period.
- Geographical coverage. Most of the studies have been undertaken along the Catalan coast, but also the Western Mediterranean coast (France, North Italy, Corsica, Sardinia) through our participation to several EU funding projects and cruises. The analyses of fish samples (muscle, bile) allowed the detection of hot-spots near big cities (Barcelona, Marseilles) and rivers (Ebro, Rhone), and indicated the elevated bioavailability of some pollutants to coastal fish (Porte *et al*., 2002; Escartin and Porte, 1999a), but also to mesopelagic fish (Garcia *et al*., 2000), and deep-sea fauna fish (Porte *et al*., 2000; Escartin and Porte, 1999b).
- Temporal coverage. Temporal coverage is limited by the non-existence of long term funding projects. Nonetheless, some fragmentary data are available for areas, such as the Ebro

Delta, which indicate a general decline of organochlorinated pesticides body burden in cultured mussels during the period 1980-1992 (Solé *et al*., 1994b)., and a less evident decline of PCBs. Some other analyses were carried out in the late 90's, and are currently under evaluation.

Increasing efforts have been addressed to the measurement of biological effects, particularly in terms of biochemical responses, that could in most cases be linked to contaminants present in the marine environment. Hence, a good relationship was observed between biomarker responses (EROD activity, CYPIA content, catalase) measured in the red mullet (*Mullus barbatus*) and tissue levels of PCBs[4]; or between degree of imposex in *Bolinus brandaris* and tissue residues of organotin compounds (Solé *et al*., 1998). These biological effects, as the bioaccumulation process itself, are induced by contaminants, but can be influenced by a variety of other natural (temperature, salinity, etc.) and anthropogenic factors (mixtures of pollutants, and pollutants availability).

To our opinion, future efforts in the field should be devoted: (a) to get comparable and representative data on concentrations of hazardous substances in a wide regional level (e.g. the whole Mediterranean basin), and (b) to establish long-term series from fixed stations representative of the area. The existence of those temporal and geographical trends will be an important management tool to establish ecotoxicological criteria, and will certainly speed-up the evaluation process of hazardous substances in the marine environment.

French experience in matter of coastal monitoring of trace contaminants, a short review

Didier Sauzade, Bruno Andral and Jean Yves Stanisière

Ifremer, Laboratoire Côtier de Toulon, La Seyne sur Mer, France

Trace contaminants are monitored in France since 1979 by the means of the "Réseau National d'Observation" (National Monitoring Network) – RNO, operated by IFREMER at the request of the Environment Ministry. RNO aims at assessing levels and trends for pollutants and general parameters of coastal waters quality (Claisse, 1989, 1999). RNO data contribute to the French commitments within International Conventions (OSPAR, Barcelone Convention) to which these data are forwarded. Sampling is carried out all along the French coastal shores, on water column, (physicochemical parameters, nutritive elements and chlorophyll), sediment and biota (heavy metals, DDT, lindane, PCB, PAH). Settled at the national scale, the spatial sampling of RNO is relatively scattered, especially on the Mediterranean coast (mean distance between stations monitoring contaminants in biota : 85 km). Consequently, the RNO cannot provide a sufficient assessment of the mean contamination of the 50 coastal units established by the Water Management Masterplan for the French Mediterranean coast.

For this reason, the RNO has been completed by a regional network, the "Réseau Indicateurs Biologiques"– RINBIO, comprising one or two stations per coastal units. This network has been developed by Ifremer, with the co-operation of IPSN * (Thébault *et al.*, 1998b) at request of the French Water Agency. Like RNO, RINBIO measures trace contaminants in biota, but RNO uses natural or cultivated mussels, or oysters when mussels are lacking, while RINBIO is based on the active biomonitoring strategy (de Koch and Kramer, 1994), using transplanted mussels.

After a pilot study carried out in 1996, two RINBIO campaigns have been performed in 1998 and 2000, with respectively 40 and 93 artificial stations, settled along the French Mediterranean coast (Andral *et al.*, 1997, 2000, 2001). After a three months immersion, the samples recovery for the last campaigns was satisfactory (respectively 85% and 97% of the moored ones) and gave confidence in the method. This network is now considered as operational and will be run in routine every 2 or 3 years.

Operational experiences issued from these two networks appear to be significant enough to contribute to the design of a Mediterranean musselwatch, as promoted by CIESM. This short review presents briefly their main characteristics (Table 1), introduces the normalisation efforts made to interpret results at spatial scale and compares their respective advantages.

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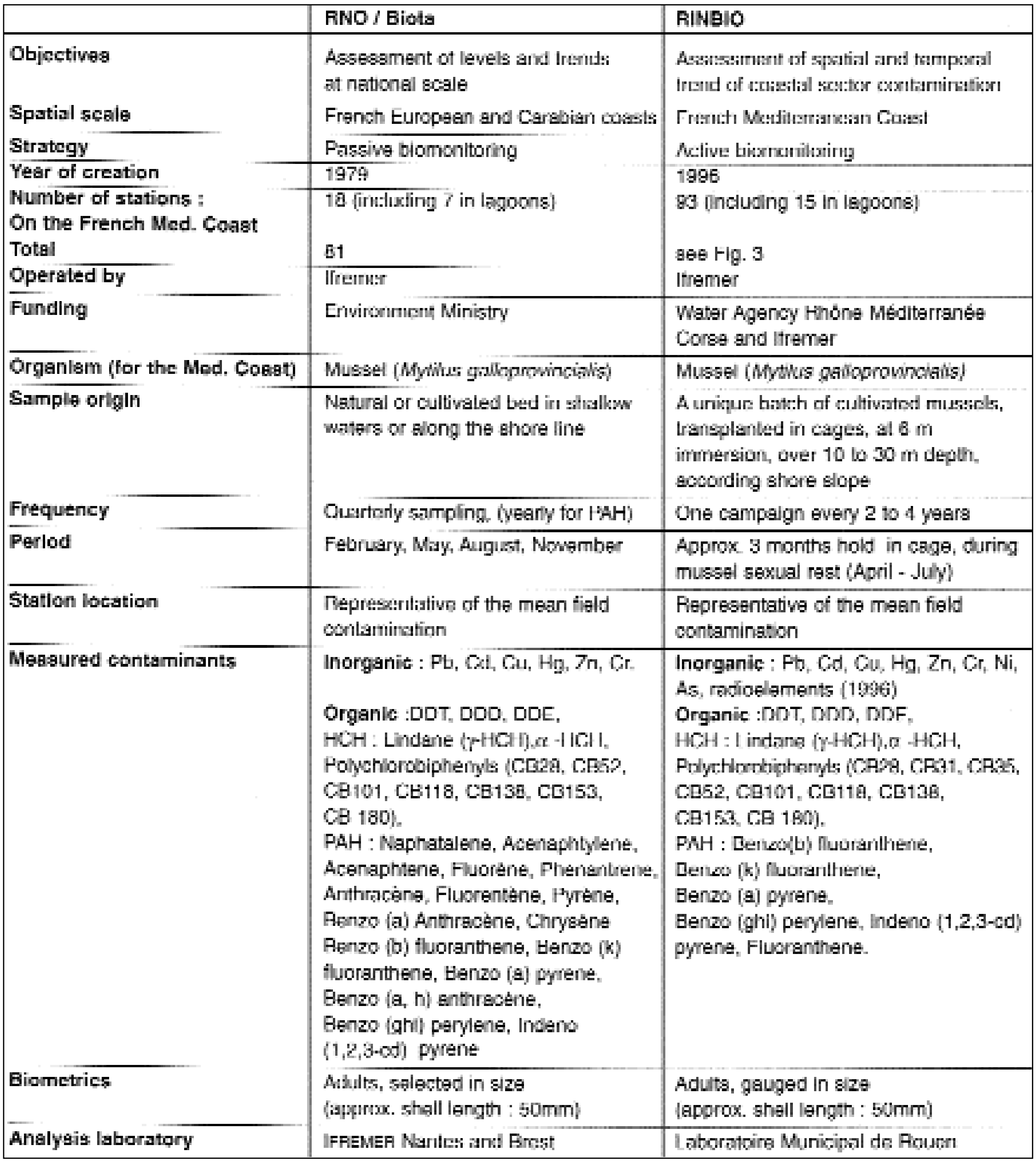
* Institut de Protection et de Sûreté Nucléaire.

Table 1. Characteristics of the two main French networks monitoring trace contaminants in biota.



	RNO / Biota	**RINBIO**
Objectives	Assessment of levels and trends at national scale	Assessment of spatial and temporal trend of coastal sector contamination
Spatial scale	French European and Carabian coasts	French Mediterranean Coast
Strategy	Passive biomonitoring	Active biomonitoring
Year of creation	1979	1996
Number of stations : **On the French Med. Coast**	18 (including 7 in lagoons)	93 (including 15 in lagoons)
Total	81	see Fig. 3
Operated by	Ifremer	Ifremer
Funding	Environment Ministry	Water Agency Rhône Méditerranée Corse and Ifremer
Organism (for the Med. Coast)	Mussel (*Mytilus galloprovincialis*)	Mussel (*Mytilus galloprovincialis)*
Sample origin	Natural or cultivated bed in shallow waters or along the shore line	A unique batch of cultivated mussels, transplanted in cages, at 6 m immersion, over 10 to 30 m depth, according shore slope
Frequency	Quarterly sampling, (yearly for PAH)	One campaign every 2 to 4 years
Period	February, May, August, November	Approx. 3 months hold in cage, during mussel sexual rest (April - July)
Station location	Representative of the mean field contamination	Representative of the mean field contamination
Measured contaminants	**Inorganic** : Pb, Cd, Cu, Hg, Zn, Cr. **Organic** :DDT, DDD, DDE, HCH : Lindane (γ-HCH),α -HCH, Polychlorobiphenyls (CB28, CB52, CB101, CB118, CB138, CB153, CB 180), PAH : Naphatalene, Acenaphtylene, Acenaphtene, Fluorène, Phenantrene, Anthracène, Fluorentène, Pyrène, Benzo (a) Anthracène, Chrysène Benzo (b) fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrene, Benzo (a, h) anthracène, Benzo (ghi) perylene, Indeno (1,2,3-cd) pyrene	**Inorganic** : Pb, Cd, Cu, Hg, Zn, Cr, Ni, As, radioelements (1996) **Organic** :DDT, DDD, DDE, HCH : Lindane (γ-HCH),α -HCH, Polychlorobiphenyls (CB28, CB31, CB35, CB52, CB101, CB118, CB138, CB153, CB 180), PAH : Benzo(b) fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrene, Benzo (ghi) perylene, Indeno (1,2,3-cd) pyrene, Fluoranthene.
Biometrics	Adults, selected in size (approx. shell length : 50mm)	Adults, gauged in size (approx. shell length : 50mm)
Analysis laboratory	IFREMER Nantes and Brest	Laboratoire Municipal de Rouen

NORMALISATION OF THE RESULTS

Pollution assessment from contaminant concentration in mussels supposes that the concentration factor (ratio between concentration at steady state and bioavailable concentration in sea water) is constant over the monitored area. However several studies have shown that contaminant bioaccumulation by mussels is influenced by both environmental conditions, such as salinity, temperature, primary production, and biotic conditions such as age, size or reproduction (ref. in Cossa, 1989). Concentration factor variability should be particularly taken into consideration in case of large scale networks such as RINBIO, designated to monitor a long coast line presenting a high range of trophic and physico-chemical conditions. from laguna and area under the Rhone plume influence to oligotrophic coastal water. In order to minimise variations due to biotic conditions, the RINBIO campaigns were performed using transplanted mussels, originated from a sole cultivated bed located in a low contamination area and selected in a narrow size range. Despite these precautions, it has been found that contaminant concentrations measured in mussels of stations exposed to the contamination "background noise" present a variability of 2 to 4. depending on the contaminant which leads to suspect an equivalent variability of the concentration factor. In particular, the growth rate during the transplantation period cannot be neglected and normalisation techniques are definitely required.

The condition index (CI), defined as the ratio of the soft tissues dry weight over shell weight, appears to be a good indicator of physiological status of mussels and is reported to be have been proposed for normalisation to compare contamination between different areas (Fisher, 1984; Soto, 1995). In the frame of the research program supporting the RINBIO network development, several regression analysis have been performed on network results and specific in situ experiments to statistically infer that tissue concentration under steady state conditions can be a function of the sample mean CI.

Two types of linear regressions link the contaminant concentration in soft tissues (Cm) and the CI of the sample: Cm is inversely proportional to CI for Zn, Hg, Cd, As, Cu, Ni, Cr and Ld. Cm is directly proportional to CI for DDT, DDD, DDE and PCB. These relations are not valid for stations located in polluted areas, when concentrations are higher than the contamination background noise (see Fig. 1). Relations are less statistically significant for As, Cu, Hg, and PAH. Models established from regressions can be used to normalise concentrations to an individual mussel characterised by a standard CI (calculated as the geometric mean of the data set used for the regression). The normalised concentrations are then comparable at the scale of the network, independently of the trophic conditions prevailing around the sampling stations. This normalisation method has been applied for interpretation of the RINBIO results, for the whole French Mediterranean coast. Reflections have started on similar normalisation for the RNO.

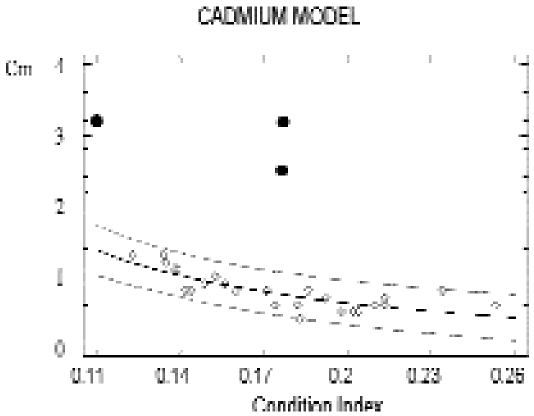


Fig. 1. Linear regression model for Cadmium. Cm in mg/g soft tissues. Confidence interval (95%) in dotted lines. The three black dots outside this interval correspond to three stations in Bages lagoons.

COMPARED ADVANTAGES OF ACTIVE AND PASSIVE BIOMONITORING

Musselwatch based on passive monitoring generally meets methodological constraints having direct consequences on sampling strategies: i) aquatic organism selected as biomonitor could not be found all along the coast; ii) use of different organisms greatly reduces signification of result comparisons; iii) for a given individual, several variation factors have been reported as susceptible to influence bioaccumulation, such as size or age of animals, seasons, or even genetic differences… Moreover, within natural populations, animals of equivalent size could present large variations in age, especially when sampling sites present differences in environmental conditions. Such variations are expected to be lower for transplanted population issued from a unique breeding site, and screened out in a narrow size range.

Main advantages of active biomonitoring are: i) known exposure period to contaminants; ii) location of stations without constraint regarding presence or not of natural or cultivated populations, whatever the distance to the shore; iii) more homogenous and less influenced by variation factors associated with natural populations bioaccumulation. From the RINBIO experience, main drawbacks are: i) logistic efforts for the mooring and recovery of cages; ii) mooring damages or losses due to climatic hazard or fishing activities.

Regarding this last point, depending on the network objectives, meteo-oceanic conditions, bathymetry and depth of moorings, many solutions are possible and generally, at reasonable cost, e.g. the RINBIO typical mooring (Fig. 2).

CONCLUSION

RINBIO, the first bioactive monitoring network to be carried out in France on such a large scale, gave a good idea of the advantages and limits of the caging technique in comparison with the use of shellfish natural populations. More specifically, it was shown that i) sample recovery rate can be very good with cheap but well designed and located moorings; ii) physiological state of the animals (through biometry) need to be considered to compare soft tissue concentrations, when sampling stations present large differences in trophic levels.

More studies are needed to complete the results gained, to know more about limits of the method, specially in sites where environmental conditions are not favourable to the natural growth of mussels.

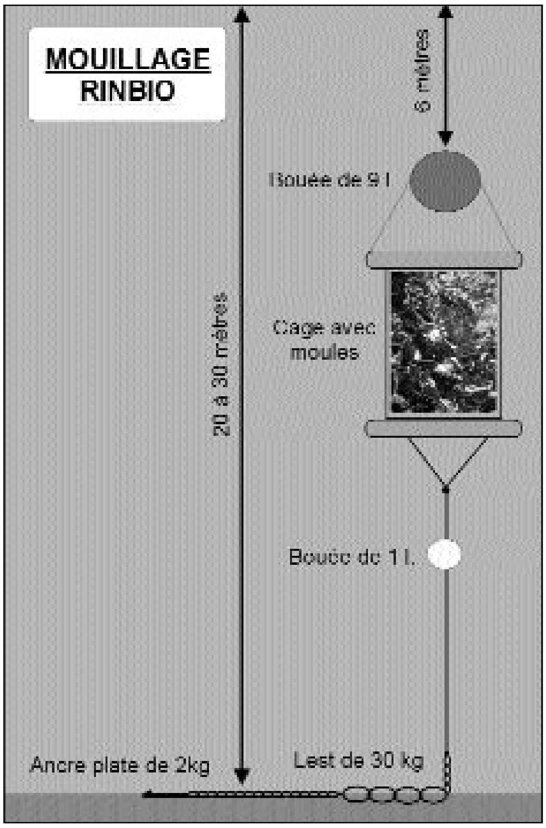


Fig. 2. Typical RINBIO mooring system.

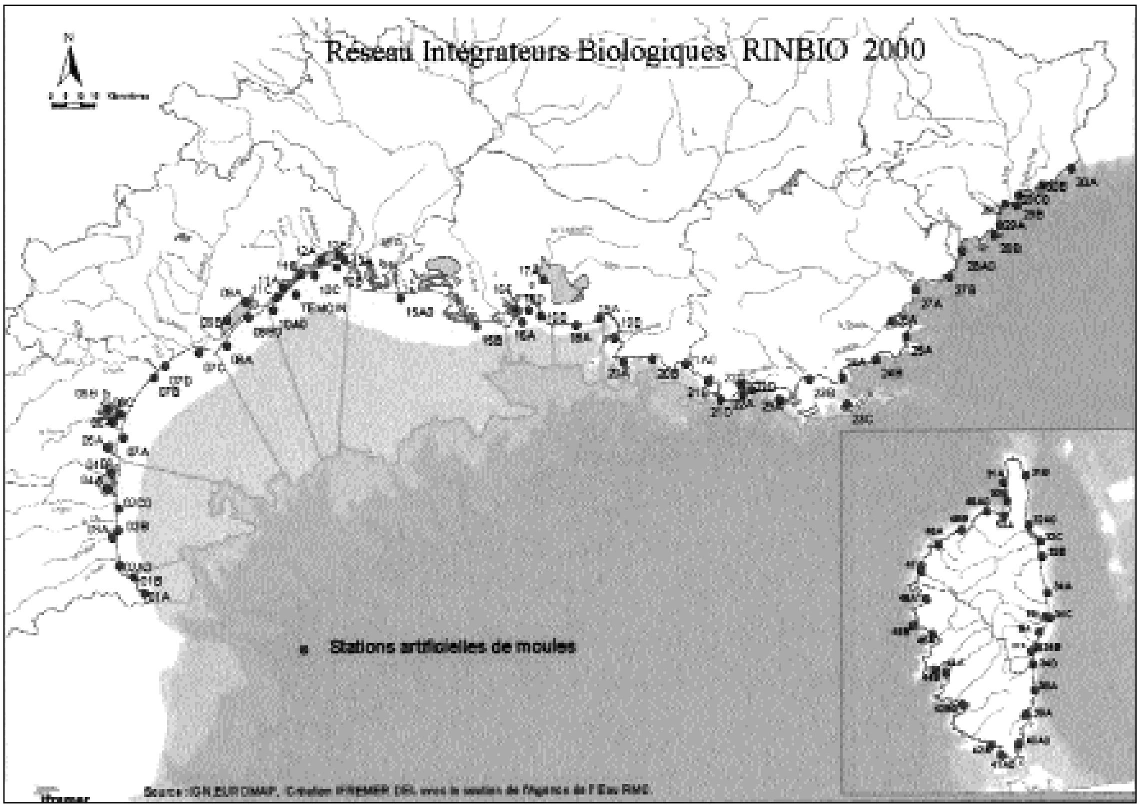


Fig. 3. Map of the RINBIO stations (2000), each dot representing one station.

Research works are in progress to develop a bioaccumulation model more realistic regarding mussel physiology, taking into account growth and sexual maturation, applicable to a Mediterranean environment.

In spite of its present limits, the use of such a technique gave way, for the first time, to a global vision of chemical contamination at a large scale in the riparian infralittoral zone. Similarly mussels immersed in cages along the Mediterranean coastline, by the riparian monitoring institutions acting within an international concerted action, could provide an instantaneous "photography" of the trace contaminant bioavailable at the scale of the Mediterranean Sea.

Monitoring of radionuclides in Libyan coastal waters

Mohamed Shenber

Tajoura Nuclear Research Centre, Radiation Protection and Health Physics Department, Environmental Radiation Measurements Division, Tripoli, Libya

OBJECTIVE

The main objectives of the workshop are to consolidate adequate regional technical capabilities and expertise for assessing radioactive and heavy metal contaminants in the marine environment with bioindicators and to develop a regional cooperation between the Mediterranean countries.

STRATEGY

In terms of marine environment protection, radionuclides are of concern, not only because of their potential effects, but also because high signal activity concentrations can be a signal of the presence of other types of pollution (e.g. heavy metals and hydrocarbons). The programme has to be divided in two parts: 1. sampling strategy; 2. quality assurance of data.

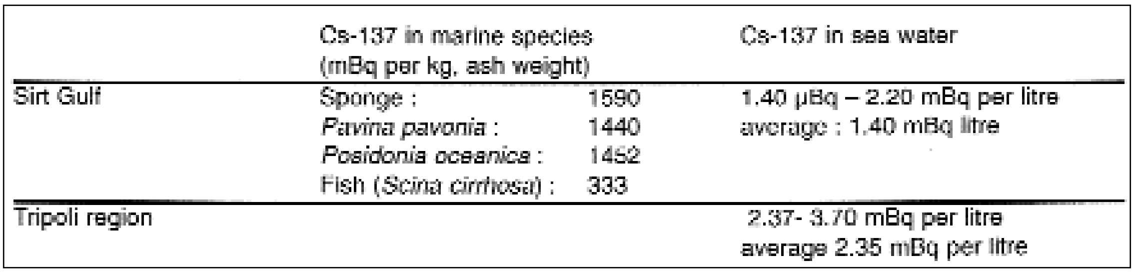
MONITORING ACTIVITIES

Tajoura Nuclear Research Centre, Radiation Protection and Health Physics Department, Environmental Radiation Measurements Division operates an on-going regional Project RAF/7/004 (1999-2003) under International Atomic Energy Agency assistance. We have started sampling in the northern coast of Libya from Zwara City till Misuratah City for about 210-km (water, sediment, fish and mollusks). We have started the preparation of the samples.

PROPOSITION

We have to create the appropriate framework for implementing and co-ordinations between the Mediterranean countries by exchanging the experience, selecting the same bioindicators for the region and exchanging the data.

RESULTS



	Cs-137 in marine species (mBq per kg, ash weight)		Cs-137 in sea water
Sirt Gulf	Sponge :	1590	1.40 µBq – 2.20 mBq per litre average : 1.40 mBq litre
	Pavina pavonia :	1440	
	Posidonia oceanica :	1452	
	Fish (*Scina cirrhosa*) :	333	
Tripoli region			2.37- 3.70 mBq per litre average 2.35 mBq per litre

The concentration factor (CF) in fish (*Scina cirrhosa*) was calculated to be 132 and the ingestion dose 19 nSv per year.

Radionuclide and heavy metal content in marine ecosystems on the Bulgarian Black Sea coast

A.S. Strezov, Tz.P. Nonova, T.T. Stoilova, P.S. Petkov

Institute for Nuclear Research and Nuclear Energy, Sofia, Bulgaria

The radionuclide and heavy metal content is investigated in Bulgarian Black Sea bottom sediment and algae samples, taken from 35 reference Black Sea coast locations, from the Romanian to Turkish border by low-level gamma spectrometry. The technogenic and natural radionuclides content in sediments is compared with the levels in algae and the obtained data show that radionuclide concentrations strongly depend on the sediment nature (slime, silt or sand) and algal species type (Figs. 1, 2) (Strezov *et al*., 1996, 1998, 1999; Jordanoca *et al*., 1999). The radionuclide concentrations were studied in different seasons but clear seasonal dependence was not established. It should be noted that *Bryopsis plumosa* (collected from three different locations) accumulates natural radionuclides (daughters of Th-230 and Th-232) with three orders of magnitude higher that the other species at the same location. Next the highest radionuclide content was obtained for the slime sediments and for certain red and green algae species.

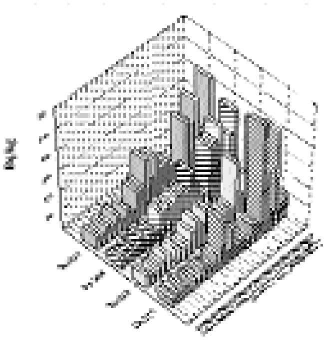


Fig. 1. Radionuclide contents in Black Sea sediments.

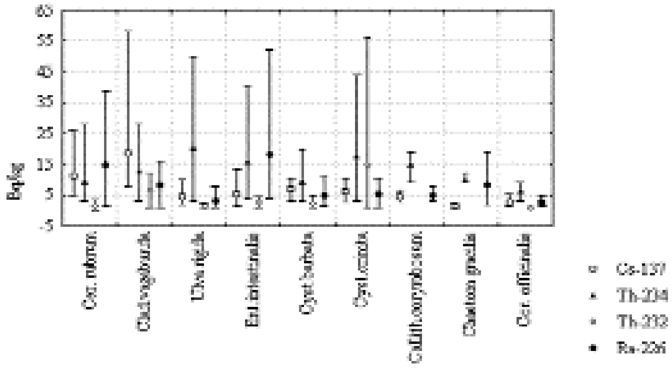


Fig. 2. Nuclide contents in Black Sea algae.

As illustrated in the Fig. 3 , Cs-137 content in algae varies in the order green:red:brown = 1:6:7 Bq/kg. The range of radionuclide accumulation is close in red algae while Ra-226 and Pb-210 concentrations clearly prevail in the green and brown algae.

Heavy metal (HM) content was measured by Atomic Absorption Spectrometry and the data obtained show that Fe and Mn concentrations are highest and that red algae species accumulate higher – Fe (red) 100-2000 ppm; (green) 140-2600 ppm; (brown) 10-700 ppm, Mn (red) 20-400 ppm; (green) 10-90 ppm; (brown) 5-55 ppm. The other HM content (presented in the Figures in

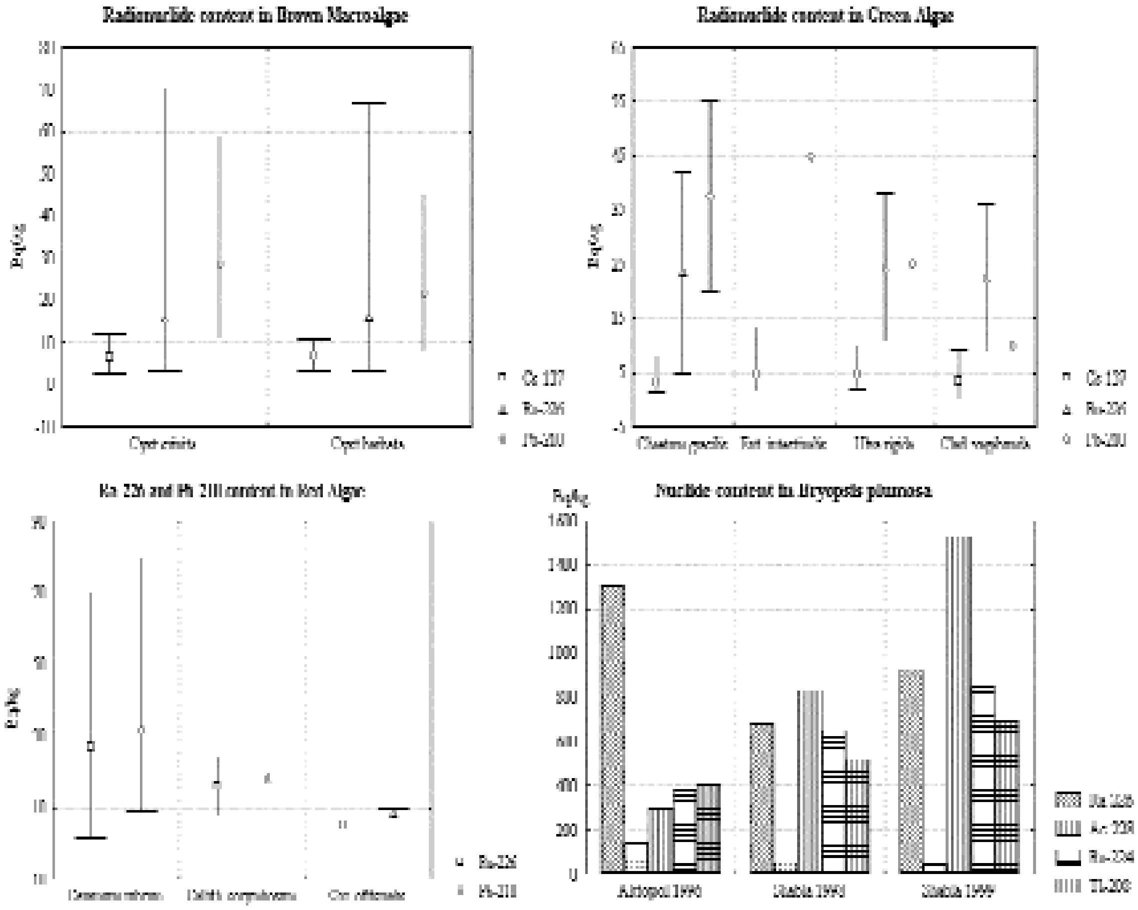


Fig. 3

means) shows that the corresponding species behave similarly except *Enteromorpha intestinalis* (Green) in the south regions and *Ceramium rubrum* (Red) in all locations which accumulate more than other species. Other authors have obtained similar to our data (Haridonidis and Malea, 1995, 1999; Topcuoglu *et al*., 1998; Guven *et al*., 1992; Campanella *et al*., 2001).

As seen from Fig. 4, Green algae (*Chaetomorpha gracilis, Enteromorpha intestinalis, Ulva rigida* and *Cladophora vagabunda*) contain similar mean quantities Pb and Cd, while Cu content varies. For the Brown (*Cystoseira crinita* and *C. barbata*) similar level is obtained for Cd and Cr,

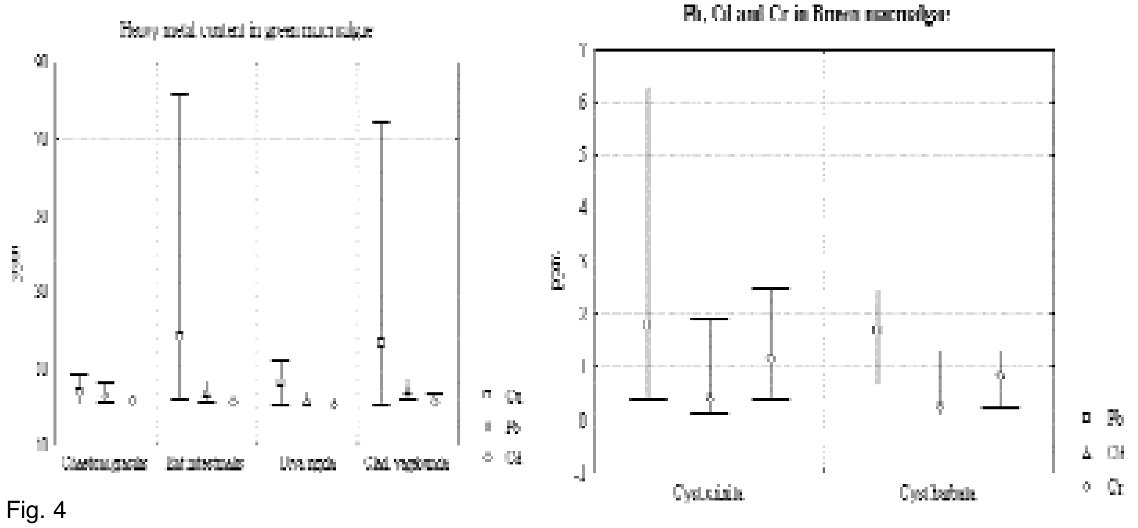


Fig. 4

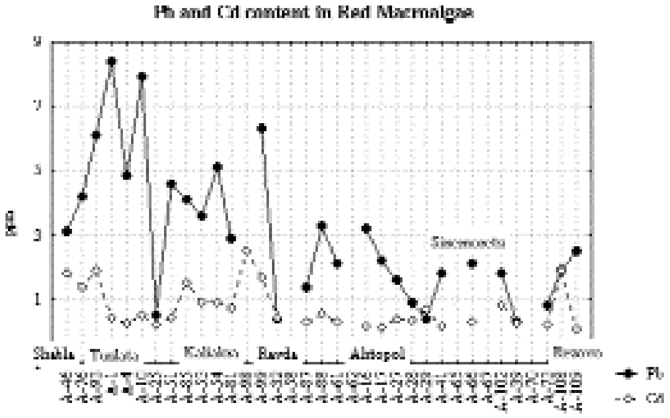


Fig. 4 (continued)

while Pb varies. For the Red macroalgae (*Ceramium rubrum, Calithamnion corymbosum* and *Corallina officinalis*) Cd content range is on the average 1 ± 1 ppm for all locations, while Pb content varies more (1-10 ppm) in the different years.

Our data indicate that there is no serious contamination in the Bulgarian Black Sea coastal waters with radionuclides and heavy metals and also which algal species can be appointed as candidates for biomonitors after statistical treatment of the obtained data and also studying the correlation between sediments – water – algae radionuclide and heavy metal content.

Comparison of heavy metal levels in native and cultured mussel *Mytilus galloprovincialis* (L, 1758) from the Bay of Izmir (Aegean Sea/Turkey)

Ugur Sunlu

Ege University, Faculty of Fisheries, Dept. of Hydrobiology, Izmir, Turkiye

INTRODUCTION

In the marine ecosystem, heavy metals occur in the water mass, suspended particles, sediment and biota. The use of biological indicators to monitor environmental contamination by trace metals has many advantages over the measurement of metals in water samples, since their content of pollutants harmful to marine life represents a time integrated image of the bioavailability of a pollutant which is not affected by short term fluctuations in sea water. Trace elements, when discharged to the marine environment, will be transported by prevailing currents and removed by either physical, chemical or biological processes. Through absorption, ion exchange and complexing of chelation, trace elements are filtered by marine bivalves and picked up by particulate organic matter and settle on the bottom. So marine bivalves and their environment, i.e. bottom sediments are known to accumulate high levels of metals, and are commonly used in monitoring studies.

More than 3 million people live around Izmir, which is located at the western end of Anotolia. Parallel to the population inflation, there is a rapid increase, in fisheries, industrial and commercial activities.

The domestic and industrial wastes of this densely populated settlement enter to the Bay water. Since 1999 50-60% of these wastes are treated in the sewage plant called “Big Channel”. The untreated waste waters of Izmir city consist of factory discharges (leather, textiles, food, detergents, beverages, chemicals, etc.). All these wastes dumped into the sea have an adverse effect both on the sea organisms and the water quality. Izmir Bay has become an important marine pollution focus point in Turkey.

The aim of this study is to compare the present status of heavy metals in the economically important native and cultured mussel *Mytilus galloprovincialis* (L, 1758) and in their living environment, i.e. bottom sediments.

STUDY AREA AND METHODOLOGY

Izmir Bay, situated in the western coast of the Aegean Sea, lies between the latitudes 28°20’ 38°42’ N and longitudes 29°25’ 27°10’ E. From the topographic and hydrographic point of view it is divided into the inner, middle and outer, bay regions (Fig. 1). During October 1998-September 2000, samples of mussel (*M. galloprovincialis)* of about 6 cm length were collected

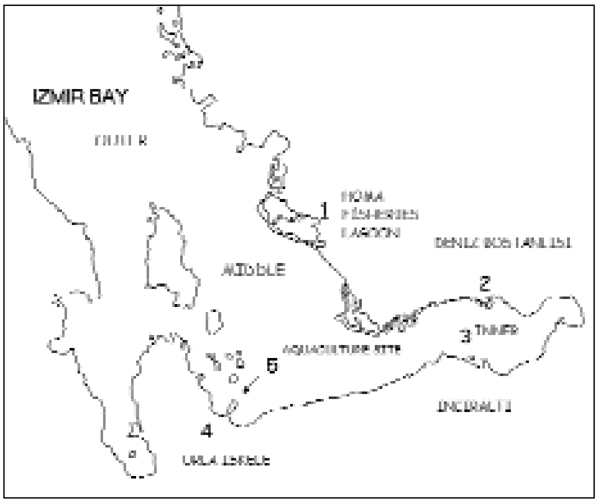


Fig. I. Map of sampling locations.

monthly by hand from the ropes at aquaculture site and four different sampling stations in the Bay of Izmir and on rocks in the shoreline of Izmir bay. Immediately after collection, the shell was cleaned and the soft part samples were made from 20 individuals, which had been carefully rinsed with abundant distilled water in order to eliminate sediment and other impurities. Then these samples were kept in a deep freeze (-21°C) until analysis and were prepared according to international standard methods (Bernhard, 1976). The composite samples of mussels were weighed and digested with conc. HNO_3: $HClO_4$ (5:1) (extra pure Merck) under reflux and filtered.

Sediments were collected monthly with an "Orange peel" grab of capacity 4,5 l from these sites at the same time and then stored plastic bags at -21°C. Each sediment sample was oven dried at -60°C for 24h and then sieved using a mesh. From the dried sediment samples an aliquot of 1g. (< 160 μ) was oxidized with 10ml., conc. HCl:HNO_3 (3:1) (extra pure Merck) under reflux at 120°C for two hours and then filtered through Whatman 40 filter paper. All samples were diluted with bi-distilled water and analyzed (Arnoux *et al.*, 1981). Metal samples were analyzed by Atomic Absorption Spectrophotometer using a 2380 Perkin-Elmer (AAS). Metals were determined by direct aspiration using an air acetylene flame. Intercalibration homogenate samples (IAEA-142/TM for mussel, IAEA-314 for sediment samples from the IAEA, Monaco Laboratory) were used as a quality control for the analytical methodology.

RESULTS IN MUSSEL TISSUES

The concentrations of some heavy metals (Pb, Cd, Cu, Zn, Ni) in the tissues of the Mussel *M. galloprovincialis* were determined separately from different regions of Izmir Bay. Minimum, maximum and mean levels of heavy metal in native and cultured mussel tissues are given in Table 1.

Table I. Minimum, maximum and average levels of heavy metal in M. galloprovincialis (L, 1758) from different regions of Izmir Bay (mg/g.wet weight).

Stations	Pb	Cd	Cu	Zn	Ni
Homa Fisheries Lagoon (1)	0.61-1.40 1.08	0.15-0.56 0.40	0.32-2.30 1.40	9.55-26,05 18.72	0.38-1.19 0.68
Deniz Bostanlisi (2)	0.58-1.82 1.20	0.41-1.12 0.68	0.82-3.25 1.54	13.20-30.60 21.75	0.30-1.32 0.82
Inciralti (3)	0.62-1.70 1.18	0.04-0.48 0.26	0.71-2.10 1.42	10.05-58.50 29.36	0.50-0.92 0.71
Urla Iskele (4)	0.60-0.86 0.73	0.16-0.34 0.22	0.90-2.09 1.29	11.10-26.60 18.20	0.35-0.90 0.54
Aquaculture Site (5)	0.60-0.85 0.70	0.09-0.30 0.19	0.81-2.04 1.20	9.90-24.41 16.85	0.20-0.70 0.48

It can be seen from the tables that there are differences in the metal concentrations according to the localities. The heavy metal concentrations of native *M. galloprovincialis* is slightly higher than cultured ones.

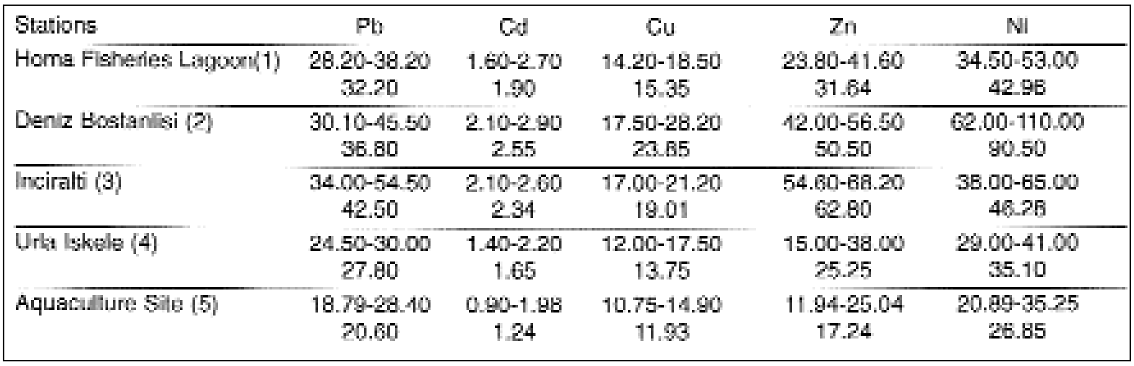
RESULTS IN SEDIMENT SAMPLES

Table II presents minimum, maximum and mean values obtained from the superficial sediments of Izmir Bay.

According to the results obtained, sediments show a contaminations of the metals such as Pb, Cd, Cu, Zn, Ni. The level of trace metals decreases from Ni to Cd.

Levels of contaminations by heavy metals in the Inner Bay are more important due to the factories, harbor activity and domestical discharges, but we note a clear decrease at the exit of the area.

Table II. Minimum, maximum and average levels of heavy metals in sediment samples from different regions of Izmir Bay (mg/g.dry weight).



Stations	Pb	Cd	Cu	Zn	Ni
Homa Fisheries Lagoon(1)	28.20-38.20 32.20	1.60-2.70 1.90	14.20-18.50 15.35	23.80-41.60 31.64	34.50-53.00 42.96
Deniz Bostanlisi (2)	30.10-45.50 36.80	2.10-2.90 2.55	17.50-28.20 23.85	42.00-56.50 50.50	62.00-110.00 90.50
Inciralti (3)	34.00-54.50 42.50	2.10-2.60 2.34	17.00-21.20 19.01	54.60-68.20 62.80	38.00-65.00 46.28
Urla Iskele (4)	24.50-30.00 27.80	1.40-2.20 1.65	12.00-17.50 13.75	15.00-38.00 25.25	29.00-41.00 35.10
Aquaculture Site (5)	18.79-28.40 20.60	0.90-1.98 1.24	10.75-14.90 11.93	11.94-25.04 17.24	20.89-35.25 26.85

CONCLUSIONS

Some mollusk species (especially *M. galloprovincialis, Ostrea edulis*) are known to accumulate high levels of trace metals in their soft tissues and these species are commonly used in biomonitoring studies. Bioaccumulation in mollusk species adequately reflects the changing levels in the marine environment for trace metals. The degree of their accumulation depends on their metabolic activity, growth, biochemical composition, reproductive and feeding condition.

According to our results, a dominant source of metal contamination is from urban and industrial activities and less important inputs are from continental and agricultural origins. Metal concentrations distribution in native mussels indicate higher levels in inner parts of Izmir Bay and the Homa fisheries lagoon is affected by the heavy polluted river of Gediz. *M. galloprovincialis* seems to be much more adapted to the environmental conditions of the polluted bay waters than the other mollusk species (Egemen *et al*., 1998; Sunlu *et al*., 1998). Aquaculture site relatively far from anthropogenic sources is affected very little by chemical contamination. In our present studies, heavy metal levels in *M. galloprovincialis* and sediment samples show the similarity with the previous studies in the Mediterranean Sea. (UNEP, 1996).

In Izmir Bay, here are still no toxic levels in the tissues of M. galloprovincialis and values lie just within the safety level for human consumption. On the other hand the average consumption of these mollusk in our region is generally low.

Consequently, Inner part of Izmir Bay needs to be monitored quite closely in the future until the Big Channel wastewater treatment project will be completed.

Monitoring of radionuclides on the French Mediterranean coast

Hervé Thébault

Institut de Radioprotection et de Sûreté Nucléaire (IRSN), Laboratoire d'Etudes Radioécologiques Continentales et de la Méditerranée, Centre IFREMER, La Seyne sur mer, France

GENERAL CONTEXT AND OBJECTIVES

The IRSN carries out radioactivity level measurements in samples collected in the environment, as part of its research activities. The objective is to determine and understand processes governing distributions and transfers of radionuclides from natural and artificial origins in different environmental media, in space and time. Knowledge of radioactivity levels and trends enables IRSN to design, support and validate modeling of processes for the transfer of radionuclides into the environment.

Environmental research at IRSN is based on the permanent structure of the OPERA (Observatoires Permanents de la RAdioactivité de l'environnement - Permanent Environmental Radioactivity Observatories) program. This work helps to determine and monitor the variation of radioactivity levels in the environment in France. Every month, the state of natural and artificial radioactivity in the environment is analysed. Information collected by IRSN is based on a network of more than 30 stations throughout the country which are representative of the main media in the biosphere. This system was set up in 1959 for the atmospheric domain (aerosols and rainfalls) (Calmet *et al*., 1998). Since 1984 it also covered the French Atlantic and Mediterranean coasts (coastal species and sediments) and was extended to cover land in 1993 (soil, plants, animals) and then rivers in 1998 (water, suspended solids). This multi-annual program was used to create chronological series essential for scientific studies on the variability of observed levels. Since 2000, all data are available on-line at the following Web address: <www.ipsn.fr/opera>.

SAMPLING STRATEGY

On the French Mediterranean coast, most of the sampling stations were chosen close to the Rhone delta into which radionuclides were released from nuclear facilities built on the Rhone River banks and its tributaries (Calmet *et al*., 1994). After the recent drastic reduction of industrial discharges, in particular with the end of spent fuel reprocessing activities in Marcoule, sampling stations were re-arranged to monitor the coastal zone from the Spanish to the Italian borders and Corsica (Fig. 1).

The sampling frequency is adapted to variations in the inputs and the seasonal cycle of the indicators. Thus, marine samples are collected monthly from species known to accumulate radionuclides in their tissues. The most frequently collected samples are mussels (*Mytilus galloprovincialis*) and in some sites, benthic fish like *Mullus* sp. Sediments are sampled quarterly in

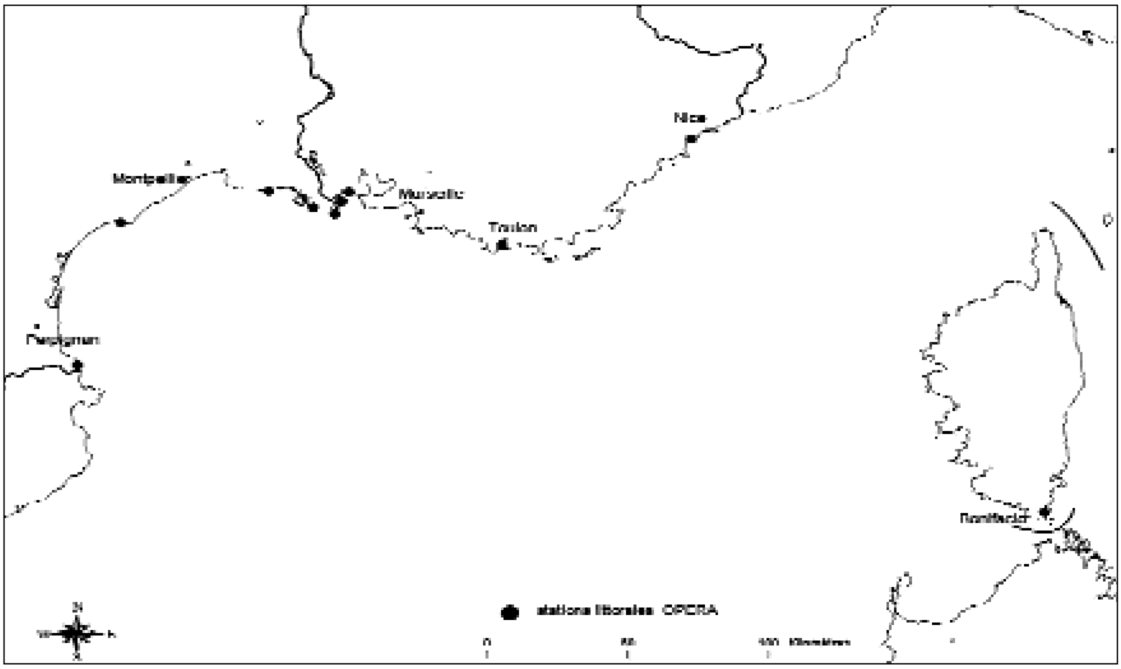


Figure 1. Sampling stations along the French Mediterranean coast.

two stations close to the mouth of the Rhone, where high sedimentation rates are observed. Mussels are collected from natural mussel beds except at Carteau where samples are taken from shellfish farming facilities

SAMPLE PROCESSING AND MEASUREMENTS

About 3 kg of mussels are processed, the intervalvar content (soft part + water) is separated from shell after heating, dried at 105°C to reach constant weight and ashed at 560°C for 12 hours.

The remaining ashes are ground to powder and homogenized to fill calibrated 20 ml counting containers. At the beginning of the year 2000, due to the decrease of environmental levels of artificial radionuclides, the sample size was raised up to 5 kg of mussels in order to fill a 60 ml counting container. This enables to lower the detection limit of some radionuclides by a 2.5 of factor.

High resolution direct gamma spectrometry is performed by IRSN/LMRE through N-type hyper-pure Germanium detectors with a relative efficiency between 50 and 70% (Bouisset et Calmet, 1997). To reduce the impact of cosmic rays on trace-level measurements, the counting room is set up two storeys below ground level under a 3 m thick borium concrete slab, cladded with 10 cm thick lead bricks and lined with 5 mm thick copper tiles.

RESULTS AND DISCUSSION

For ^{137}Cs, the time series for all sampling sites shows a similar trend with a 100-fold decrease from levels observed just after the Chernobyl accident (Charmasson *et al.*, 1999) and 10-fold decrease during the past ten years (Fig. 2) to reach levels between 0.02 and 0.04 Bq.kg^{-1} wet weight of intervalvar content. The evolution over time of ^{137}Cs in mussels collected in the coastal zone follows the steady decrease of inputs from the global and Chernobyl fallout and the reduction of industrial discharges along the Rhone river. Present levels in benthic fish are slightly higher than in mussel, ranging from 0.07 to 0.2 Bq.kg^{-1} wet weight, in relation with the position of this species in the food web and with the tissue analysed (muscle in fish).

^{60}Co and ^{106}Ru are detected in mussels only in stations influenced by the Rhone river outflow, with decreasing values from the river mouth to the west, which corresponds to the prevailing direction of the advective transport of the Rhone plume along the coast. Releases of ^{60}Co from nuclear power were constantly reduced in the last 10 years, leading to an equivalent decrease in levels observed in mussels reaching now 0.02 to 0.06 Bq.kg^{-1} wet weight. Variations of ^{106}Ru

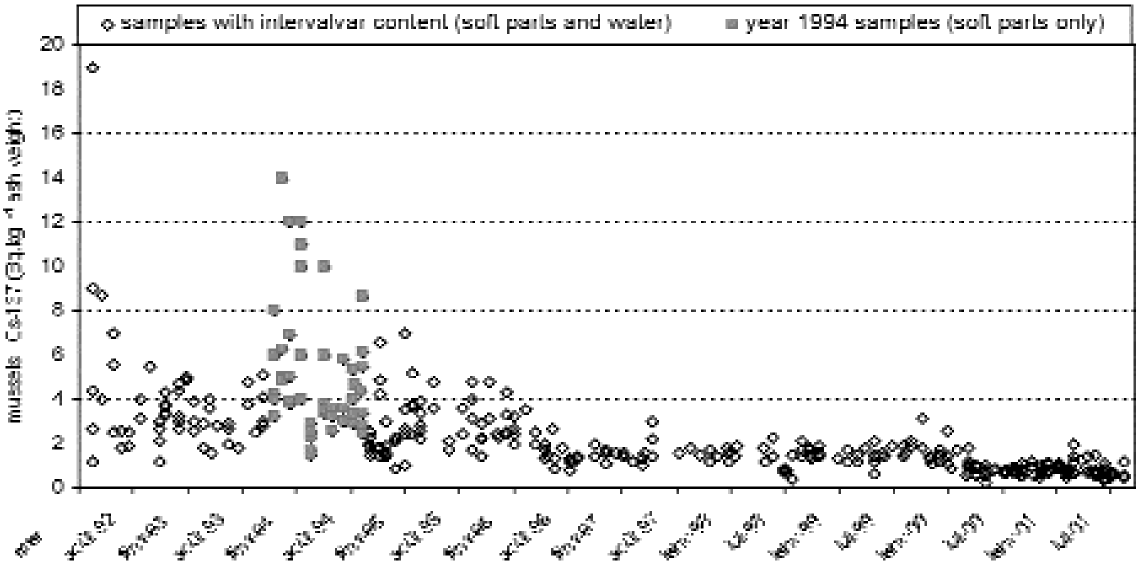


Figure 2. Evolution of ^{137}Cs in mussels from all stations along the French Mediterranean coast 1992-2001.

levels in mussels sampled in the Rhone delta follow changes in ^{106}Ru discharges from the reprocessing facilities in Marcoule and the river seasonal flow (Fig. 3). When the reprocessed activities stopped, ^{106}Ru levels quickly decreased below detection limit in all environmental samples from the coastal zone.

ADDITIONAL SURVEY

In the OPERA network, the constant monitoring on a monthly basis to set up time series restricts the sampling sites to a few. At any rate, extension of this network is anyway limited by the lack of natural or cultivated mussels beds along large sections of the coast. Besides, differences in mussel size and physiological condition between stations lead to variations in the bioaccumulation in mussel tissues, for a given contaminant ambient load. This makes for difficult direct comparison of results on a spatial scale. Therefore, a new tool for the so-called “active” bio monitoring is jointly developed by IFREMER, the Water Agency and IRSN using transplantation of mussels from a unique origin in the mean field of the coastal zone to produce an overview

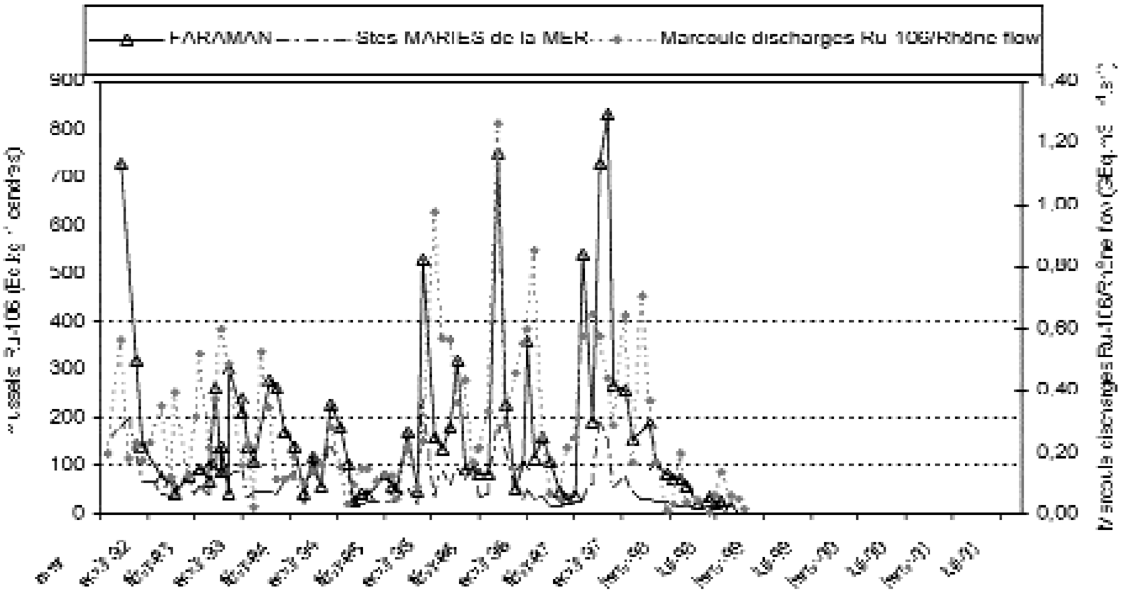


Figure 3. Evolution of ^{106}Ru in mussels in the Rhone delta compared to levels in the river.

of the pollution situation (Thébaut *et al*., 1998). In this program, called RINBIO, calibrated mussels from a single batch are deployed for four months (April to July) in 40 stations by the mooring of mussel caging devices at a mean distance from the shoreline of about 2 km. The results show that ^{137}Cs levels in mussels rapidly decrease off the coast to range between 0.02 to 0.07 Bq.kg^{-1} wet weight and confirm the impact of the Rhone plume in the Gulf of Lions, west of the river mouth (Andral *et al*., 2001).

CONCLUSIONS

The use of mussels for biomonitoring of long-term trends in radioactive contamination of coastal waters appears to be well founded. In the framework of the Mediterranean Mussel Watch, we proposed to contribute to the regional network with data from monthly samples of mussels collected in stations that appear on Fig. 1, namely: Nice, Toulon, Carteau, Faraman, Stes Maries, Sète, Banyuls and Bonifacio. The proposed measured parameters are the radionuclides detected by trace-level gamma spectrometry and probably ^{210}Po and Pu isotopes.

Monitoring of radionuclides and heavy metals in Turkish Black Sea Coast, Bosphorus and Marmara Sea using mussels *(Mytilus galloprovincialis)* as a bioindicator

Sayhan Topcuoglu

Çekmece Nuclear Research and Training Center, Istanbul, Turkey

Our scientific knowledge of pollution problems in the marine environment promises to expand in years ahead. Nowadays, it is necessary to advance in the integration of biokinetic, ecotoxicology and monitoring studies for determination of sensitivity to pollutants of human populations and marine organisms. Such integrated studies are being conducted by the Radioecology Laboratory of Çekmece Nuclear Research and Training Center of Turkey. The main objectives of the Radioecology laboratory on the monitoring study were as follows:

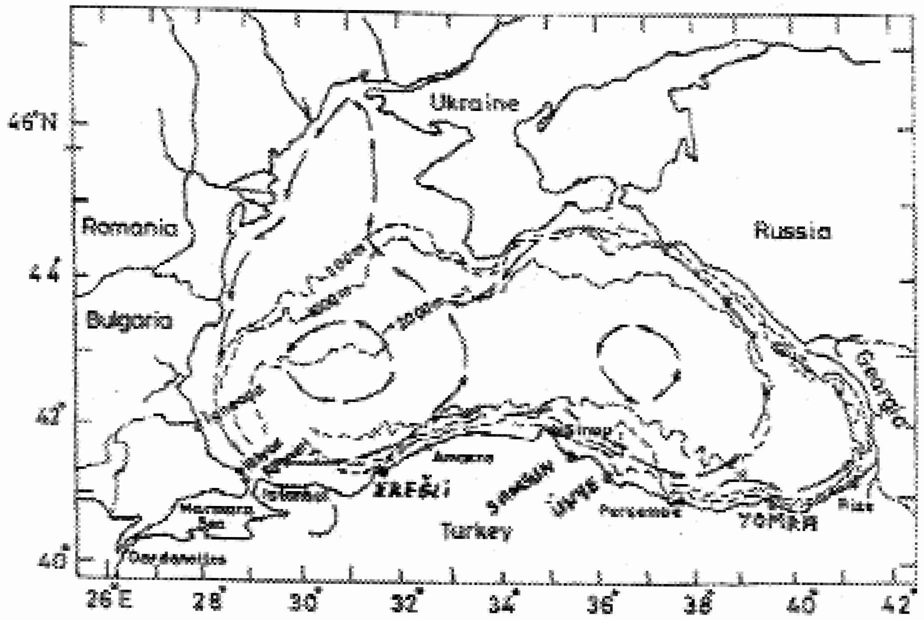
- to determine: a) anthropogenic and natural radionuclide concentrations in algae, mussel, sea snail, limpet, fish and sediment samples in Turkish coast of the Black Sea, Bosphorus and Marmara Sea; and b) the heavy metal levels in the same samples;
- the natural (^{210}Po and ^{238}U) and anthropogenic (^{137}Cs) radionuclides results will be used for estimation doses from marine radioactivity through ingestion of marine food. In addition, the heavy metal concentrations in the marine food will be evaluated in connection with FAO/WHO regulation for provisional tolerable intake;
- determination of some physical, chemical and biological parameters of the marine environments will be continued.

This abstract highlights selected mussel studies of the Black Sea, Bosphorus and Marmara Sea related to both radioactive and metal pollutions.

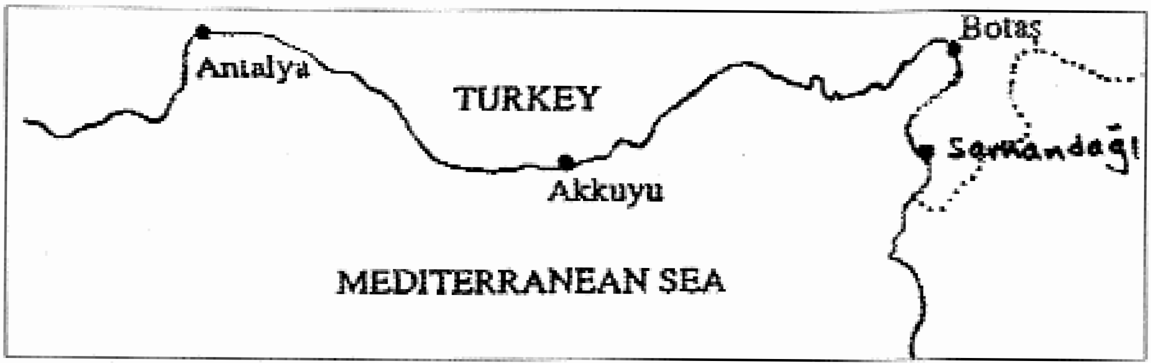
RADIOACTIVE POLLUTION

It is well known that after the Chernobyl accident, many anthropogenic radionuclides entered into the Black Sea and Marmara Sea. At the same time, nuclear weapons tests have also spread artificial radionuclides to the Turkish marine environment during the global fallout contribution. Nowadays, the artificial radionuclides in the Black Sea marine environment originated from riverin born radionuclides from the Chernobyl accident site or contaminated region and further inputs from nuclear power plants in countries around the Black Sea. The Marmara Sea has been likewise influenced to some degree by all these sources. Black Sea water flows by the Bosphorus into the Marmara Sea, carrying contaminated water.

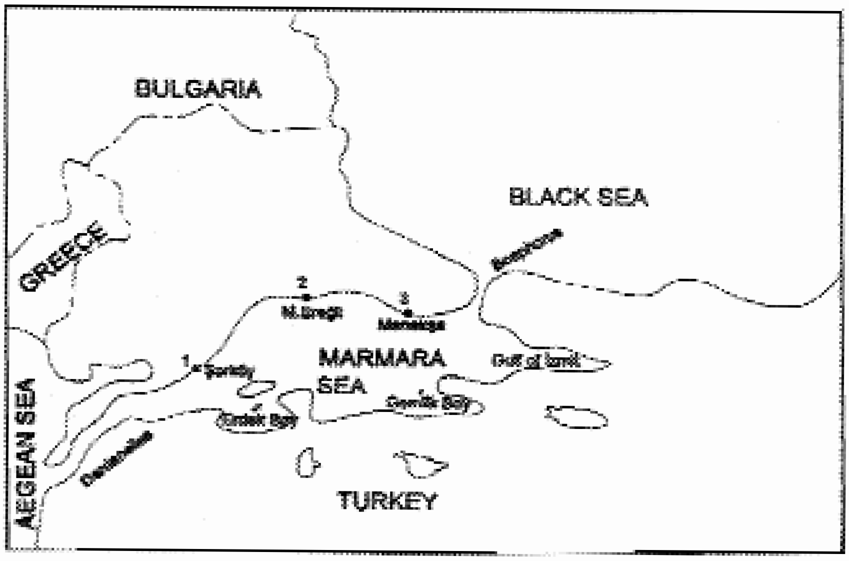
Following the Chernobyl accident in 1986, the fallout radionuclides in the Bosphorus mussel samples were determined monthly for two years (Topcuoglu *et al.*, 1988). High level of total gamma activity (^{131}I, ^{106}Ru, ^{134}Cs, ^{137}Cs) was found to be 120 Bq/kg wet weight during May 1986. The similar sized mussels were also collected from the Rumeli station in the Bosphorus



Present stations in the Black Sea : Igneada, Kilyos, Amasra, Persembe, Rize
Present stations in the Bosphorus : R. Feneri
Proposed stations in the Black Sea : Eregli (near the chemical treatment plant; and iron and steel complex)
Samsun, Unye (between a fertilizer plant and a copper smelter station)
Yomra (selected as uncontaminated area)



Present stations in the Mediterranean Sea: Antalya, Akkuyu and Botas
Proposed stations in the Mediterranean Sea: Samandagi



Present stations in the Marmara Sea : Igneada, Sarköy, Menekse, M. Eregli
Proposed stations in the Marmara Sea : Cannakale

from May 1986 to February 1987. The highest activities were found for ^{134}Cs and ^{137}Cs are 142 and 289 Bq/kg (dry weight) in soft tissues during June 1986. The ^{134}Cs/^{137}Cs activity ratio was calculated averaging as 0.48 (Topcuoglu and Van Dowen, 1997). Strontium-90 activity was found to be below 0.1 Bq/kg dry weight in the mussel samples collected from Bosphorus during the period of 1986-1988 (Topcuoglu *et al*., 1989). The ^{137}Cs activity of the mussel samples in Kilyos station on the Black Sea was found to be 7.3 Bq/kg dry weight in 1993 (Topcuoglu *et al*., 1998). During the period of 1997-1998, mussel samples were collected from different stations on the Turkish Black Sea Coast in order to determine activity levels of ^{137}Cs radionuclide. ^{137}Cs activity in soft parts of the mussel samples was found to be below the lower limit of detection (Topcuoglu *et al*., 2001). The concentrations of the ^{137}Cs activity in the Marmara mussels were also determined to be <3, 6.1 and <3 Bq/kg dry weight at Menekse, M.Ereglisi and Sarköy stations in 2000 samples (Topcuoglu *et al*., 2000). The limpet (*Patella* sp.) sample was collected from Akkuyu station at the Turkish coast of the Mediterranean during summer 1989. The ^{137}Cs activity was found to be 2 Bq/kg dry weight (Topcuoglu *et al*., 1993).

Nowadays, the study of natural radionuclides in marine environment has received increasing attention due to enhanced levels of some natural radionuclides from use of fertilizers, fossil fuel industry, detergent or phosphate industry and use of pesticides. The specialists in our laboratory have been working on the determination of natural radionuclides since 1997. The preliminary results showed that the ^{238}U, ^{210}Po and ^{210}Pb concentrations in mussel samples collected from seven stations at the Black Sea were within the ranges of 140 to 240 Bq/kg, 100 to 162 Bq/kg and 14 to 35 Bq/kg dry weight, respectively (Topcuoglu, 2000b; Güngör *et al*., 2001). The ^{210}Po activity level in the soft parts of the Marmara mussels ranged from 149 to 167 Bq/kg dry weight (Topcuoglu *et al*., 2000).

HEAVY METAL POLLUTION

Heavy metals are introduced into the Black Sea and Marmara Sea via rivers or direct discharge of industrial wastes and agricultural and municipal usage. Moreover, the metal levels in the marine environment have increased due to oil pollution and airborne contaminants. It is well known that the contaminants are also introduced through water way into the Bosphorus and Marmara Sea by a surface current from the Black Sea and deep current from the Mediterranean Sea. In a previous study, about 17 elements in the Bosphorus mussels have been analyzed by using the techniques of instrumental thermal neutron activation analysis, atomic absorption spectroscopy and x-ray fluorescence during the period of 1986-1988 (Kut *et al*., 1989). The highest levels of Ag, As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se and Zn were found to be 0.4, 32.5, 4.5, 2.5, 9.5, 15.5, 7.6, 3.8, 8.1, 4.3 and 345 μg/g dry weight, respectively. In another study, the heavy metal concentrations of the mussels in the Kilyos station at the Black Sea were investigated after collection in 1993 (Topcuoglu *et al*., 1998). The metal concentrations were as follows: As, 8.1; Se, 13.6; Zn, 314.2; Cr, 26.4; Co, 5.5 and Fe, 3967 μg/g dry weight. During the period 1997-1998, mussel samples were collected in different stations of the Black Sea Turkish Coast in order to establish the levels of selected heavy metals (Topcuoglu *et al*., 2002). The heavy metal occurrences in order of decreasing contents were Pb>Co>Cd>Cr>Cu>Mn>Ni>Zn>Fe> for soft part of the mussel samples. The Cu, Co and Ni concentrations were higher in mussel species collected from the Rize station than other stations. On the other hand, Zn, Cd and Pb were detected at higher levels in Amasra mussel samples. In unpublished study, the heavy metal concentrations (Pb excepted) in Marmara sea mussels are generally lower than those in Black Sea mussels (Topcuoglu and Kırbasoglu, submitted).

MUSSEL BEDS AND CAPTURE PRODUCTION

The areas between Karaburun and Kefken island, Çatlı burun and Yosun burun and Fener burun and Rize are important mussel beds on the Turkish Coast of the Black Sea. The Rumeli Feneri location in the Bosphorus is also very important for mussel production. In the Marmara Sea, mussel beds (with no commercial value) are located in M.Ereglisi, Sarköy, Erdek, Bandırma and Çanakkale regions. The production of mussels from the Black Sea are 2950, 2435 and 1584 tons annually in 1997, 1998 and 1999, respectively (Publication of Turkish State Institute of Statistics, 2001). These amounts were found to be 3275, 849 and 2 tons annually at the same dates for the Marmara Sea (include Bosphorus region). The mussel production in the Marmara Sea significantly decreased in 1999 due to inhibitory law for mussel capture in the Bosphorus. The cultivation of the mussel species carried out in Kefken and Yomra regions at the Black Sea and Dardenelles area for scientific purposes.

The annual production of the other kind of mussel species (*Venus gallina*) was 2400 tons from the Black Sea and 1028 from the Marmara Sea in 1999. Unfortunately, we have no data for radioactive and chemical pollution on this particular species. The European flat oyster (*Ostrea edulis*) species produced about 800 tons for exportation in Marmara Sea in 1999. Heavy metal levels were determined in Dardanelles and Bosphorus oyster samples during the period 1987-1988 (Topcuoglu *et al*., 1994).

ADDITIONAL STUDIES

We plan to investigate the radioactive and metal levels in the mussels after collection from Eregli, Samsun, Ünye and Yomra regions beside the present stations (Igneada, Kilyos, Amasra, Sinop, Persembe and Rize) in the Black Sea.

We plan also to investigate the pollutants in mussel or other biota samples after collection from Çannakale (beside Sarköy, M.Eregli and Menekse in Marmara Sea) and Samandagi (beside Antalya, Akkuyu and Botas in the Mediterranean Sea).

The radioactivity and metal concentrations will be measured in the Bosphorus mussels periodically (every 3 months). At the same time, the effects of body size and cooking will be investigated on the pollutant concentrations.

The radioactivity and metal levels will be determined in the other mussel species, *Venus gallina* in Marmara Sea stations .

We will find a collaborative Institute for measurement of pesticide concentrations in our prepared samples.