Heavy metals in seawater and surface sediments of the Gulf of Bourgas

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The Gulf of Bourgas is one of the highly polluted regions of the Black-sea coasts. The pollutants include petroleum products as well as heavy metals. The pollution is due to the geomorphological charateristics of the gulf as well as the presence of a large number of chemical industries in the vicinity of the sea. The investigations on the heavy metals present in the sea-water and the surface sediment are rather scarce. Therefore such a study is useful.

In May and September, 1991, samples were done from the sea water and the surface sediment at three different stations : namely Bourgas Port (I), Petroleum Port (II) and the bulk of the gulf (III). The samples were analysed for the concentrations of Cd, Cu, Pb and Zn by a suitable electroanalytical method.

The sediments were collected from the surface by mechanical drilling, dried at a temperature of 105° C, grinded and fractioned. A preweighed dry sample (d < 0.06 mm) was dissolved in HCl (purified by isothermal distillation) on heating in a closed container at 250°C for 10 h, filtered out and analysed by a polarographic method with standard addition (1). The completeness of the extraction process was controlled by the atomic emission spectral analysis of the insoluble residue.

The Pb content was determined directly in the filtrate. A part of the solution was evaporated to complete drying, then dissolved in excess NH_3 and filtered again. Cu, Cd and Zn contents in a samplewere determined simultaneously The half-wave potentials of the elements under these conditions were respectively -0.3V, -0.8V and -1.4V vs SCE.

The samples from the sea water were collected in plastic bottles pretreated with HNO_3 and filtered through a membrane filter (pore-diameter < 0.45 μ). In order to eliminate organic impurities, the samples were subjected to electrochemical anodic oxidation at + 1.4 V for 10 min. in a graphite container.

The mentioned heavy metals in the sea-water were determined by a method of anodic stripping voltametry (ASV) [2] under the following conditions : stripping process on HMDE at 1.2V vs SCE for 10 min., deoxidation with N_2 and anodic dissolution of the deposited impurities at a rate of 400 mV/min. The metals under investigation show well-defined sigle peaks and their concentrations were determined by a method of standard addition.

Table 1. - Heavy metals in sea-water (sw), mg/l and sediments (sed) mg/g dw

Station	elements	Cu		Zn		Cd		Pb	
	month	V	IX	V	IX	V	IX	V	IX
I	sw	10.3	11.2	9.3	9.9	0.25	0.23	0.05	0.05
	sed	93.4	99.9	52.4	53.3	3.70	3.90	73.40	78.80
II	sw	15.1	16.8	10.4	10.5	0.28	0.30	0.06	0.07
	sed	115.2	107.6	53.3	61.4	4.80	4.70	79.50	78.10
III	sw	8.7	9.1	10.1	12.0	0.21	0.23	0.04	0.07
	sed	82.2	82.1	50.8	52.2	3.60	3.70	70.40	73.30

* The mentioned concentrations are the averages of 6 parallel measurements.

The experimental results show that the concentrations of Cu and Cd at station II are the highest - a fact most probably attributed to the presence of Copper mine nearby. The Pb concentrations is comparatively higher at the stations I and II, however Zn concentration is almost constant . Although no general conclusions can be drawn for the seasonal changes in the heavy metal contents, it may be noted that in September (IX), their concentrations are higher than in May (V). There exists some correlation between the heavy metal contents in the sediments and that in the corresponding sea-water. Due to the limited number of investigations, the obtained data for the concentrations show the relative pollution in the investigated regions of the gulf .

The electrochemical methods proposed for the analysis of Cu, Cd, Pb and Zn are rapid, sensitive and selective. Relative standard deviation for the polarographic determination is 2-5 % and that for ASV is 8-12 % (n = 6).

REFERENCES

KUTA J., TRIFONOV A., 1955. - Introduction in Polarography, Sofia, Nauka i Izkustvo, pp. 252 (in Bulgarian).

BRAININA Ch., NEYMAN E., SLEPUSHKIN W., 1988. - Inv. Electroanal. methods, Moscow. Mir, pp. 106 (in Russian).

Effects of pollution on the physiology of *Callianassa tyrrhena* (Crustacea : Decapoda) from Saronikos gulf

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This study concerns the effect of pollution on the physiology of the decapod crustacean *Callianassa tyrrhena*, which is a widely distributed callianassid along the european coast of the Mediterranean and is present in coastal polluted and non polluted areas of the Saronikos gulf (gulf of Athens). This species forms dense populations on sand flats and its influence on redox potential and nutrient cycling of the sediment has been documented by OTT*et al.* (1976). Its ecology and biology in greek seas has been the subject of some recent studies (THESSALOU-LEGAKI, 1988, 1990).

The physiological condition of the organisms was examined by means of a dual approach : metabolic (respiration rate) and enzymatic, in order to obtain a pluridimentional view of the effects of pollution at the physiological level.

Two sampling areas were chosen : Elefsis bay (eutrophic, one of the most polluted areas in the eastern Mediterranean) and Vouliagmeni bay and Fleves Islands (oligotrophic, non polluted area). Animals were collected from the shore of both areas during spring and summer 1991, using a hand-operated pump.

In each sample, males, ovigerous and non-ovigerous females were immediately selected and the individuals were placed separately. Animals were then divided into two groups. The first one was preserved in a freezer (-25°C) and transported by air to Marseille (France) in a dry ice container for the enzymatic tests. The API-ZYM system (Bio-Merieux, France) was used as a semiquantitative micromethod to test the enzymatic activity of 19 hydrolases from digestive gland extracts. Oxygen consumption both on individual (total respiration rate) and per biomass unit (weight-specific respiration rate) basis was measured from the second group using a dissolved oxygen meter (YSI model 51B) according to OMORI and IKEDA (1984). Dry weight of the individuals was determined only in a few cases directly (for 72h at 70°C) while for the majority of the specimens it was calculated from length-weight relationships that already existed (THESSALOU-LEGAKI, 1988). The statistical treatment of the results was mainly based on a multifactor analysis of variance taking into account the relationships between the respiration rate and the season, the locality, the sex (males, ovigerous and non ovigerous females) and the dry weight.

From the biochemical point of view, the results showed a large individual variability according to the physiological state of each specimen. Figure 1 gives the comparative mean enzymatic activities observed by the API-ZYM technique in males and non ovigerous females from the two areas. A statistically significant increase was observed in the total respiration rate with increase of the dry weight, whereas the weight-specific respiration rate decreased significantly with increasing individual dry weight (P<0.001). A seasonal variation was also observed (P=0.01): the mean total respiration rate was 70.5±5.2μl in spring and 55.5±3.7μl $O_2.ind^{-1}.hr^{-1}$ in summer. No statistically significant variation was found in the total respiration rate regarding sex, locality as well all the interactions of the factors concerned. A significant geographical variation was observed for the weight-specific respiration rate (P=0.05): 0.12+0.01μl O_2.mg DW^{-1} hr^{-1} for specimens from Elefsis bay, and 0.20±0.02μl O2.mg $DW^{-1}.hr^{-1}$ for those from Vouliagrneni.

The results obtained both by enzymatic tests and respiration experiments, show that *C. tyrrhena* exhibits different physiological behavior according to sampling areas. As hydrolases are acting mainly on substrates provided by the environment, the differences observed for most of them may be attributed to the trophic conditions of the sampling areas. Therefore, they appear to be an ecophysiological response to the environmental conditions. Out of the 19 enzymes tested, only 3 exhibit a higher activity in Elefsis than in Vouliagmeni specimens.

The general tendency is, therefore, an "inhibitory" effect of the pollution on enzymatic activities. Such observations are in agreement with several literature data (AMIARD *et al.*, 1982; RIVIERE et KERAMBRUN, 1983) and may be related to the benthic way of life, which is more directly affected by pollution.

As far as respiration metabolism is concerned, season and locality significantly affect total and weight-specific respiration rate respectively : the total respiration rate of the individuals collected in summer is about 1.3 times lower than those collected in spring while the animals of the non polluted area (Vouliagmeni) have a two times higher weight-specific respiration rate than those of the polluted eutrophic area (Elefsis bay).

The low rates of respiration of *C. tyrrhena* obtained from the present study are in good agreement with the literature data concerning thalassinids from coastal or intertidal sediments, the respiration rates of which are among the lowest observed for decapods. This is interpreted as an adaptation allowing them to maintain aerobic respiration even in almost anoxic conditions. The decrease in the activity of digestive enzymes agrees with this interpretation and suggests that the whole metabolism is involved in the adaptation to pollution.

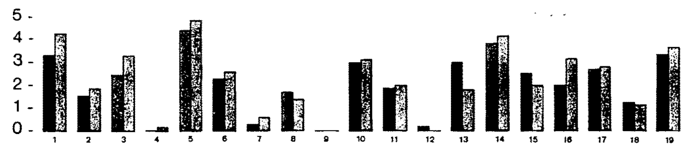

Figure 1. *Callianassa tyrrhena*. Comparative enzymatic activities mean obtained from API- ZYM (arbitrary units from 0 to 5). Dark : Elefsis bay samples; Light: Fleves islands samples.
1, acid phosphatase; 2, esterase (C4); 3, esterase lipase (C8); 4, lipase (C14); 5, leucine arylamidase ; 6, valine arylamidase; 7, cystine arylamidase; 8, trypsine; 9, α-chymotrypsine; 10, acid phosphatase; 11, phosphoamidase; 12, α-galactosidase; 13, β-galactosidase; 14, β-glucuronidase; 15, α-glucosidase; 16, β-glucosidase; 17, N-acetyl-β-glucosaminidase; 18, α-mannosidase; 19, α-fucosidase

REFERENCES

AMIARD T.C., AMIARD-TRIQUET C. et DENUIT C., 1982. - *Publs Cent. natn. Exploit. Océans* (CNEXO), 14: 385-398.

OMORI M. and IKEDA T., 1984. - *John Wiley & Sons*, 311 pp.

OTT J.A., FUCHS B. and MALASEK A., 1976. - *Senckenbergiana Maritima*, 8: 61-79.

RIVIERE D. et KERAMBRUN P., 1983. - *Mar. Biol.*, 75: 25-35.

THESSALOU-LEGAKI M., 1988. - Ph.D. Thesis, Univ. Athens, 229 pp.

THESSALOU-LEGAKI M., 1990. - *J. Crust. Biol.*, 10 (4): 659-666.

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