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Influence of Accumulation of Cadmium on the Content of Other Microelements of Two Species of Black Sea Decapods

Bogdan Skwarzec,[1] Anna Kentzer-Baczewska,[1] Ewa Styczynska-Jurewicz,[1] and Ewa Neugebauer[2]

[1]Institute of Oceanology, Polish Academy of Sciences, Powstancow Warszawy 55, 81-967 Sopot and [2]Institute of Oceanography, University of Gdansk, Czolgistow 46, 81-378 Gdynia, Poland

Heavy metals are natural chemical components of the marine environment. They are present there in small amounts as trace elements, but progressing industralization of the continents evokes a rapid increase of contamination, especially in coastal waters and estuaries. This increasing contamination may be hazardous for all forms of life in the marine environment. Living organisms need for their normal development and function some amount of heavy metals. Nevertheless, the excess introduction of such contaminants to the sea, even from small, local sources, may increase their concentration to an undesired level. Consequently, the dose of toxic metals accumulated by plants or animals may be harmful or even lethal.

One of the most toxic heavy metals is cadmium (Friberg et al., 1976). The potential threat resulting from the contamination with this metal was described for many groups of marine animals (D'Agostino and Finney, 1974; George et al., 1978; Kayser and Sperling, 1980; Ray et al., 1980; Roed, 1980; Theede, 1980; O'Neill, 1981; Muramato, 1981). Studies were conducted on the effects of cadmium on growth and reproduction of marine animals (Stebbing, 1976), blood composition (Larsson, 1975), and the development and structure of skeleton (Bengtsson, 1975). Because the accumulation of cadmium by an organism may be a progressive and long-term process, the investigation of the influence of this trace metal on the balance of other micoelements, especially those of key significance for normal biological functioning, is of great interest. Estimation of the accumulation capacity of organs and tissues involved in this process is of great importance as well.

MATERIALS AND METHODS

The accumulation of cadmium was investigated under laboratory conditions in two Black Sea decapod crustacean species: benthic crab Xantho hydrophilus and nektonic coastal shrimp Palaemon elegans. All specimens were collected in one site of constant physico-chemical parameters inside a small rocky bay "Rayski Zaliv" at Sozopol near Burgas, Bulgaria and acclimated without feeding for 3 days to the laboratory conditions. The average natural content of cadmium in the waters of this area of the Black Sea is a constant value of about 0.2 $\mu g/l$. Natural concentrations of other microelements are as follows: Fe about 13 $\mu g/l$; Mn 0.8 $\mu g/l$; Cu 2.75 $\mu g/l$; Co 0.32 $\mu g/l$ and Pb 1.3 $\mu g/l$ (Spencer et al., 1972; Morozov et al., 1976). The LD_{50} for shrimp established experimentally for 3 and 5 days treatment at temperature 22°C was 1.5 mg/l, but crabs survived well even at twice this concentration. Therefore, we accepted concentrations equal to 1 mg/l and lower as sublethal for shrimp and equal to 2 mg/l and lower as sublethal for crabs.

The accumulation of cadmium was examined in sublethal concentrations, 1 and 2 mg Cd/l for crabs and 0.01 up to 1 mg/l Cd for shrimp under the same conditions.

In order to determine the relation between the content of microelements and body size, i.e. age, of specimens, the control shrimp were selected into four size groups, depending on the individual dry weight.

To find the tissue of especially high accumulation, pools of the following organs were selected from 5 cadmium-treated experimental gravid shrimp belonging to the IV th size group: gills, hepatopancreas, ovaries, external eggs glued under the abdomen, and abdomen muscles.

After the treatment with cadmium, the experimental animals as well as sampled tissues, were dried to a constant weight at 60°C; then homogenized in agath mortar and mineralized.

In the case of crab material (dry weight range of 1 to 2.5 g), mineralization was performed with 15 ml of concentrated HNO_3 in a 50 ml Erlenmeyer flask. Solutions remained under the fume hood for 3 days, were evaporated, treated with 5 ml of 70% $HClO_4$ and heated at about 200°C till the disappearence of white fumes. The last procedure was repeated and 2 ml of concentrated HCl were added to the remaining dry substance.

The mineralization of shrimp material (dry mass 4 to 600 mg) was performed in a similar way but using 5 ml of concentrated HNO_3 and 2 ml of 70% $HClO_4$. After evaporation of HCl, mineralized samples of crab and shrimp material were dissolved in 10 ml of 0.1M HCl. Cd, Cu, Fe, Pb, Mn and Co content determinations were performed with a Varian-Techtron 1200 atomic absorption spectrophotometer (AAS). All determinations were made using the air-acetylene absorption technique. The calibration of the apparatus was done using mixed standards which contained from 0.02 to 5 mg of a given element per 100 ml.

RESULTS AND DISCUSSION

Among the microelements which were determined Fe, Cu, Mn, and Co are indispensable for normal life processes, while cadmium and lead are not involved in normal biological metabolism. When present in the organism they can, however, influence the content of other biologically important trace elements. This kind of influence is shown in Table 1 with regard to the crab.

Table 1. The content of microelements (ppm d.w.) of *Xantho hydrophilus* after the treatment with 2 different concentrations of cadmium.
(means ± S.D., n = number of individuals)

	n	Cd	Cu	Pb	Fe	Mn	Co	% Moisture of fresh material
Control	8	3.2±0.3	49.1±3.1	18.8±1.2	77.9±6.2	40.3±5.2	25.2±2.8	61.8
1 mg/l Cd	8	46.8±5.3	37.9±3.0	19.9±1.5	86.0±6.8	43.9±4.9	27.3±2.2	62.2
2 mg/l Cd	4	118.4±10.1	56.7±5.2	21.1±1.7	218.5±11.6	60.2±4.2	26.1±1.2	61.4

Table 2. The natural content of several microelements (ppm d.w.) of *Palaemon elegans* in relation to size of non-treated animals.
(means ± S.D., n = number of individuals)

Size class	Range of specimen dry weight (mg)	n	% Moisture fresh material	Cd	Cu	Pb	Fe	Mn	Co
I	23-48	8	73.3	4.9±0.5	71.5±4.5	28.2±1.7	73.4±8.4	20.7±2.6	12.1±0.5
II	51-79	5	73.6	4.3±0.4	71.0±4.5	14.0±0.9	85.3±9.7	23.3±4.1	8.7±0.4
III	113-171	5	69.4	1.9±0.2	59.7±3.8	7.8±0.5	45.3±5.1	19.7±1.3	7.0±0.3
IV	229-391	5	81.5	1.9±0.2	74.3±4.7	6.1±0.4	18.6±2.1	13.7±1.7	11.0±0.5
Weighted mean			75.8	2.7±0.3	68.0±4.1	11.2±0.7	47.3±5.0	18.5±1.8	9.3±0.4

Table 3. The content of several microelements (ppm d.w.) in *Palaemon elegans*. The results are means of three samples. Each sample was a pool of 4 specimens of size class I and II.

	Cd		Cu		Pb		Fe		Mn		Co	
	*1	**2	1	2	1	2	1	2	1	2	1	2
Control	2.1	2.3	85.5	84.4	22.5	-	50.4	32.5	13.5	13.3	8.8	10.5
0.01 mg/1 Cd	3.2	5.5	75.2	71.6	-	15.1	34.7	35.7	-	13.3	17.0	27.3
0.05 mg/1 Cd	6.2	12.0	70.2	65.6	-	16.5	39.6	36.5	-	15.3	51.2	89.9
0.1 mg/1 Cd	10.3	19.7	63.1	53.7	-	18.0	37.7	35.5	-	16.1	-	42.9
0.5 mg/1 Cd	15.2	50.2	62.4	44.3	-	22.5	42.4	40.0	-	18.5	18.3	-
1.0 mg/1 Cd	29.0	80.4	56.6	37.0	23.5	-	54.9	37.3	20.9	21.6	8.4	-

*1 - after 72 hour treatment
**2 - after 120 hour treatment with different concentrations of cadmium

Table 4. Accumulation of Cd, Cu and Fe (ppm d.w.) in internal organs of gravid females of *Palaemon elegans* after 72 hours incubation of specimens in sea water with 1 mg/1 cadmium content.

Organs (pool of 5 specimens of class IV)	Cd	Cu	Fe
Gills	138.9	-	902.8
Hepatopancreas	97.6	-	487.8
Ovaries	27.8	54.3	104.2
Eggs outside	2.6	57.9	132.4
Muscles	1.2	4.7	32.9

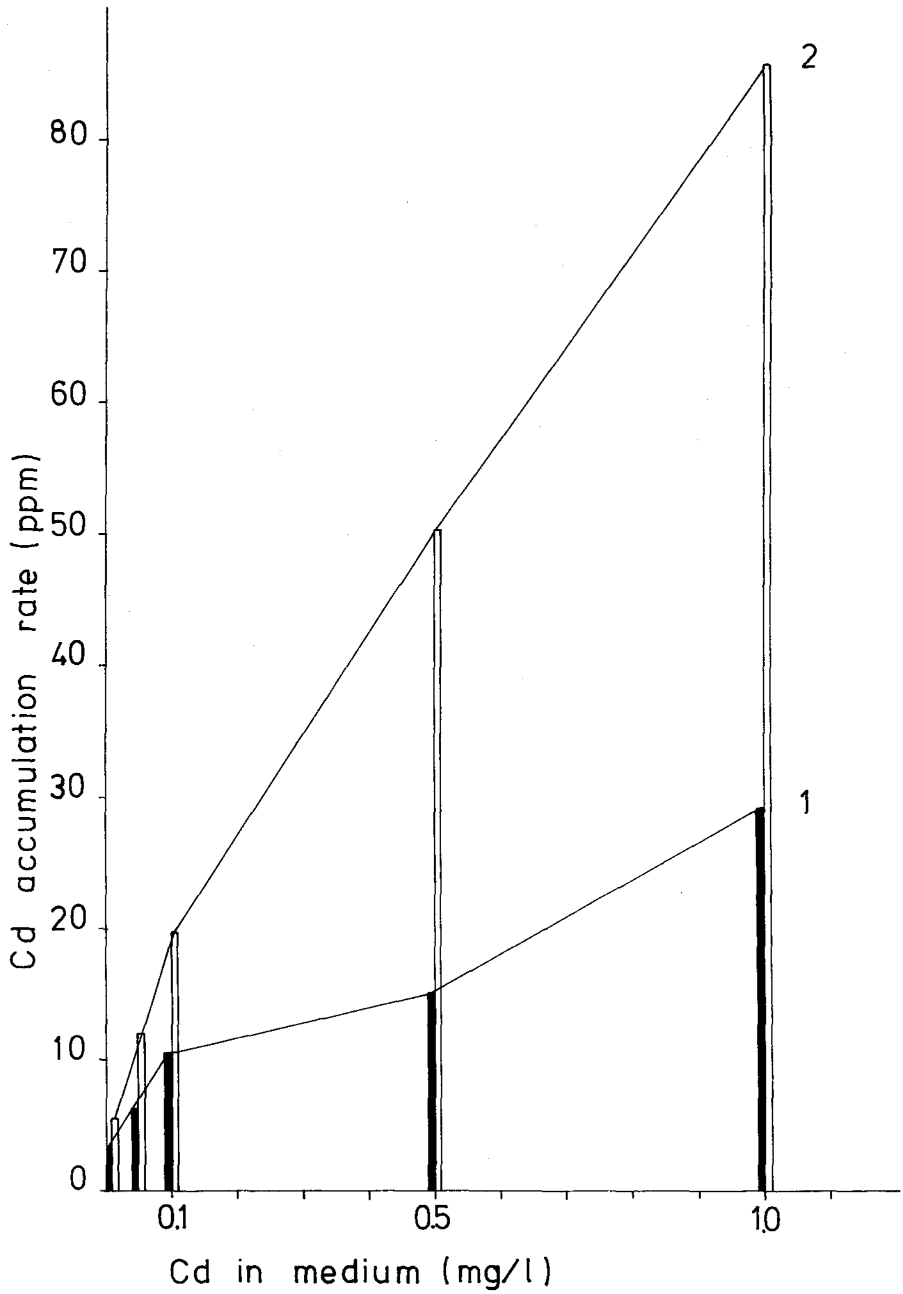

Figure 1. Accumulation rate of cadmium in Palaemon elegans after treatment with different concentrations. 1. After 72 hours; 2. After 120 hours

The content of cobalt in the control samples was relatively high (Karbe et al., 1977). Fluctuations in the cobalt level were irregular and no uniform conclusion can be drawn from the data in Table 1 and 3. The icrease of cadmium content in treated animals was accompanied by a very slight increase of Pb and Mn, whereas fluctuations in the level of remaining microelements were rather small.

Especially interesting size-dependent relationships regarding the natural content of microelements were found in Palaemon elegans. The whole collection of control specimens was divided into four size groups depending on individual dry weights (Table 2). Trace elements were determined for each group separately. Apart from Cu and Co the content of Cd, Pb, Fe, and Mn in controls was significantly higher in smaller, i.e. younger, individuals. We did not find any exhaustive explanation for this regularity in the available literature, but examples of similiar dependence are given for fish (Bouquegueau et al., 1979) and for the bivalve Mytilus edulis (Theede et al., 1979).

We suppose that the higher accumulation rate in young, i.e. small individuals, results from the higher rate of their metabolism, mostly from the higher transport efficiency in assimilating organs (gills). From our data presented in Table 2, this efficency has to be about twice as high in specimens of smaller size (size class I) than in the larger (size IV) individuals. This special role of gills is well illustrated in Table 4. The Cd concentration in various fractions of soft parts decreased in the order: gills, hepatopancreas, ovaries, eggs outside, and muscle.

In higher concentrations of cadmium (0.5 and 1.0 mg/l) the accumulation rate in shrimp increased significantly with longer exposure times. Accumulation found after longer treatment (120 h) was about twice as high as after 72 h treatment in low concentrations (up to 0.1 mg/l), but about 3 fold higher in concentrations closer to the lethal dose (Fig. 1).

After a 3-day treatment with a solution of 1 mg Cd/l, the content of cadmium increased about 15 times in both species(Tables 1 & 3). The anatomic sites of especially intense accumulation of Cd in Palaemon elegans were gills and hepatopancreas (Table 4). Undoubtedly, these two organs play a decisive role in the process of physiological accumulation of this heavy metal. According to Bouquegueau et al., (1979) gills were the main site of cadmium transport in fish. In the case of legthly exposure, cadmium accumulated in internal organs, mostly in the liver and kidney, while its concentration in muscles was always found to be very low (below 1 ppm). In a decapod crustacean, Homarus americanus, after a prolonged treatment (30 or 60 days) the content of cadmium was highest in the digestive gland, followed by the gills (Thurberg et al., 1977). In bivalves and shrimp which were held for 21 days in a concentration of 10 μg/l Cd, the cadmium level in selected tissues was higher than the average for the whole organism (Eisler et al., 1972). According to Overnell and Trewhella (1979) the digestive gland of Cancer pagurus accumulated the highest amount of cadmium.

The direct absorption of cadmium from the sea water is more important than from food (Kerfoot and Jacobs, 1976). The reaction of cadmium with biologically active sites of enzymes, carboxyl, phenoxyl, sulfhydryl, disulphide, phosphate, (Friberg et al., 1976) inhibits ATP-ase, phosphatase, carbohydrase, peptidase, and aldolase (Vallee and Ulmer, 1972; Schröder and Alsen, 1976). According to Haberer and Norrmann (1971) crustaceans are capable of assimilating cadmium up to the concentration of 3 mg/l in the surrounding medium. The results obtained by the former authors indicated the synergistic interaction of copper with cadmium. In our experiment this synergism was revealed as a visible decrease in copper content when the accumulation of cadmium was increased (Table 3).

We suspect that in the presence of high amounts of cadmium, the assimilation and gill transport of copper from the surrounding water was depressed or inhibited, while the elimination of copper continued. Similar interpretation was given by Ahsanullah et al. (1981) for cadmium-copper interaction in the shrimp Callianassa australiensis.

Interpreting irregular changes in cobalt content (Table 3) in treated Palaemon elegans and an increase of iron (Table 1) in Xantho hydrophilus, we must take into consideration that the presence of a toxic agent, cadmium, may stimulate some mechanisms of physiological resistance, e.g. synthesis of some enzymes of the defense system, synthesis of metallothioneins (Köhler and Riisgard, 1982) and other processes in which these microelements my be involved. Such regulatory mechanism may work only in case of a short-term sublethal impact of low concentrations of cadmium. In highly toxic concentrations other physiological effects dominate, like pathological deviations of physiological function or behavior, agonal effects, etc.

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