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Uptake and Excretion of Cadmium, CdEDTA, and Zinc by *Macoma balthica*

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The accumulation of cadmium from sea water by marine invertebrates has been shown for crabs (Hutcheson 1974), oysters (Zaroogian and Cheer 1976), mussels (George and Coombs 1977), lobsters (Thurberg et al. 1977), polychaete worm (Ray et al. 1980a), and shrimp (Nimmo et al. 1977; Ray et al. 1980b).

Chelation of Cd with ethylenediaminetetraacetic acid (EDTA) was reported to double the rate of Cd uptake and the final tissue concentration in mussels, Mytilus edulis (George and Coombs 1977). In contrast, Ray et al. (1979) found that the 14-d concentration factor for CdEDTA compared with that for $CdCl_2$ was about 40% less for the marine worm, Nereis virens, and about 25% less for the marine shrimp, Pandalus montagui. Similarly, Hung (1982) reported that Cd complexed with EDTA, NTA, or humic acid decreased the accumulation of Cd in American oysters by as much as 70%.

Zn concentration showed no substantial change in the marine shrimp, P. montagui, during exposure to Zn alone at 65 µg/L for 14 d. During 14-d exposure of shrimp to Zn at concentrations up to 410 µg/L and with Cd at about 40 µg/L, the Zn concentration remained relatively constant in tail muscle and in hepatopancreas but doubled in eggs, carcass, and the whole animal (Ray et al. 1980b). There was no accumulation of Zn in N. virens exposed to Zn at levels up to 1 mg/L, but accumulation occurred at higher levels (Ray et al. 1979).

The physicochemical properties of Cd and Zn are similar and it has been suggested that the two elements are biologically antagonistic (Schroeder et al. 1967; Hill and Matrone 1970; Magos and Webb 1978).

When both Cd and Zn are present, the level of Cd is reduced in polychaete worms (Bryan and Hummerstone 1973; Ray et al. 1979) and bivalve molluscs (Jackim et al. 1977). In contrast, the level of Cd remained fairly constant for most tissues of P. montagui exposed to Cd at a constant level and to Zn at different levels (Ray et al. 1980b).

Cd is not excreted by N. virens (Ray et al. 1980a), C. virginica (Zarrogian 1979), P. montagui (Ray et al. 1980b), or by lobsters,

H. americanus (McLeese et al. 1981). Dethlefsen (1977/78) found no excretion of Cd from shrimp, Crangon crangon, during 12 d following exposure to Cd at 5 and 10 µg/L, but there was a significant loss during the initial 3 d following exposure to Cd at 20 µg/L. Because of the contrasting results concerning the effects of Cd and Zn combined, and of chelated Cd, this study was undertaken to determine if exposure to Cd and Zn in combination affects uptake and excretion of either element by the deposit-feeding mollusc, Macoma balthica. In addition, the effects of Cd complexed with EDTA and the chemical form of Cd on uptake of Cd in M. balthica were examined.

MATERIALS AND METHODS

Clams, M. balthica, valve length 0.9 cm, collected from Passamaquoddy Bay, N.B., were held in flowing sea water for 1 wk before testing was initiated. In addition, clams about 0.5 and 1.3 cm in length were used to determine if animal size affected uptake of Cd or Zn.

The clams were exposed at 10°C in 2-L glass beakers to aerated solutions (800 mL) of $CdCl_2$ at 45 µg Cd/L with $ZnCl_2$ at 0, 45, and 540 µg Zn/L, or to CdEDTA at 45 µg Cd/L. The solutions were changed every 4 d, and the concentrations of the metals in the solutions were measured during the tests. The concentrations of Cd averaged 45 µg/L (range 36 to 52 µg/L, n = 42) and 25 µg/L (range 22 to 26 µg/L, n = 10). Zn concentrations averaged 45 µg/L (range 30 to 60 µg/L, n = 8) and 540 µg/L (range 500 to 560 µg/L, n = 3). In addition, some clams were exposed to Cd at 1.4 µg/L (range 1.3 to 1.6 µg/L, n = 5). Twelve medium-sized clams were exposed in a beaker except for the size-effect test where the numbers were six large or 24 small clams. Duplicate samples of six clams were taken at 2, 4, 8, and 14 d for analyses of Cd and Zn levels.

Following exposure, the remaining animals were transferred to flowing clean sea water at 10°C. Duplicate samples of six clams were taken at 4, 8, 16, and 32 d for analysis.

To determine whether Cd in the clams was in free or bound form, clams were exposed for 8 d in 4 L of $CdCl_2$ or CdEDTA solutions with the Cd level at 25 µg/L for both. The solutions were renewed every 2 d. Samples of three clams were taken from each at 2, 4, 7, and 8 d during the uptake phase and at 1, 2, 4, and 14 d during the excretion phase, for analysis.

The clams were rinsed with distilled water to remove residual test solution or sea water. The soft tissues of six, or three, animals were pooled for analysis. Pooling provided enough dry tissue to ensure analytical accuracy comparable with that obtained with NBS oyster tissue.

Methods for determining total Cd and Zn in water and animal tissues have been described previously (Ray et al. 1980b). In brief, tissues were prepared by freeze-drying, ashing for 16 h at 450°C,

and dissolving in nitric acid. Cd and Zn in the digests and water samples were analyzed by atomic absorption, using graphite furnace and flame techniques, respectively. Precision and accuracy of the analyses were confirmed against NBS reference materials number 1643a (water) and number 1566 (oyster tissue).

The chemical form of Cd in extracts from pooled tissues of three clams was determined by polarography according to the method of Chou et al. (1978). A Metrohm polarograph (Model #BM 5-03) equipped with a Metrohm E261 Polarecord was used for the analyses.

RESULTS AND DISCUSSION

There is an inverse relationship between size of clams and concentration of Cd accumulated. When exposed to Cd at 45 µg/L for 14 d, levels of Cd in small, medium, and large clams were 21.8, 12.5, and 8.5 µg/g dry weight, respectively (Table 1). The dry matter content for the tissues of medium-sized clams was 20.7% of the wet weight.

Table 1. Cd concentrations in M. balthica of different lengths exposed to Cd at 45 µg/L.

Exposure time (d)	Cd conc. (µg/g dry weight) in clams[1]		
	Small (0.5 cm)	Medium (0.9 cm)	Large (1.3 cm)
0	0.2	0.2	0.1
4	12.4	10.6	6.3
8	13.0	10.8	9.8
14	21.8	12.5	8.5

[1]One sample.

Cd uptake was not observed in clams exposed to 1.4 µg Cd/L, the level in clams averaging 0.3 µg Cd/g dry weight (range 0.2 to 0.4, n = 7) during 14-d exposure and 14-d post-exposure. After exposure to 25 µg Cd/L for 8 d, the level in clams was 3.2 µg Cd/g dry weight (Table 2). When exposed to 45 µg Cd/L, the level in clams varied from 6.2 to 10.8 µg Cd/g at 8 d and from 8.5 to 12.5 µg Cd/g at 14 d (Tables 1, 3).

The presence of Zn may or may not have altered Cd accumulation by clams. The average Cd concentration for those exposed to Cd at 45 µg/L with Zn at 0, 45 or 540 µg/L at 8 d was 6.2, 5.6, and 5.1 µg/g dry weight, respectively, and at 14 d was 8.5, 7.4, 6.7 µg/g, respectively (Table 3). However, variation between duplicate samples was as much as 2 µg/g. Jackim et al. (1977) noted that Zn reduced Cd uptake by M. edulis and by Mulina lateralis. However,

Table 2. Cd concentration (μg/g dry weight) in M. balthica exposed to $CdCl_2$ and to CdEDTA at 25 μg Cd/L.

Time (d)	$CdCl_2$[1]		CdEDTA[2]
Uptake			
0	0.3	0.3	0.3
2	1.4	1.5	1.1
5	2.3	3.7	2.0
7	2.3	3.5	2.1
8	3.2	3.5	2.7
Excretion			
1	2.9	3.5	2.5
2	3.2	3.3	1.6
4	2.3	2.9	1.8
14	2.4	3.1	2.2

[1]Two samples. [2]One sample.

Table 3. Cd concentration (μg/g dry weight) in M. balthica exposed to Cd at 45 μg/L with Zn at 0, 45 and 540 μg/L, or to CdEDTA.

Time (d)	Cd at 45 μg/L		Cd at 45 μg/L Zn at 45 μg/L		Cd at 45 μg/L Zn at 540 μg/L		Cd at 45 μg/L Zn[1]		CdEDTA at 45 μg/L	
Uptake										
0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
2	2.1	2.2	1.8	1.8	1.8	1.8	-	-	1.1	1.7
4	3.9	4.0	2.2	2.3	2.9	2.4	-	-	2.2	2.3
8	7.0	5.4	6.0	5.2	5.5	4.6	3.4	4.8	3.4	2.8
14	8.2	8.8	6.4	8.4	7.1	6.3	10.6	10.1	4.9	4.5
Excretion										
4	5.8	7.1	8.4	7.9	6.9	6.7			4.5	4.7
8	6.1	5.9	6.2	6.1	5.9	6.2			4.7	5.0
16	5.3	6.5	5.5	8.3	4.2	5.1			3.5	4.3
32	6.5	4.9	5.8	6.6	5.6	4.4			3.2	3.5

[1]Clams pretreated by exposure to Zn at 45 μg/L for 4 d.

high levels of Zn were required and they concluded the process is not ecologically significant.

A 4-d exposure of Macoma to Zn at 45 µg/L before exposure to Cd alone at 45 µg/L did not alter the uptake of Cd (Table 3). The mean Cd level in clams at 14-d exposure of 10.4 µg/g dry weight is intermediate between 12.5 µg/g (Table 1) and 8.5 µg/g (Table 3) for similar sized clams exposed to Cd at the same level.

Chelation of Cd with EDTA reduces the uptake of Cd by clams. Those exposed to 25 µg Cd/L either as Cd or as CdEDTA accumulated 3.2 and 2.7 µg Cd/g dry weight respectively by 8 d (Table 2). When exposed to 45 µg Cd/L as $CdCl_2$ or as CdEDTA, they accumulated 8.5 and 4.7 µg/g, respectively (Table 3). Presumably, chelation of Cd with EDTA caused the lower uptake of Cd, as shown previously for marine worms and shrimp (Ray et al. 1979) and oysters (Hung 1982) and in contrast with data for mussels (George and Coombs 1977).

Zn concentration in the clams did not change with exposure to Zn alone, to Cd alone, or to Zn and Cd in combination. The mean Zn content of the clams was 0.27 with a range from 0.13-0.51 µg/g dry weight for 83 determinations. In contrast, when shrimp were exposed to Cd at 40 µg/L and to increasing levels of Zn (70 to 410 µg/L), Cd level in the shrimp remained relatively constant but Zn level doubled (Ray et al. 1980b).

Cooke et al. (1979) found that the Zn:Cd ratio in cockles decreased as Cd was accumulated. They concluded that there was an inverse relationship between Cd and Zn concentrations. However, the mean Cd concentration in cockles rose steadily from 0.55 µg/g initially to 7.3 µg/g at 4 d exposure to Cd and the mean Zn concentration varied from 81 µg/g initially to 62 at 2 d, to 65 at 3 d and increased to 103 µg/g at 4 d. It appears that increase in Cd was the main reason for the decrease in the Zn:Cd ratio.

During the excretion phase of the experiments, average Cd concentration decreased by 16 to 18% during 14 d for clams that had been exposed to $CdCl_2$ or to CdEDTA at 25 µg/L (Table 2). Similarly, the decrease ranged from 16 to 34% during 32 d for those that were exposed to CdEDTA or to $CdCl_2$ with or without Zn present (Table 3). However, considering the ranges of the values for Cd concentration, it is not clear whether there was excretion of Cd. If so, in most cases, the decline occurred during the early part of the excretion phase, within 4 d (Table 2) or within about 8 d (Table 3). The exception may be the CdEDTA test (Table 3) where a decline may have occurred between 8 and 16 d.

Analysis of Macoma exposed to Cd at 25 µg/L (Table 2) to determine the chemical form of Cd indicated that more than 95% of the Cd was in a bound form. Chou et al. (1978) found Cd in scallop tissue to be in bound form but that 67% of Cd in oyster tissue was free. Noël-Lambot (1976) presented evidence for a Cd-binding protein in

mussels which may be similar to mammalian metallothionein. The presence of a binding protein would explain the rapid accumulation and perhaps the low excretion of Cd by Macoma.

In uncontaminated coastal sea water, Cd levels are 0.1 µg/L or less and Zn levels are 2.0 µg/L or less. Cd levels up to 125 µg/L and Zn up to 250 µg/L have been reported for contaminated areas (Loring et al. 1980). In relation to these levels, the concentrations of Cd used in this study, 25 and 45 µg/L, represent low to moderate contamination. Zn at 45 µg/L represents moderate and at 540 µg/L represents severe contamination.

Cd level in M. balthica did not increase when they were exposed to 1.4 µg Cd/L in sea water for 14 d but increased when exposed to 25 and 45 µg Cd/L for 8 and 14 d, respectively. Smaller animals accumulated higher amounts of Cd per unit weight than larger animals. Both Cd and Zn levels remained constant in M. balthica when the animals were exposed to Cd in association with Zn at concentrations up to 540 µg Zn/L. The level of Cd was 45% lower at 14 d when exposed to CdEDTA (45 µg Cd/L) compared with exposure to the same concentration of Cd as $CdCl_2$. More than 95% of Cd in M. balthica was in a bound form, regardless of the mode of exposure to Cd.

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