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Heavy metal accumulation and calcium content in the bivalve *Donacilla cornea*

Francesco Regoli[1], Enzo Orlando[1], Marina Mauri[2], Marco Nigro[1], Giulia Alfinito Cognetti[1]

[1] Dipartimento di Biomedicina Sperimentale Infettiva e Pubblica, Università di Pisa, Via Volta 4, I-56100 Pisa, Italy
[2] Dipartimento di Biologia Animale, Università di Modena, Via Università 4, I-41100 Modena, Italy

ABSTRACT: Accumulation of heavy metals (Cu, Cd and Mn) and their effects on total Ca content were studied under laboratory conditions in sandy-bottom bivalves *Donacilla cornea* from an unpolluted shore in northern Sardinia. The bivalves were found to accumulate the 3 metals. Exposure at 2 different temperatures (18 and 25 °C) showed significant differences only for Cu accumulation. Following decontamination in clean seawater, the body content of Cu and Mn decreased strongly, whereas that of Cd remained practically unchanged. Among organs, gills contained the highest metal concentrations. A net increase of total Ca concentration in the body was observed during metal accumulation, and the digestive gland seems the most suitable organ for studies on the effects of heavy metals on calcium homeostasis.

INTRODUCTION

Marine bivalves are known to accumulate and tolerate high concentrations of heavy metals (Graham 1972, Windom & Smith 1972, Alexander & Young 1976, Okazaki 1976, Goldberg 1980). Therefore they have been used as monitors of pollution of the marine environment (Goldberg 1986). Moreover, some species are widely employed in laboratory studies on uptake, loss and biological effects of heavy metals, petroleum compounds and other chemicals (Bayne 1980, Widdows et al. 1981, Bayne et al. 1982). Although heavy metals are known to interfere with calcium homeostasis in mammals and fishes (Ma et al. 1974, Shephard & Simkiss 1978, Bansal et al. 1985, Reddy et al. 1988, Zhang et al. 1990), very few data are available for marine invertebrates (Viarengo et al. 1988a, b).

In this study we investigated the ability of the seawedge shell *Donacilla cornea* (Poli) to concentrate copper, cadmium and manganese and the effect of these heavy metals on total calcium content in gills, digestive gland and whole soft parts.

MATERIAL AND METHODS

Donacilla cornea (1.5 ± 0.5 cm shell length) were collected in April 1989 from Porto Pozzo, an unpolluted sandy shore in northern Sardinia. The bivalves were acclimatized for 7 d in synthetic aerated seawater (37 ‰ salinity) without sediment before exposure to Cu, Cd and Mn, supplied separately.

Metal exposures were carried out at 18 and 25 °C. Concentrations of Cu in seawater were 150 and 300 µg l^{-1} at 18 °C and 100 and 150 µg l^{-1} at 25 °C. At this latter temperature levels of Cu in seawater higher than 150 µg l^{-1} resulted in a too high mortality. Cd and Mn concentrations were respectively 200 µg l^{-1} and 10 mg l^{-1} at both temperatures. The concentration of Mn used in this work was in the range of values measured in interstitial waters of the estuarine area of the Arno river, Italy (Mauri & Orlando 1982). Seawater (0.5 l ind.$^{-1}$) was changed daily and specimens were not fed during the experiments. Bivalves were supplied with heavy metals and then allowed to depurate in clean seawater.

For each treatment 15 individuals were sampled at different times and groups of 3 were used for metal analysis. The entire soft parts, separated from the shells, were weighed wet and dry and then digested with nitric acid (Aristar BDH) in closed teflon vessels, first at room temperature for 8 h and then at 120 °C for 10.5 h. The analytical procedure was checked with standard reference material (lobster hepatopancreas) provided by the National Research Council of Canada.

Bivalves exposed at 18 °C to Cu (150 µg l^{-1}), Cd (200 µg l^{-1}) and Mn (10 mg l^{-1}) were also used for measur-

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ing concentrations of Ca in digestive gland, gills and total soft parts. Some bivalves were exposed to metals for 14 d; others were transferred to clean seawater after 7 d of exposure. Samples were collected after 7 and 14 d. Each sample consisted of 25 individuals: the organs, dissected from these specimens, were grouped into 5 pools, rinsed in a Ca-free physiological solution, and weighed before acid digestion.

Metals were determined by flame atomic absorption spectrophotometer (IL mod. S11 equipped with deuterium background corrector). Lanthanum nitrate (0.1 %) was added before Ca determination to control chemical and ionization interferences. The differences between groups of means were tested firstly with the analysis of variance (ANOVA) and then with the multiple range test of Scheffe.

RESULTS

Accumulation of heavy metals

Laboratory experiments revealed that copper was strongly accumulated by *Donacilla cornea* (Fig. 1), with maximum body concentrations at 25 °C. At this temperature the body concentrations for 150 µg l^{-1} of Cu in seawater were significantly higher than for 100 µg l^{-1}. No significant differences in Cu accumulation were observed at 18 °C between individuals exposed to 150 and 300 µg Cu l^{-1}. Loss of Cu occurred when exposed individuals were transferred to clean seawater. After 6 d of decontamination, Cu decreased by about 50 %, and at the 15th day values similar to those found in controls were measured (Fig. 1).

No differences in cadmium and manganese accumulation were observed between 18 and 25 °C. Cd concentration progressively increased throughout the period of exposure. After 15 d in clean seawater, the loss of the metal was not statistically significant (Fig. 2). Mn was rapidly accumulated, the whole-body concentration reaching a maximum at the 3rd day and then remaining almost constant (Fig. 3). Following 3 d in clean seawater the decrease of the metal was 60 %. However control organisms also showed a significant loss of Mn with a decrease of about 50 % after 13 d.

Data on the content of heavy metals in the organs (Tables 1 to 3) showed the highest concentrations of Cu, Cd and Mn in the gills in which, following decontamination, an important loss of these 3 metals was observed. Decontamination did not significantly

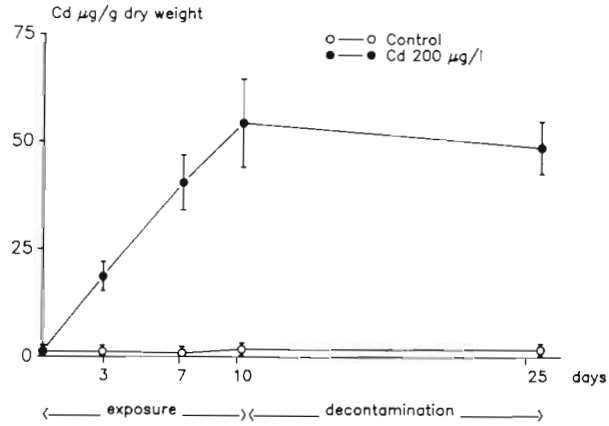

Fig. 2. *Donacilla cornea*. Cadmium concentrations (means of 10 samples ± SD) in the total soft parts of bivalves exposed to Cd and allowed to decontaminate in clean seawater for 15 d

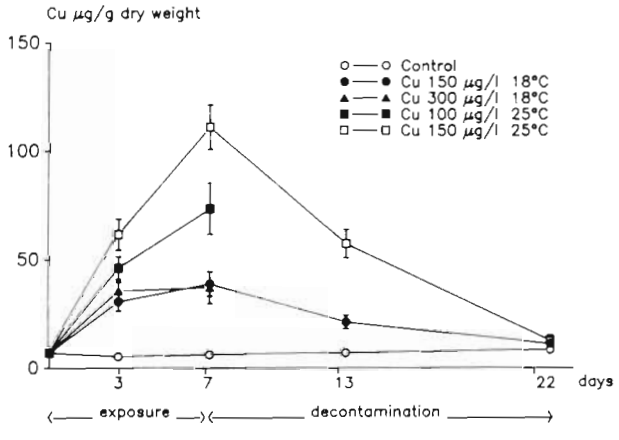

Fig. 1. *Donacilla cornea*. Copper concentrations (means of 5 samples ± SD) in the total soft parts of bivalves exposed to Cu and allowed to decontaminate in clean seawater at 18 and 25 °C for 15 d

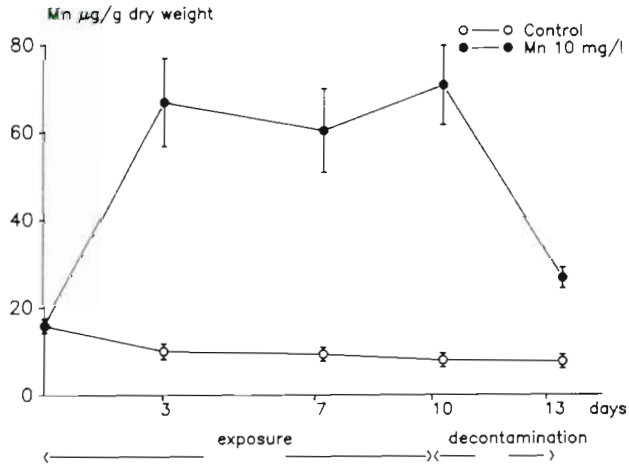

Fig. 3. *Donacilla cornea*. Manganese concentrations (means of 10 samples ± SD) in the total soft parts of bivalves exposed to Mn and allowed to decontaminate in clean seawater for 3 d

change the concentrations of Cu and Cd in the digestive gland, whereas Mn levels here returned to control values after 7 d in clean seawater.

Variations of total calcium content

No significant differences in calcium levels were observed in controls during the experiment, whereas in general a net increase of the metal was observed in the total soft parts and in the organs of *Donacilla cornea* exposed to heavy metals (Tables 1 to 3).

In whole individuals (Tables 1 & 2) large increases in Ca concentrations were observed following exposure to Cu (+89.8 % after 7 d) and to Cd (+252 % after 14 d). Similarly, in the digestive gland, exposure to heavy metals significantly increased the Ca concentration (Tables 1 to 3). In individuals exposed to Cu or Cd this organ did not lose Ca (Figs. 4b & 5b), nor heavy metals (Figs. 4a & 5a), after 7 d decontamination. On the contrary a net decrease of Ca (Fig. 6b) accompanied the loss of Mn (Fig. 6a).

In bivalves exposed to the 3 metals separately, the gills showed Ca increases which, when significant, were smaller than those observed in total soft parts or in the digestive gland (Tables 1 to 3).

DISCUSSION AND CONCLUSIONS

From our results it appears clearly that *Donacilla cornea* is able to accumulate copper, cadmium and

Table 1. *Donacilla cornea*. Mean values and standard deviations (n = 5) for Cu and Ca (μg g^{-1} dry wt) in the digestive gland, gills and total soft parts of bivalves exposed to Cu (150 μg l^{-1}, 18 °C). Also shown are percentage variations of Ca and significance with respect to the mean value at Day 0 (* $p > 0.05$; ** $p < 0.05$). Exp.: days of exposure to the metal; Decon.: days of decontamination in clean seawater

Exp.	Decon. (d)	Body part	Cu	Ca	Ca %
0	–	Dig. gland	16.2 ± 1.8	960 ± 100	–
0	–	Gills	22.5 ± 3.2	1240 ± 190	–
0	–	Soft parts	7.1 ± 1.6	1080 ± 180	–
7	–	Dig. gland	38.2 ± 3.9	1200 ± 140	+ 25.0**
7	–	Gills	64.2 ± 8.5	1520 ± 100	+ 22.6**
7	–	Soft parts	38.8 ± 5.7	2050 ± 150	+ 89.8**
14	–	Dig. gland	61.3 ± 7.8	2300 ± 220	+139 **
14	–	Gills	120 ± 15	1430 ± 80	+ 15.3*
14	–	Soft parts	68.1 ± 5.7	1670 ± 110	+ 54.6**
7	7	Dig. gland	40.2 ± 3.5	1340 ± 80	+ 39.6**
7	7	Gills	36.2 ± 3.3	1100 ± 100	− 11.3*
7	7	Soft parts	22.8 ± 3.7	1260 ± 200	+ 16.7*

Table 2. *Donacilla cornea*. Mean values and standard deviations (n = 5) for Cd and Ca (μg g^{-1} dry wt) in the digestive gland, gills and total soft parts of bivalves exposed to Cd (200 μg l^{-1}, 18 °C). See Table 1 for explanation

Exp.	Decon. (d)	Body part	Cd	Ca	Ca %
0	–	Dig. gland	1.0 ± 0.2	960 ± 100	–
0	–	Gills	2.3 ± 0.3	1240 ± 190	–
0	–	Soft parts	1.2 ± 0.2	1080 ± 180	–
7	–	Dig. gland	33.2 ± 5.9	1190 ± 180	+ 24.0**
7	–	Gills	56.7 ± 5.5	1300 ± 190	+ 4.8*
7	–	Soft parts	40.4 ± 6.4	2130 ± 290	+ 97.2**
14	–	Dig. gland	57.3 ± 10.3	1370 ± 160	+ 43.1**
14	–	Gills	105 ± 20	1670 ± 140	+ 34.8**
14	–	Soft parts	73.5 ± 13.6	3800 ± 510	+252 **
7	7	Dig. gland	35.1 ± 6.3	1270 ± 210	+ 32.3**
7	7	Gills	34.8 ± 6.2	1290 ± 70	+ 4.0*
7	7	Soft parts	37.4 ± 6.9	2350 ± 420	+118 **

Table 3. *Donacilla cornea*. Mean values and standard deviations (n = 5) for Mn and Ca (μg g^{-1} dry wt) in the digestive gland and gills of bivalves exposed to Mn (10 mg l^{-1}, 18 °C). See Table 1 for explanation

Exp. (d)	Decon.	Body part	Mn	Ca	Ca %
0	–	Dig. gland	7.9 ± 1.4	960 ± 100	–
0	–	Gills	7.0 ± 0.9	1240 ± 190	–
7	–	Dig. gland	25.7 ± 2.6	2370 ± 310	+147 **
7	–	Gills	46.7 ± 8.3	1320 ± 130	+ 6.5*
7	7	Dig. gland	6.1 ± 1.1	1030 ± 130	+ 7.3*
7	7	Gills	7.1 ± 0.9	1490 ± 120	+ 20.2*

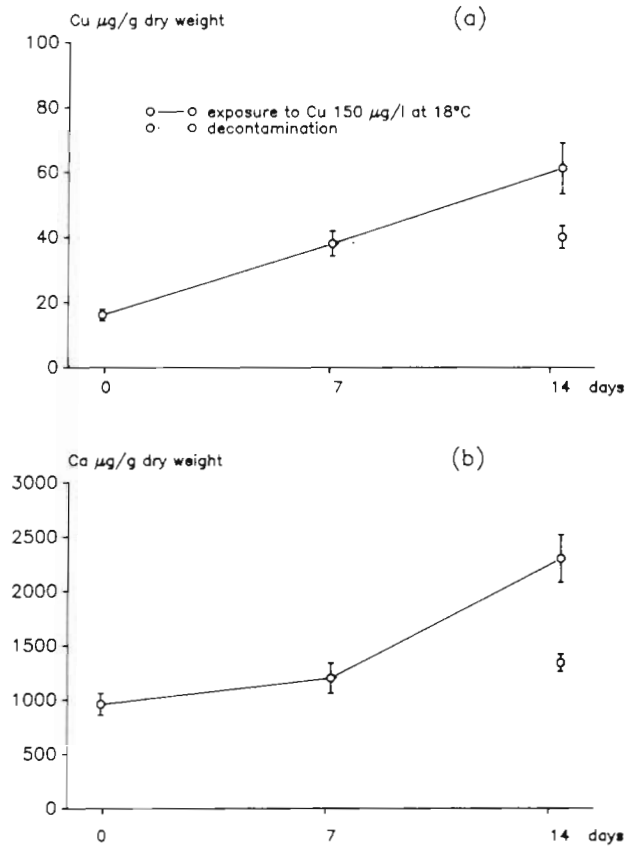

Fig. 4. *Donacilla cornea*. (a) Copper and (b) calcium concentrations (means of 5 samples ± SD) in the digestive gland of bivalves exposed to Cu (150 μg l^{-1} at 18 °C) and allowed to decontaminate in clean seawater for 7 d

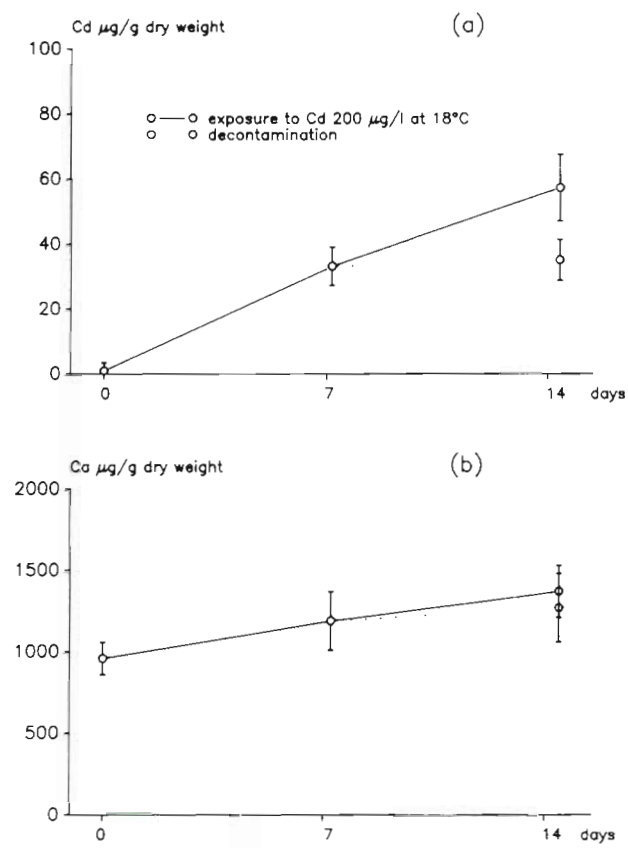

Fig. 5. *Donacilla cornea*. (a) Cadmium and (b) calcium concentrations (means of 5 samples ± SD) in the digestive gland of bivalves exposed to Cd (200 μg l^{-1} at 18 °C) and allowed to decontaminate in clean seawater for 7 d

manganese, with the highest concentrations occurring in the gills.

Temperature does not seem to affect Cd and Mn uptake in *Donacilla cornea*, but copper concentrations were significantly higher at 25 °C than at 18 °C. In contrast, in the gills of *Mytilus galloprovincialis* accumulation of Cu at 13 °C was higher than at 23° (Viarengo et al. 1988b). Moreover it was reported that Cd uptake in *Mytilus edulis* was affected by temperature only at low (12‰) salinity (Phillips 1976). According to the latter author, temperature influences Cu accumulation causing variations in either uptake or excretion of the metal, often leading to erratic results.

Since our experiments were carried out in the absence of food and sediments, the accumulation of heavy metals probably occurred via a direct uptake mechanism from water. Our findings do not exclude that bacteria, introduced with the bivalves, might influ-

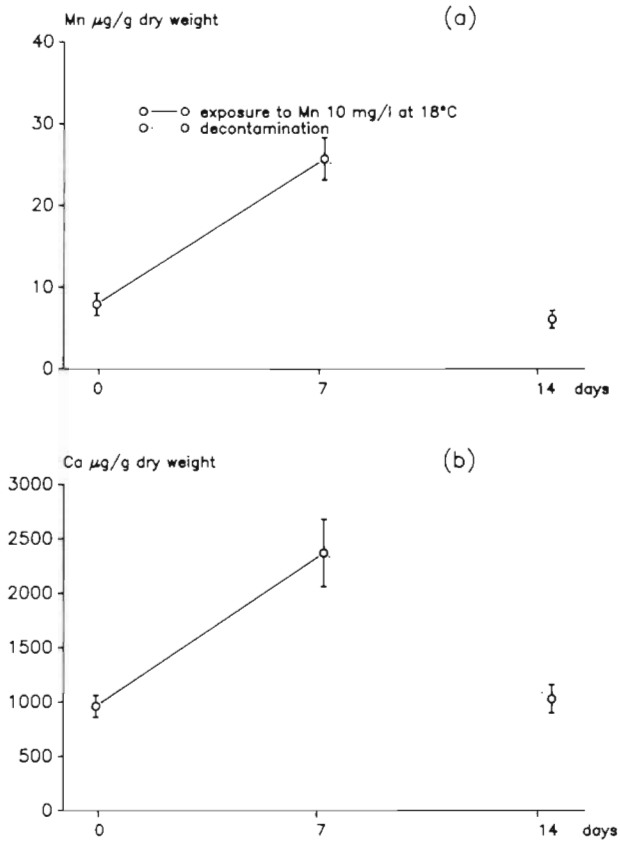

Fig. 6. *Donacilla cornea*. (a) Manganese and (b) calcium concentrations (means of 5 samples ± SD) in the digestive gland of bivalves exposed to Mn (10 mg l^{-1} at 18 °C) and allowed to decontaminate in clean seawater for 7 d

ence heavy metal uptake. This aspect should be investigated in future studies.

Following decontamination whole-body Cu levels lowered to control values, whereas total Cd decrease was not statistically significant. This result might be related to the different capacity of the cells to eliminate these 2 metals, when they are bound to thioneins (Viarengo et al. 1985). In fact, in another bivalve (*Mytilus galloprovincialis*), Cu bound to thioneins was accumulated within the lysosomal system and then eliminated by exocitosis of the residual bodies (Viarengo et al. 1984). For Cd, such a mechanism does not seem to occur: only a small percentage of this metal was eliminated by excretion of residual bodies, probably bound to the peroxidated lysosomal matrix (George 1983a, b). Considering that the gills lost 38 % of Cd following decontamination, it might be speculated that only part of the metal found in this organ was bound to metallothioneins. Practically no loss of Cu was observed in the digestive gland after a 7 d recovery in clean seawater. This suggests that Cu is firmly bound and that depuration of this metal requires a longer period of time. Pringle et al. (1968) suggested that the depletion rate from a tissue is directly related to metal concentration. Likewise, Schulz-Baldes (1974) reported that in *Mytilus edulis* the loss of lead was closely correlated with the internal metal concentration.

The marked and fast loss of Mn following decontamination of *Donacilla cornea*, and observed also in control organisms, is probably due to metal unbound or loosely bound in the tissues. In this respect, it is of interest to note that Bryan & Hummerstone (1973) found rapid Mn depletion in the polychaete *Nereis diversicolor*.

The sharp increases in Ca levels observed in bivalves exposed to heavy metals, but not in the controls, indicate an alteration of Ca homeostasis as a result of heavy metals accumulation. A significant increase of total Ca content was also found in the gills of mussels exposed to Cu (Viarengo et al. 1988b) and in the digestive gland after exposure to Cu in presence of hydrocarbons (Viarengo et al. 1988a). The pattern of Ca variations in the digestive gland were rather similar to those of the heavy metals (Figs. 4 to 6).

The increases in Ca concentrations in whole organisms exposed to Cu or to Cd, much larger than those observed in some organs, probably reflect the high content of Ca in the kidney, where numerous granules containing Ca phosphate were found to accumulate in renal cells during exposure to metals (authors unpubl.).

From our results, it appears that, while the gills of *Donacilla cornea* are good material for monitoring heavy metals in seawater, its digestive gland is particularly suitable for studies on the effect of heavy metals on Ca homeostasis.

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