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Depuration Effects on Trace Metals in *Anomalocardia brasiliana* (Gmelin, 1791)

M. Wallner-Kersanach,[1] S. E. Lobo,[2] E. M. da Silva[2]

[1]Meereszoologie, University of Bremen, FB 2, Bürgermeister Smidt-Strasse 20, 27568 Bremerhaven, Germany
[2]Instituto de Biologia da UFBa, Campus de Ondina, 40170-110, Salvador-Ba, Brazil

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Bivalves have been regarded as suitable bioindicators of metal pollution in the marine and estuarine environments (Phillips 1977; Goldberg et al. 1978; Theede et al. 1979). However, the metal concentrations of the soft parts of individual bivalves can vary considerably depending on size variations, geographic and genetic differences, individual variability in metal uptake, ingestion of sediment particles and induction of metal-binding proteins (Waldichuck 1985; Phillips and Segar 1986). Metal-containing particulate matter in the gut can also significantly influence the determination of tissue metal concentrations in invertebrates (Flegal and Martin 1977; Chapman 1985; Mo and Neilson 1991) and in fish (Lobel et al. 1991). For example, the sediment content in the gut of mussels could represent 36 to 39% of the total body load of copper, thus the period of depuration in the laboratory can minimize its influence and it is now considered as part of the routine methodology (Goldberg et al. 1978). Indeed, these authors showed that if depuration is not carried out, then large variations in metal concentrations are more likely to occur. Latouche and Mix (1982) reported that Cu and Ni concentrations increased in somatic tissues of Mytilus edulis even after depuration, while Mn decreased significantly.

Anomalocardia brasiliana (Gmelin 1791) (Bivalvia-Veneridae) occurs in the upper layers of sediment in marine intertidal regions from the West Indies to Southern Brazil. In the Todos os Santos Bay located on the northeastern coast of Brazil, the cockle A. brasiliana is the most abundant bivalve and it is well adapted to the conditions in the area. It is an important source of food for many local communities (Peso-Aguiar 1980). This species has been frequently used as an indicator for heavy metals pollution (Tavares et al. 1983; CRA 1984); however, no data have been published on metal concentrations of A. brasiliana of different size classes and on the effects of depuration on overall concentration. It is therefore important to determine the metal concentrations in A. brasiliana both from the point of view of how the concentrations relate to metal loading of the area. The effect of size upon metal concentration of shellfish has been examined by Boyden (1977), who found that zinc in

Correspondence to: M. Wallner-Kersanach

Mytilus edulis was greater in smaller individuals while cadmium was independent of size. The objective of this study was to determine concentrations of copper, zinc, cadmium and lead in A. brasiliana collected from a site with a relatively low impact of heavy metal contamination, to examine metals in different size classes and to assess the effect of depuration on tissue concentration of the metals. These elements were selected due to their toxicity to marine organisms, when their excess as free metal may interact with cell structures and/or enzymes affecting metabolic activities (Viarengo, 1989).

Materials and Methods

Individuals of A. brasiliana were collected in September 1990, after the rainy season, from an intertidal area at Mapele in Aratu Bay located in the Todos os Santos Bay, Salvador, Brazil (12° 46' N and 38° 26' W). The sampling was carried out only at the end of the rainy season because of the highest metal concentrations found in organims of this tropical area (Wallner and Freitas 1991). The sampling site was located near an industrial area on the northeastern side of the bay, which is part of the larger Todos os Santos Bay.

The organisms were collected manually from the upper 2 cm of the sediment. Individuals from Mapele station were separated into three different shell-length classes of 1.20-1.70, 1.70-2.20 and 2.20-2.80 cm, and classed as small, medium and large, respectively. A total of 100 individuals collected were separated into two groups of 50 organisms and each group classed in the same way as above with 17 small, 16 medium and 17 large individuals.

In the laboratory, one group of 50 organisms were maintained separately to depurate for 18 hr in a 1000 ml beaker containing clear non-filtered seawater with continuous aeration and a seawater beaker as a blank. At the same time, each of the other 50 individuals from the three different classes of Mapele station had its shell length measured and total fresh weight recorded. The same procedure was also carried out for the other group of cockles after they had undergone depuration. The soft body of the organisms were dried in an oven at 60° C to constant weight. The cockles were individually digested with HNO_3 and $HClO_4$ (1:1) and fumed to near dryness on a sand bath to about 190° C for 12 hr, sufficient time to leave the samples colorless. Cu, Zn, Cd and Pb were determined by graphite furnace AAS (Perkin-Elmer, Zeeman/3030).

To assess the metal concentration of particulates in the guts of the animals, the seawater used to depurate each group of Mapele cockles was filtered through an acid-washed membrane filter (Millipore HAWP, 0,45 µ, ø 45 mm) to recover the material expelled by the organisms during depuration. A further set of filters through which seawater (without

cockles) had been passed served as blank values. The filters were dried at 60 °C until constant weight was reached and then mineralized in the same way as the organisms. Afterwards they were analyzed with AAS for the metals given above. The results found were subtracted from the seawater and the filter blank values as well. The quality of the measurements was routinely checked by frequent analysis of standard solutions of known concentrations. As reference materials lobster hepatopancreas (TORT-1) and coastal sediments (BCSS-1) were used. The percent recoveries of analysed TORT-1 were 100% for Cu, 96% for Zn, 100% for Cd and 95% for Pb. The standard reference BCSS-1 presented percent recoveries of 91% for Cu and 86% for Zn. Unsatisfactory recoveries were obtained for Cd and Pb in BCSS-1. This was probably due to an inherent problem regarding these metals in the reference material, as suggested by the good recoveries of all metals in TORT-1 and Cu and Zn in BCSS-1.

A statistical comparison employing Student's t-test at the 5% level of significance was carried out between the non-depurated and depurated group of cockles. Differences between size classes and heavy metal accumulation in the organisms were tested by the Kruskal-Wallis test at the 5% level of significance.

Results and Discussion

The mean concentrations of Cu, Zn, Cd and Pb (µg/g ± sd dry weight) in cockles for both treatments and with all size classes grouped together are shown in Table 1. The mean concentrations of all trace metals analyzed in non-depurated cockles were significantly greater than in the depurated group (t-Test $P < 0.05$).

Table 1. Trace metal concentrations (µg/g ± sd dry weight) in non-depurated and depurated A. brasiliana (t-test comparison) and the percentage decrease of heavy metals after depuration at Mapele station.

Metal	Non-Depurated (n=50)	Depurated (n=50)	t-test (P)	Decrease (%)
Cu	8.31 ± 1.69	7.70 ± 1.33	0.0303	7.34
Zn	65.55 ± 9.04	52.85 ± 6.29	0.0001	19.37
Cd	1.66 ± 0.64	1.05 ± 0.28	0.0001	36.75
Pb	0.77 ± 0.32	0.47 ± 0.18	0.0001	38.96

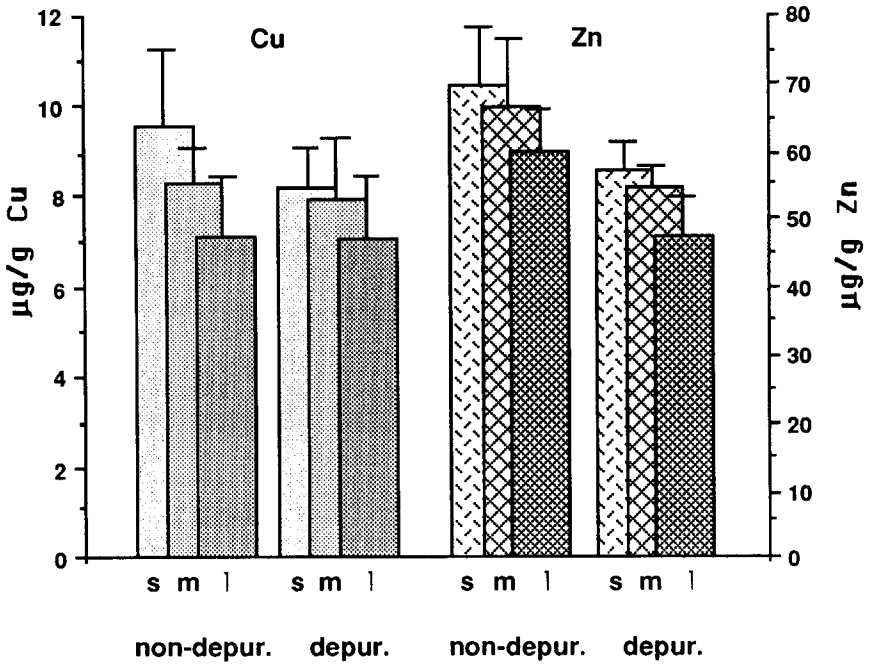

Figure 1. Concentrations of copper and zinc (µg/g dry weight) and standard deviation in different sizes (s=small; m=medium; l=large) of non-depurated and depurated A. brasiliana. Number of organisms: s=17, m=16 and l=17.

After depuration metal concentrations in cockles had decreased. The depurated individuals presented a decrease of 7.3, 19.4, 36.8 and 39.0 % for Cu, Zn, Cd and Pb, respectively. Goldberg et al. (1978) found that Cu and Zn had decreased by 9 and 12 %, respectively, in Mercenaria mercenaria after 35 hr of depuration. Otherwise, the standard deviations of metal concentrations of non-depurated individuals in Mapele were relatively higher than depurated ones, which suggests the variable nature of metal-containing particles in the gut.

The higher percentage loss of Cd and Pb after depuration (Table 1) in A. brasiliana can probably be related to the low concentration of these elements in the organisms, which tends to present more variations during the analysis and/or because of the size difference of the organisms.

Both the depurated and non-depurated cockles showed a decrease of Cu and Zn concentrations with increasing size ($P < 0.05$) (Figure 1). On the other hand, Cd concentrations were significantly higher in larger depurated ($P < 0.05$) and non-depurated individuals ($0.05 > P > 0.01$) (Figure 2). A similar pattern has also been observed for Cd in oysters (Boyden 1977) and in Mytilus edulis (Fischer 1983). This may indicate that bivalves cannot regulate Cd concentrations in their bodies as they do for

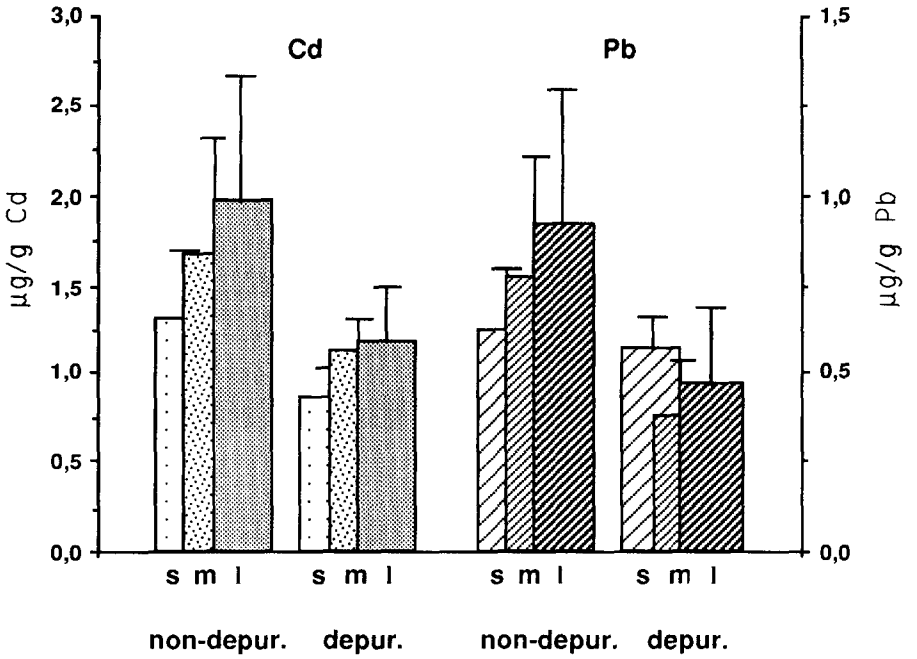

Figure 2. Concentrations of cadmium and lead (µg/g dry weight) and standard deviation in different sizes (s=small; m=medium; l=large) of non-depurated and depurated A. brasiliana. Number of organisms: s=17, m=16 and l=17.

copper and zinc.

The results as a whole indicate that for essential metals (Cu and Zn) the depuration rate is smaller than for non-essential metals (Cd and Pb). Another valid consideration is that as the organisms grow larger they seem to become more able to regulate Cu and Zn, using the needed amounts, but on the other hand, non-essential metals, such as Cd and Pb, are still very difficult to regulate. However, there exist processes of re-arrangement of these metals which may change the internal distribution in the course of the time (Theede and ter Jung 1989).

There was no significant difference ($P > 0.05$) for Pb concentrations in non-depurated A. brasiliana between different size classes due to the large standard deviations (Figure 2). However, Pb presented some variations in depurated individuals, which were significantly different ($P < 0.05$), probably due to the low concentrations of this element in the cockle tissue.

It was observed in the freshwater mussel Anadonta anatina that while Cd and Pb concentrations increased with tissue weight, those of Cu decreased; this has been suggested to occur in conditions of extreme

contamination (Phillips 1980). Concentrations of cadmium in A. brasiliana in this study were not very high.

The amount of particles retained on the filters was not estimated. It was obvious, however, that the larger and therefore older organisms expeled more particles than the smaller ones. The trace metal concentrations of the particulate material in the water also showed an increase with the size class of cockles (Table 2).

Table 2. Trace metal concentrations of the particulate material in water (µg/g) of the different size classes of depurated specimens of A. brasiliana.

Metal	Size (cm)	
	1.18 - 1.71 (small-class)	1.72 - 2.12 (medium-class)
Cu	7.45	11.73
Zn	17.41	28.54
Cd	0.02	0.04
Pb	6.31	7.45

In spite of having lost the filter containing the particulate material from the large-class of organisms during the digestion procedure due to an over-flow, there was a similar trend for the metal concentration and animal size. Because lead in the environment is strongly adsorbed onto sediment and soil particles, its bioavailability is very low to organisms (WHO 1989). This may explain the high concentration of Pb found in the particles retained on the filters and the low concentration found in the cockles.

It is assumed that the metals found in the intestinal tract of cockles originated mostly from sediment ingestion. In cases where organisms can control their uptake rate, and/or the metal-bound sediment is not absorbed very much, the amount in ingested particles may have a greater influence on its total concentration of metals, especially in sites with high metal concentrations. Results from Bertine & Goldberg (1972) and Romeril (1979) indicate that there was a good relationship between heavy metals in the sediment and in the guts of bivalves.

Metals in bivalves are already inherently variable, dependent on many factors like species and age. This study highlights the different responses of cockles, with a tendency for essential elements to decrease and non-essential elements to increase with size of the cockles. The use of depuration may be necessary to avoid measurement variations of the total metal load in the tissue.

The influence of size and depuration on metal concentrations of cockles

has shown that these factors are important in the design of biomonitoring programs in the Todos os Santos Bay. Thus, for further studies, it should be important to consider cockles size in the population and apply the normalization of data based on relationships developed by Boyden (1977). Complementary studies should be carried out to investigate the effects of sex and seasonal variations on trace metal concentrations in A. brasiliana.

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