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Accumulation and Depuration of Cu and Zn in the Blood Cockle *Anadara granosa* (Linnaeus) under Laboratory Conditions

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ABSTRACT

Studies on the accumulation (4 days of single metal exposure) and depuration (6 days in natural seawater) of Cu and Zn were conducted in the blood cockle *Anadara granosa* under laboratory conditions. Different rates of accumulation and depuration between the soft and hard tissues probably reflect the different mechanisms of binding and regulation of Cu and Zn between the soft tissues and shells of cockles. At the end of depuration, the concentrations of Cu and Zn in the soft tissues were only 1.71 and 1.75 times higher than prior to the exposure, respectively. Thus, no significant difference was found in the depuration level between Cu and Zn. Hard tissues showed that the levels of Cu and Zn are similar to those before the exposure. This indicated the slow rates of accumulation and depuration in the shells as compared to the soft tissues of *A. granosa*. The condition index of *A. granosa* could be used as a potential physiological indicator of metal pollution. The capabilities to accumulate Cu and Zn and to depurate both metals in the soft tissues indicate that *A. granosa* is a potential biomonitoring organism for its health assessment using the condition index.

Keywords: ***Anadara granosa*, Cu and Zn exposure, laboratory studies, accumulation and depuration of Cu and Zn in the cockles**

INTRODUCTION

Cu and Zn have attracted a lot of attention in ecotoxicological studies in the literature. For example, Yap *et al*. (2003b: 2004a) proposed the use of mussel shell as a biomonitoring material for Zn, while Yap *et al*. (2003c) suggested the use of different soft tissues of *Perna viridis* as biomonitoring agents for the Cu pollution in coastal waters.

The cockle, *Anadara granosa* (Order: Arcoida; Family: Arcidae), exhibits characteristics as a good biomonitor of heavy metal pollution. They have a sessile life style, have a wide geographical distribution in the tropical intertidal muddy sediments (Lowe and Kendall, 1990; Dame, 1996), and are filter feeders ingesting both zooplankton and phytoplankton, in addition to being easily collected. They are important to local commercial fisheries and are easy to maintain in the laboratory (Ong and Din, 2001). Cockles thrive best under calm conditions, especially in shallow inlet bays, with soft and flocculent mud with salinity ranging from 18 to 30 ppt (part per thousand) (Ng, 1984; Panthasali and Soong, 1985).

Some previous studies have shown that *A. granosa* are able to accumulate Cd and Cu to a significant level in their tissues (Mat, 1994;

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Mat *et al*., 1994a, b; Noorddin, 1995). The exposure to Cd causes significant increases in the concentrations of Cd in the gills, hepatopancreas and the total soft tissues of cockles as compared to the controls (Chan *et al*., 2002). However, little is known about the distributions of Cu and Zn in the soft and hard tissues of *A. granosa*. Therefore, the objective of this study was to determine the accumulation and depuration of Cu and Zn in the soft and hard tissues of *A. granosa* under laboratory conditions.

MATERIALS AND METHODS

Sampling and Acclimatization of the Cockles

Individual *A. granosa* were purchased from the wet market in Port Dickson, Negeri Sembilan, Malaysia (collected from Teluk Intan, Perak). For acclimatization purpose, the cockles were then transplanted to the mudflat areas in Pasir Panjang, Negeri Sembilan for 7 days. The cockles were checked everyday and they were considered dead if they did not show any tactile stimulus and gape their shell valves wide. Similarly, salinity and temperature were also checked every day. The Pasir Panjang area has a salinity range of 26 to 30 ppt and a temperature range of about 25 to 30°C. Later, all of the cockles were transported to Hatchery 1, at the Center of Marine Science Research, Universiti Putra Malaysia, Teluk Kemang, Port Dickson, Negeri Sembilan, Malaysia.

All the experimental seawater was sand filtered. Healthy acclimatized cockles were selected for the exposure study. Sixty cockles were exposed in each plastic aquarium. All the experimental treatments were based on a single metal exposure study. Prior to the metal exposure, 20 cockles were collected to analyse the background metal concentrations. The cockles were exposed to sublethal concentrations of Cu (nominal: 0.10 mg/L; measured 0.133 mg/L) and Zn (nominal; 1.00 mg/L; measured 1.323 mg/L) for 6 days. A control aquarium, with cockles but without the addition of metal solution to sand filtered seawater, was also simultaneously set up. The measured concentrations were close to the nominal concentrations, as shown in Table 1. The test solutions (10 L) were constantly changed once every two days to new seawater spiked with standard solutions of Cu and Zn on Days 0, 2 and 4. On Day 6, the cockles were rinsed with clean seawater and were transferred into a clean seawater aquarium for the depuration study. Samplings (12 cockles at each sampling) were conducted on Days 2, 4 and 6 during the metal accumulation period and on Days 8 and 10 during the depuration period. All the samples were stored at -10°C until further analysis. The test seawater was constantly aerated and held at room temperature (26-30°C) and salinity 28-30 ppt.

Sample Preparation

The samples of cockles were then thawed at room temperature (27°C) on a clean tissue paper to drain away the excess water before being analyzed, dried for 72 h at 105ºC in an oven

TABLE 1
The nominal and measured test concentration (mg/L) of Cu and Zn during exposure periods

Metals	Nominal	Day of exposure	Measured Mean ± SE
Cu	0.1	0	0.137 ± 0.004
		2	0.162 ± 0.015
		4	0.122 ± 0.020
Zn	1.0	0	1.320 ± 0.033
		2	1.262 ± 0.024
		4	0.987 ± 0.040

to constant dry weight. After that, the samples were digested in concentrated nitric acid (Ajax Chemicals, HNO_3 65 %, Australia) in a hot-block digester, first at a low temperature (40°C) for 1 hour and then at a high temperature (140°C) for at least 3 hours. The samples were assumed to be digested after the acidic solutions had became clear (Yap *et al*., 2003b). The digested samples were then diluted with double distilled water.

Determination of Cu and Zn in Cockles

Cu and Zn were determined using the air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin Elmer model AAnalyst 800. The data were calculated on μg/g dry weight basis. All the glassware and equipment used were acid-washed to avoid possible contamination, while procedural blanks were analysed in every five sample to check for contamination. Finally, the quality control samples (made from the standard solutions of Cu and Zn) were analyzed in every five sample to check for their recoveries (Yap *et al*., 2002).

Data Analysis

The bioconcentration factor (BCF) was calculated in relation to the metal concentration in seawater according to Taylor (1983) and Yap *et al*. (2003a: 2004b):

$$BCF = \frac{Ce - Ci}{Cs}$$

Ce = he metal concentration in the cockle tissue during metal exposure (g/g wet weight)

Ci = the initial metal concentration in the cockle tissue before metal exposure (g/g wet weight)

Cs = the experimental metal concentration in the aquarium test seawater

The concentration factor (CF) was calculated at the end of depuration (Day 6) in comparison with the level of metal before exposure, as follows (Yap *et al*., 2004b):

$$CF = \frac{\text{Metal level}_{\text{end of metal depuration}}}{\text{Metal level}_{\text{pre-exposure of metal}}}$$

In order to measure the percentage of metal reduction, metal concentrations in the soft tissues and shells of *A. granosa* were taken as the end of exposure (Day 4), i.e. (after 4 days of the exposure period. At the end of the depuration period, the percentage of metal reduction in the soft tissues and shells of *A. granosa* (Yap *et al*., 2003a) was characterized based on the following equation:

Percentages of metal reduction =

$$\frac{\text{Metal level}_{\text{end of metal exposure}} - \text{Metal level}_{\text{end of metal depuration}}}{\text{Metal level}_{\text{end of metal exposure}} - \text{Metal level}_{\text{pre-exposure of metal}}} \times 100\%$$

The rate of metal accumulation was calculated according to the following formula (Yap *et al*., 2003a):

Rate of metal accumulation =

$$\frac{\text{Metal level}_{\text{exposed}} - \text{Metal level}_{\text{control}}}{\text{Day(s) of metal exposure}}$$

The rate of metal depuration was calculated according to the following formula (Yap *et al*., 2003a):

Rate of metal depuration =

$$\frac{\text{Metal level}_{\text{end of metal exposure}} - \text{Metal level}_{\text{end of metal depuration}}}{\text{Day(s) of metal depuration}}$$

T-test of metal levels between the end of accumulation (Day 6) and control treatment, and between the end of depuration (Day 10) and the control treatments were done by using STATISTICA.

RESULTS AND DISCUSSION

Accumulation and Depuration in the Soft Tissues of Cockles

Table 2 shows the accumulation and depuration of Cu and Zn in the soft tissues of cockles. For both metals, the metal levels were found to increase during the accumulation period but they decreased during the depuration period. Basically, there were only slightly differences in the accumulation and depuration patterns of these two metals. Both metals were found in significantly ($P< 0.05$) higher levels in the soft tissues at the end of accumulation (Day 6) and at the end of depuration (Day 10) as compared to those in the control treatments (Table 3).

Table 4 shows the rates of accumulation and depuration of Cu and Zn. The rates of metal accumulation in the soft tissues were faster at Day 2 than at Days 4 and 6. In general, the accumulation rates in the heavy metal exposure were higher than in the control exposure. Exposures to Cu and Zn singly showed the highest BCF values at the end of the accumulation period (i.e. Day 6), as shown in Table 4. However, the levels of Cu and Zn in the soft tissues of cockles, exposed to both the metals at the end of depuration, were close to the levels prior to the exposure (CF: < 2 times).

During the depuration period, their levels were found to decrease (Table 2). According to the accumulation rate, the cockles have a higher capability to accumulate Zn (highest rate = 37.98) than Cu (highest rate = 2.91) from ambient water. This conclusion was also supported by the BCF values (Table 4).

The high accumulations of Cu and Zn in the soft tissues of *A. granosa* were able to

TABLE 2
Concentrations (mean μg/g dry weight ± standard error) of Cu and Zn during the accumulation and depuration in the soft and hard tissues (shell) of *Anadara granosa* (N=3)

Metal/Tissue		During accumulation period				During depuration period	
		Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Cu	Soft tissue	6.92	10.99 ± 0.20	14.27 ± 0.42	18.20 ± 0.16	14.59 ± 0.05	11.83 ± 0.15
	Shells	6.56	6.69 ± 0.09	6.85 ± 0.06	6.94 ± 0.06	6.70 ± 0.03	6.63 ± 0.03
Control	Soft tissue	6.92	5.17 ±0.09	4.60 ± 0.03	5.30 ± 0.01	5.75 ± 0.10	5.93 ± 0.10
	Shells	6.56	6.59 ± 0.04	6.52 ± 0.11	6.51 ± 0.07	6.45 ± 0.14	6.39 ± 0.05
Zn	Soft tissue	106.9	182.04 ±2.32	258.3 ± 0.68	264.6 ± 1.25	201.8 ± 1.72	187.0 ± 0.87
	Shells	4.17	5.05 ±0.08	5.22 ± 0.06	5.57 ± 0.05	5.40 ± 0.05	5.30 ± 0.06
Control	Soft tissue	106.9	106.08 ± 0.39	107.9 ± 0.46	106.1 ± 0.37	109.2 ± 0.50	107.5 ± 0.51
	Shells	4.17	4.16 ± 0.03	4.22 ± 0.04	4.43 ± 0.07	4.20 ± 0.09	4.12 ± 0.04

TABLE 3
T-test results of the metals between the end of accumulation and the control treatment, and between end of depuration and control treatment

		End of accumulation (Day 6)	End of depuration (Day 10)
Cu	Soft tissues	$P< 0.05$	$P< 0.05$
	Shells	$P> 0.05$	$P> 0.05$
Zn	Soft tissues	$P< 0.05$	$P< 0.05$
	Shells	$P> 0.05$	$P> 0.05$

TABLE 4
Bioconcentration factor (BCF) and concentration factor (CF) and the accumulation and depuration rates (µg/g per day) of Cu and Zn in the cockles' soft and hard tissues exposed to 0.1 mg/L of Cu and 1.0 mg/L of Zn

	Metal	BCF	CF	Rate of accumulation			Rate of depuration	
				Day 2	Day 4	Day 6	Day 8	Day 10
Zn	Soft tissue	157.99	1.75	37.98	37.58	26.42	31.43	19.4
	Hard tissue	1.41	1.27	0.45	0.25	0.19	0.09	0.07
Cu	Soft tissue	112.80	1.71	2.91	2.42	2.15	1.81	1.60
	Hard tissue	3.40	1.01	0.05	0.08	0.07	0.12	0.08

induce metallothionein-like protein in high metal concentrations (Viarengo *et al*., 1985; Chan *et al*., 2002). Secretion of metallothionein in high metal concentrations could be a mechanism to counteract metal toxicity (Phillips and Rainbow, 1993). Although the function of this protein is still not fully understood, MT is generally accepted to involve in the regulation of essential metals, such as Cu and Zn for cell growth and development (Mackay *et al*., 1993). Meanwhile, Viarengo *et al*. (1985) reported that higher levels of accumulated metals in soft tissues were correlated to the metals binding to metallothionein and they could therefore be stored in detoxified forms.

The rate of accumulation and BCF showed the ability of cockles to accumulate metals to certain levels. Their accumulations over time in the bodies of organisms may cause toxicity (Gorell *et al*., 1997). In more specific, all heavy metals are potentially harmful to most organisms at some levels of exposure and absorption (Yilmaz, 2003). In order to survive in this metallic stress environment, cockles have to counteract metal toxicity. This could be done by regulating the intracellular concentrations of the metal and removing it to prevent deleterious effects on the functions of cell (Phillips, 1985). Results showing the depuration rate of metals during the depuration period are presented in Table 4. During the depuration period, a more rapid loss of Zn than Cu was observed. The CF values showed the ability of cockles to retain the accumulated metals during the depuration period (Table 4). Based on the CF values (Zn: 1.75 and Cu: 1.71), the soft tissues of cockles have almost similar abilities to retain the accumulated Cu and Zn.

Meanwhile, Zn and Cu are known as essential metals for metabolic functions (Mackay *et al*., 1993) and they can be regulated in bivalves (Yap *et al*., 2003a: 2004b). However, since the CF values for Zn (1.75) was almost similar to Cu (1.71), a rapid loss of Zn reported for the green-lipped mussel, *Perna viridis* (Watson *et al*., 1995), was not observed for cockles. Therefore, whether Zn was bound to the easily mobilized compartment was not evidently shown based on the present findings. Yap *et al*. (2003a) showed that in *P. virirdis,* Zn was possibly bound to the easily mobilized compartment in comparison to the non-essential Cd which was clearly shown to be not easily lost possibly due to its metallothionein binding. Since the present work focused only on Zn and Cu, such a comparison with a non-essential metal is not possible. In addition, although Zn was reported to be regulated in the soft tissues of many bivalves in the literature, this is not shown in the present study.

Accumulation and Depuration in the Shells of Cockles

Table 2 shows the levels of metals accumulated in the shells during the accumulation period. However, the increases in the Cu and Zn levels in the shells at the end of accumulation (Day 6)

and at the end of depuration (Day 10) are not significantly higher (P>0.05) when compared to the control treatment (Table 3). Based on the metal accumulation rates, the shells of cockles were found to have a slightly higher capability to accumulate Zn (i.e. highest rate = 0.45) than Cu (highest rate = 0.08) from the ambient seawater, as depicted in Table 4. This result is also similar to that in the soft tissues of *A. granosa* and was supported by the BCF values of shells (Table 4).

Table 4 shows the different patterns of metals depuration in the shell and soft tissues. A rapid depuration was observed in the soft tissues, but there was a slow depuration of metals in the shells. Meanwhile, a biodeposition in the crystalline lattices of the shells would cause the metals to permanently stay there (Yap *et al*., 2003b). This also caused the ability of the shell to act as a sink for metals (Yap *et al*., 2004a).

Based on the CF values (Zn: 1.27 and Cu: 1.01), the shells of cockles have higher ability to retain the accumulated Zn than Cu. Both the rates of accumulation and depuration in the shells were significantly (P< 0.05) lower than in the soft tissues of *A. granosa*. However, in long-term exposure, shells might accumulate higher levels of metal as compared to the soft tissues.

Based on the findings of the present study, the shells could possibly be good biomonitoring materials for Cu and Zn. Moreover, most previous studies have shown that shells (hard tissues) could be a potential biomonitoring material of heavy metals due to some of their characteristics (Yap *et al*., 2003b: 2004b). Firstly, different mineralogies and chemistries in the shells are the characteristics that cause them to have the ability to accumulate a wide range of metals (Yap *et al*., 2003b). Secondly, some trace metals have been incorporated into the shells through the substitution of the calcium ions in the crystalline lattices of the shell. They would associate with the organic matrix during shell growth (Watson *et al*., 1995).

Condition Index

Based on the data presented in Table 5, the CI values of *A. granosa* were found to be directly proportional to exposure days. After the experimental period (6 days for the accumulation period and 4 days for the depuration period), the CI values of *A. granosa* were decreased as compared to those in the controls. During the depuration period, the CI values were slightly decreased as compared to the accumulation period. For the single metal exposure after 2 days of the accumulation period, the CI value of cockles in the Cu exposure was 18.48 g/cm^3 and this was 19.23 g/cm^3 for the Zn exposure. At the end of the depuration period (i.e. at Day 10), the CI values were reduced to 15.23 g/cm^3 for Zn and 14.59 g/cm^3 for Cu.

TABLE 5
Condition index (CI) and CI reduction in *Anadara granosa* between the control and exposed cockles

Day	CI (g/cm3)			CI reduction (%)	
	Control	Zn (1.0 mg/L) exposed	Cu (0.1mg/L) exposed	Zn (1.0mg/L) exposed	Cu (0.1 mg/L) exposed
Accumulation					
2	20.56	19.23	18.48	6.47	10.12
4	20.33	18.13	17.26	10.82	15.10
6	18.76	15.64	14.77	16.63	21.27
Depuration					
8	17.84	15.35	14.65	13.96	17.88
10	17.34	15.23	14.59	12.17	15.86

According to Yap *et al*. (2002), there are two types of mechanisms usually used by organisms to cope with pollution. First, the mechanical responses and metabolic requirement. In order to cope with pollution, cockles decrease their filtration activity, and this leads to reduction of the amount of food consumed. In addition, the cockles may have to use more food than consumed to maintain the normal metabolic activities. Sequentially, the stored glycogen, carbohydrate, protein and lipid might be utilized to maintain those activities (Yap *et al*., 2002). This situation explained the decrease in their CI values when compared to the control exposure. This could also explain the low accumulated metals in their soft tissues in the initial exposure period. Second, the induction of metallothionein (MT) (Chan *et al*., 2002). MT is involved in the regulation of essential metals, such as Cu and Zn for cell growth and development (Mackay *et al*., 1993). Metallothionein reacted to bind the uptake metals and stored them in detoxified forms (Viarengo *et al*., 1985). Although the detoxification mechanisms would not cause acute mortality, they would impose energetic costs to organisms through increased rates of metallothionein synthesis and subsequent excretion (Yap *et al*., 2002). Cockles utilize stored energy to meet this metabolic requirement for detoxification process. As a result, the declining energy stored is likely to be measured in its lower CI value.

These results indicated the increase in the percentage of CI reduction as compared to those in the control treatment. Since it can reflect changes in the nutrient state of the bivalve for the stored energy reserves and the animal's metabolic response to environmental stress (Peddicord, 1977), the CI of *A. granosa* is therefore a simple physiological index to evaluate its response to Cu and Zn stress.

CONCLUSIONS

This laboratory experimental study on the accumulation and depuration of Cu and Zn in *A. granosa* indicated two phenomena. Firstly, *A. granosa* (especially the soft tissues) is a potential biomonitor of Cu and Zn. It is proposed that the shells, together with the soft tissues of *A. granosa*, should be analysed to provide a better interpretation on the bioavailabilities of Cu and Zn. Secondly, the CI of *A. granosa* is a potential physiological indicator of Cu and Zn toxicities under laboratory conditions. However, field-based experiments using *A. granosa* should be conducted to assess the response of transplanted cockles to heavy metal pollution in mudflat intertidal areas.

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