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RESEARCH NOTES

MUDDY SEDIMENTS ACTING AS SINKS OF Cu AND Zn: EVIDENCE FROM A LABORATORY EXPERIMENTAL STUDY BY USING COCKLE *Anadara granosa* PLUS MUDDY SEDIMENTS

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Previous studies showed that red-blood cockle *Anadara granosa* was able to accumulate Cd and Cu to a significant high level in their tissues (Mat *et al*., 1994a, 1994b; Chan *et al*., 2002). However, little is known on the distribution of Cu and Zn in the soft tissues of *A. granosa* in the presence of muddy sediments, under laboratory conditions. In this study, sediment samples were analyzed because sediments have advantages to assess anthropogenic impacts on aquatic environments (Bryan & Langston, 1992). Sediment plays a major role in the transport and storage of metals (Salomons *et al*., 1987) and it can be used to identify sources of pollutants both spatially and temporally (Zwolsman *et al*., 1996; Birch *et al*., 2001). Furthermore, sediments can be used to locate the main sinks for heavy metals since these elements are persistent in the marine environment (Nriagu 1978; Hickey *et al.*, 1995). However, understanding the 'sediments can locate the main sinks for heavy metals' is sometimes difficult and very much dependent on how a researcher define it and again it is based on the researcher's comprehension. To understand that the sediment acts as a sink of heavy metals also needs evidence that can be provided through experimental studies based on proper experimental design.

Therefore, the objective of this study was to determine the accumulation and depuration of Cu and Zn by using *A. granosa* under laboratory conditions, between single metal exposure and single metal exposure plus muddy sediments, thus, to provide evidence that muddy sediments acting as sinks of Cu and Zn.

For the methodology, individuals of *A. granosa* were purchased from the wet market in Port Dickson, Negeri Sembilan, Malaysia (collected from Teluk Intan, Perak). Then, the cockles were transplanted to the muddy sediment areas at Pasir Panjang, Negeri Sembilan for 7-days acclimatization. The cockles were checked everyday and the cockles were considered dead if they showed no tactile stimulus and gaped their shell valves wide. Salinity and temperature were also checked every day. The Pasir Panjang area had a salinity range from 26 to 30 ppt and temperature range about 25 to 30C.

For laboratory experimental study, all of the cockles were transported to COMAS (Centre of Oceanograph and Marine Sciences), Universiti Putra Malaysia, Teluk Kemang, Port Dickson, Negeri Sembilan. Twenty cockles (shell lengths: 40-50mm) were collected for the analysis of background metal concentrations before the studies of accumulation and depuration. Some trials and errors experimental studies were conducted prior to the present study. Volume of seawater used for each experimental aquarium was 10 liters, based on our several preliminary studies (Yap *et al.*, 2010).

Healthy acclimatized cockles were selected for the accumulation and depuration studies of Cu and Zn. Sixty cockles (shell lengths: 40-50mm) were exposed in each plastic aquaria. For accumulation study, the cockles were exposed to individual 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. All experiments were conducted on single metal exposure. The sublethal metal concentrations used in the present study were based on our several preliminary studies (Yap *et al.*, 2010).

The test solutions (10 litres) were changed for every 2 days to new seawater with standard solutions of Cu and Zn at days 0, 2 and 4. The test water was

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constantly aerated and was held at room temperature (27-30ºC) and salinity 28-30 ppt. A control treatment with cockles but without the addition of metal solution was set up. At day 6, the cockles were transferred into the clean seawater aquarium for the depuration study. Samplings were conducted at days 2, 4 and 6 during the metal accumulation period and days 8 and 10 during the depuration period.

All samples were stored at -10ºC until metal analysis. The samples of cockles then were thawed at room temperature (27ºC) on a clean tissue paper to drain away the excess water. After that, the samples were dried for 72 h at 105ºC in an oven to constant dry weights. The samples were digested in concentrated nitric acid (Ajax Chemicals, HNO_3 65%, Australia) in a hot-block digester first at low temperature (40ºC) for 1 hour and then at high temperature (140ºC) for at least 3 hours (Yap *et al.,* 2003a). The digested samples were then diluted to a certain volume with double distilled water.

The prepared samples were determined for Cu and Zn by using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Model AAnalyst 800. The data were presented in µg/g dry weight. All the glassware used were acid-washed to avoid possible contamination and procedural blanks were analysed to check for contamination. Quality control samples were done by analysing standard solutions of Cu and Zn in every five samples to check for their recoveries to make sure their results were satisfactory (80-110%). To determine the level of significance between two variables, a t-test analysis was performed, by using STATISTICA 99" Edition (Version 5.5).

Bioconcentration factor (BCF) was to shows the availability of metals accumulation. It was calculated in relation to metal concentration in seawater according to Yap *et al.* (2003a):

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

Where,

Ce = metal concentration in the tissue at the end of metal exposure (µg/g dry weight);

Ci = metal concentration in the tissue before metal exposure (µg/g dry weight);

Cs = experimental metal concentration in the test seawater (mg/L).

Concentration factor (CF) was to shows the availability/ability to retain the accumulated metals. It was calculated at the end of depuration in comparison with the metal level before exposure as follows (Yap *et al.,* 2003a):

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

The rate of metal accumulation (RA) was calculated according to Yap *et al.* (2003a):

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

The rate of metal depuration (RD) was calculated according to Yap *et al.* (2003a):

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

Figure 1 shows the patterns of accumulation and depuration of individual Cu and Zn with and without muddy sediment (Cu+Mud and Zn+Mud) in the soft tissues of cockles. Six days were allowed for accumulation period and four days for the depuration period. The accumulation and depuration patterns of Cu and Zn with and without mud showed approximately the same pattern.

Table 1 shows the rates of accumulation and depuration of single metal and metal plus mud. It is found that, for single Cu exposure, the BCF and CF values, and all the rates of accumulations and depurations in all the days were significantly ($P< 0.05$) higher that those in Cu plus muddy sediment treatment. For Zn, the BCF value, rates of accumulations at days 4 and 6, and rate of depuration at day 8, were significantly ($P< 0.05$) higher than those in Zn plus muddy sediment treatment. Although CF value, rate of accumulation at day 2 and rate of depuration at day 10, were not significantly ($P> 0.05$) different between Zn and Zn plus mud, those values were still consistently higher than those in Zn plus muddy sediment treatment.

The results show that high metal levels were accumulated in the soft tissues during the accumulation period. The metal concentrations in the cockle soft tissue increased during the accumulation period and decreased during the depuration period. Although the test seawater concentrations were different between Cu (0.1 mg/L) and Zn (1.0 mg/L), we can conclude that cockles had high capability to accumulate Zn (highest rate; 37.98µg/g) than Cu (highest rate; 2.91µg/g) from the ambient water in the absence of muddy sediment, according to the rates of accumulation. This conclusion was also supported by the high BCF and CF values of cockles in their respectively conditions (Table 1). The rate of accumulation of metals with cockles plus muddy sediment was found lower when compared to the rate of accumulation with single metal exposure without addition of muddy sediment. Theoretically, when the metals were discharged into the environment, they were partitioned into solid and liquid phases. This was followed by the formation of chemical species by the binding of the metals with a variety of inorganic and organic ligands in the environment through the speciation process (Simkiss & Taylor, 1989). This will affect the bioavailability of the metals to the bivalves (Salomons *et al*., 1987) and will affect the amount of metals that can be accumulated by cockles. The addition of muddy sediments had decreased metal accumulation in the soft tissue of cockle. The metal ions are believed to be adsorbed onto the surface of the muddy sediments and were later brought down on the bottom aquariums. Fan and Wang (2001) reported that increasing the contact time of metals to sediment decreased assimilation of metals by clam. Yap *et al.* (2003b) noted that free cupric ions (Cu^{2+}) were strongly related to bioaccumulation of Cu. Most of test metals might bind and sink into the ligands or other organic compounds of the mud; this will reduce the free ions form and as a result it will reduce the uptake of metals by the cockles. According to a study reported by Roussieza *et al.* (2006) in the Gulf of Lions, they found that sediment dilution and particle sorting were among the factors that could cause most riverborne heavy metal contaminants rapidly reach natural levels when going seaward. This indicated that sediments (particulate sediments) can bind the metal ions from the water column and deposited to the bottom. This may indicate the sediment could act as a sink of heavy metals thus supporting the present finding.

In conclusion, the addition of muddy sediment into the exposure aquaria could possibly reduce the bioavailability of the metals to the cockle and would affect the amount of metals that could be accumulated in the cockle soft tissue. Therefore, the present experimental study provided direct evidence on the role of sediments acting as sinks for Cu and Zn. This is postulated that the bioavailability of trace metals to bivalves could be highly influenced by the muddy sediments in their habitats. Presence of muddy sediment might be important for the metal adsorption in the intertidal area. Together with the

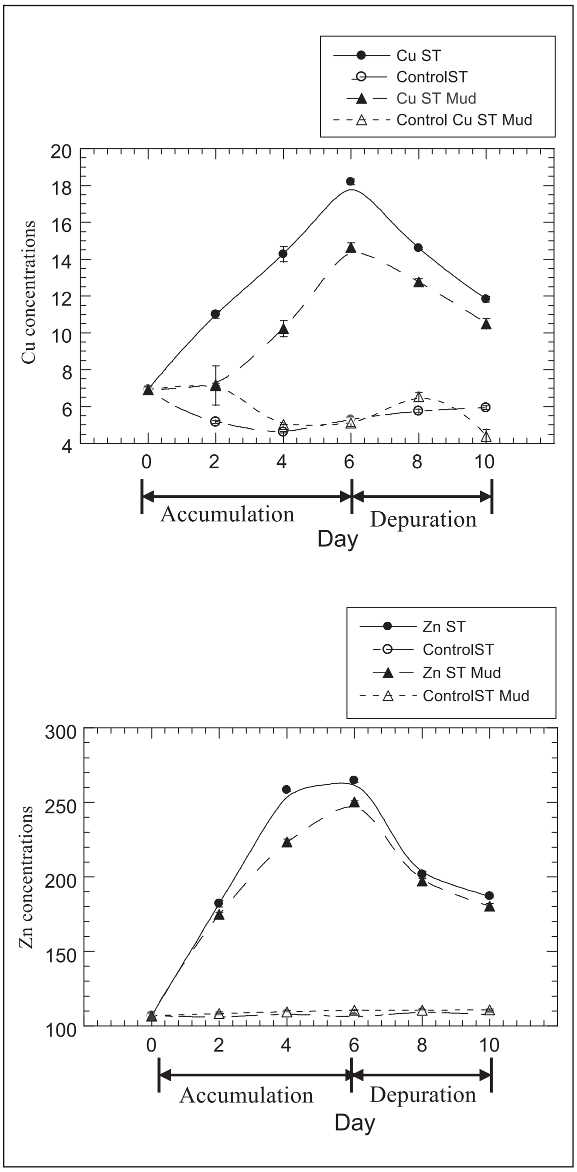

Fig. 1. Comparison of concentrations (µg/g dry weight [mean ± standard error]) of metals during accumulation and depuration periods in the total soft tissues (ST) of *Anadara granosa* between single metal exposure and single metal exposure plus muddy sediments.

Table 1. Bioconcentration factor (BCF) and concentrations factor (CF) and rates of accumulation (RA) and depuration (RD) (µg/g per day) of Zn in the total soft tissues of cockles exposed to 1.0 mg/L of Zn and 1.0 mg/L of Zn plus muddy sediment (Zn+Mud) in test seawater, of Cu in total soft tissues of cockles exposed to 0.1 mg/L of Cu and 0.1 mg/L of Cu plus muddy sediment (Cu+Mud) in test seawater

Treatments	BCF	CF	RA			RD	
			Day 2	Day 4	Day 6	Day 8	Day 10
Cu	**112.8**	**1.71**	**2.91**	**2.42**	**2.15**	**1.81**	**1.60**
Cu + Mud	77.2	1.52	0.03	1.30	1.60	0.94	1.04
Zn	**158**	1.75	37.9	**37.6**	**26.4**	**31.4**	19.4
Zn + Mud	144	1.69	33.3	28.5	23.3	26.6	17.5

Note: Values in bold indicated that the metal is significantly ($P< 0.05$) higher than those in metal plus muddy sediment (mud), using statistical T-test analysis.

estuarine circulation and biotic interactions, muddy sediment could play an important role in the metal retention between the water and sediment in the intertidal zone.

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