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Effect of Body Size on Heavy Metal Contents and Concentrations in Green-Lipped Mussel *Perna viridis* (Linnaeus) from Malaysian Coastal Waters

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ABSTRACT

The concentrations of cadmium, copper, zinc and lead, in the total soft tissues of green-lipped mussel *Perna viridis* of a wide range of sizes (2-11 cm), were determined from a population at Pasir Panjang. The metal contents (µg per individual) and concentrations (µg per g) of cadmium, lead, copper and zinc were studied in *P. viridis* to find the relationships with body sizes. Smaller and younger mussels showed higher concentrations (µg per g) of Cd, Pb and Zn than the larger and older ones. The results of the present study showed that the plotting of the metal content, against dry body flesh weight on a double logarithmic basis, gave good positive straight lines; this observation is in agreement with Boyden's formula (1977). This indicated that *P. viridis* showed a different physiological strategy for each metal being studied, which is related to age.

Keywords: *Perna viridis*, metal contents, metal concentrations, shell length, total dry body weight, shell thickness

INTRODUCTION

The green-lipped mussel, *Perna viridis,* is an established biomonitor of heavy metal contamination in the coastal waters of Asia-Pacific (Tanabe, 2000), and particularly in Malaysia (Yap *et al.*, 2003; 2006). From the literature, the heavy metal concentrations measured in the soft tissues of mussels could be used as biomonitors of heavy metal bio-availabilities and contamination in the coastal environment, in which the mussels live (Yap *et al.*, 2006). However, the accumulation of heavy metal concentrations in the tissues of mussels is also affected by a number of intrinsic and extrinsic factors. The extrinsic factors include spawning season (Dare and Edwards, 1975; Phillips, 1980; Lobel *et al.*, 1991) and mussel size (Boyden, 1977; Cossa *et al.*, 1980; Williamson, 1980; Lobel and Wright, 1982; Propham and D'Auria, 1983; Lobel *et al.*, 1991; Riget *et al.*, 1996).

Previous studies revealed that the body size might change the heavy metal uptake due to the changes in the kinetic steady-states as mussels grew (Lobel *et al.*, 1991; Riget *et al.*, 1996). Obviously, body size would affect metal bioaccumulation in the rates of uptake and excretion (Phillips and Rainbow, 1993). Besides, the effects of the body size on different physiological rates such as pumping, filtration and respiration, have been reported in the mussel, *Mytilus edulis* (Winter, 1978; Mφhlenberg and Riisgard, 1979; Bayne and Newell, 1983; Jones *et al.*, 1992).

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The relationships between heavy metal concentrations and body sizes in temperate molluscs have been well documented (Boyden, 1974, 1977; Simpson, 1979; Cossa *et al.*, 1980; Lobel and Wright, 1982), but such information for tropical and sub-tropical species is rather limited. Therefore, the present study aimed to provide comparative information in understanding the physiological strategies for the accumulation of Cd, Pb, Cu and Zn in relation to body size of a tropical mussel. The objective of the present study was to investigate the effects of the length, thickness and total dry flesh body weight of shells on metal contents (µg per individual) and concentrations (µg per g) in *P. viridis.*

MATERIALS AND METHODS

Mussels, having a wide range of body sizes (2-11 cm), were collected from a local mariculture site in Pasir Panjang between August and October, 1998. All samples were stored at -10°C until metal analysis was conducted. The samples were thawed at room temperature, on a clean tissue paper, with the posterior margins placed downwards to drain away the excess water (Chan, 1988). The total soft tissues were carefully removed by de-shelling the mussels with a stainless steel knife. The dry weight of the soft tissues was determined by drying the whole soft tissues individually for at least 72 hours at 105°C to constant weights (Mo and Neilson, 1994). The samples were pooled to get enough samples for the metal analyses.

The samples were digested in concentrated nitric acid (Yap *et al.*, 2003, 2006). They were placed in a hot-block digestor at a low temperature (40°C) for 1 hr, and digested at a high temperature (140°C) for 3 hrs. The digested samples were then diluted to a known volume with double distilled water. After filtration, the prepared samples were subjected to Cd, Cu, Pb and Zn analyses in an air-acetylene flame atomic absorption spectrophotometer (Perkin-Elmer Model 4100). The data are presented in µg g^{-1} dry weight basis. To avoid possible contamination, all glassware and equipment used were acid-washed, and the accuracy of the analysis was checked using the blank and standard addition testing procedure. The percentages of recoveries for heavy metal analyses were found to be 92% for Zn, 110% for Cd, 92.5% for Pb and 96% for Cu. The samples were also checked with the Certified Reference Material for Soil (International Atomic Energy Agency, Soil-5, Vienna, Austria). Standard solutions were prepared from 1000 mgL^{-1} stock solutions of each metal (MERCK Titrisol).

The effects of body size on heavy metal concentrations were investigated in the mussels using a linear regression analysis of logarithmic transformed data (Boyden, 1974, 1977). Regression analyses are better interpretations than simple correlations because they can predict fluctuation, shape of curve and accuracy between variables; whereas the degree of closeness between X and Y is not easy to grasp (Snedecor and Cochran, 1979). Boyden (1974, 1977) suggested that plotting the metal contents (µg per individual) or concentrations (µg g^{-1}) against body size on double logarithmic scales generally produces a straight-line relationship which can easily be defined using an equation, thus:

$$\text{Log (metal)} = \text{Log(a)} + \text{b Log(body size)}.$$

where (a) = intercept and (b) = slope. In the present study, body sizes (flesh dry weight, shell length and shell thickness) were plotted against metal contents and metal concentrations of the tested animal.

Shell thickness, which is considered as an age measure (Cossa *et al.*, 1980; Frew *et al.*, 1989), was calculated according to the following formula:

$$\text{Shell Thickness } (g.cm^{-3}) = \frac{\text{shell weight (g)}}{\text{shell length (cm)} \times \text{shell height (cm)}}$$

RESULTS AND DISCUSSION

Fig. 1 shows that all metal contents (Y) (µg per individual) were related to body dry flesh weights (X) by the following relation, $Y= aX^b$ [$Log(Y)= a + bLog(X)$], where $b< 1$. According to Boyden (1977), by plotting metal contents (Y) (µg per individual) against body size, an equation with a slope (b) less than 1 is explained by larger individuals containing less metal, and a logarithmic transformation of this equation yields a straight-line relationship. The results of present study are similar to those of other studies carried out in blue mussels, snails, fish and clams (Table 1). Cd showed the weakest relationship with an increasing dry flesh body weight ($r= 0.62$, $p< 0.01$) and it had a similar concentration (Y) (µg per g) to that of a study on mussel *M. edulis* which reported a coefficient correlation of 0.65 (Cossa *et al.*, 1980). In other aquatic organisms, this non-essential metal (Cd) was not regulated and kept accumulating until it reached a threshold level (Kraak *et al.*, 1994; Lukyanova *et al.*, 1993; Tessier *et al.*, 1994). The Cu content was positively correlated ($r= 0.94$, $p< 0.001$) with the increasing body weight. Similar positive relationships for Cu were also reported for *M. edulis*, *Mercenaria mercenaria* and *Venerupis decussata* (Boyden, 1974).

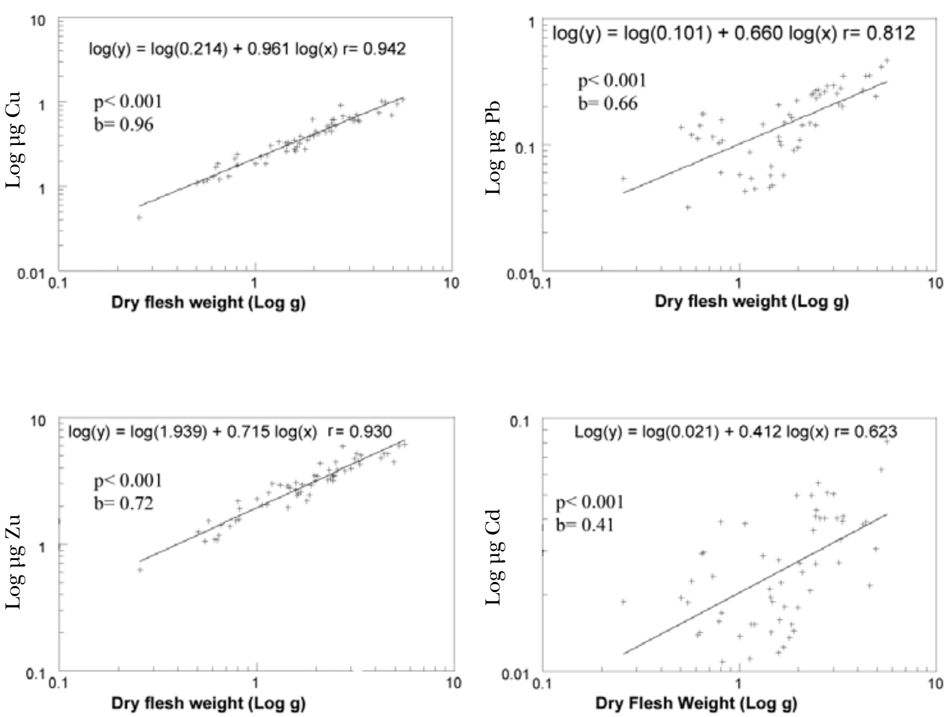

Fig. 1: The relationships between Cd, Cu, Pb and Zn content (log µg) and the total soft tissue dry weight (log g) of mussel P. viridis *from Pasir Panjang, Port Dickson, (with a positive regressive equation as $log(Y)= log(a) + b\, log(X)$, $0 < b< 1$)*

Fig. 2 shows the double logarithmic transformations, in which metal concentrations (Y) (µg per g) were found to be related to the length and shell thickness of shell, as X by the relation $Y = aX^b$, where the slopes (b) were negative. Boyden (1977) described this equation, by plotting metal concentrations (Y) (µg per g) against body size, as having negative slopes (b) when larger mussels were indicated to accumulate less heavy metal as compared to smaller ones. The concentrations of Cd and Zn showed significant ($p < 0.001$) decrease with the increasing shell length ($r = -0.69$ and $r = -0.65$) and thickness ($r = -0.68$ and $r = -0.66$).

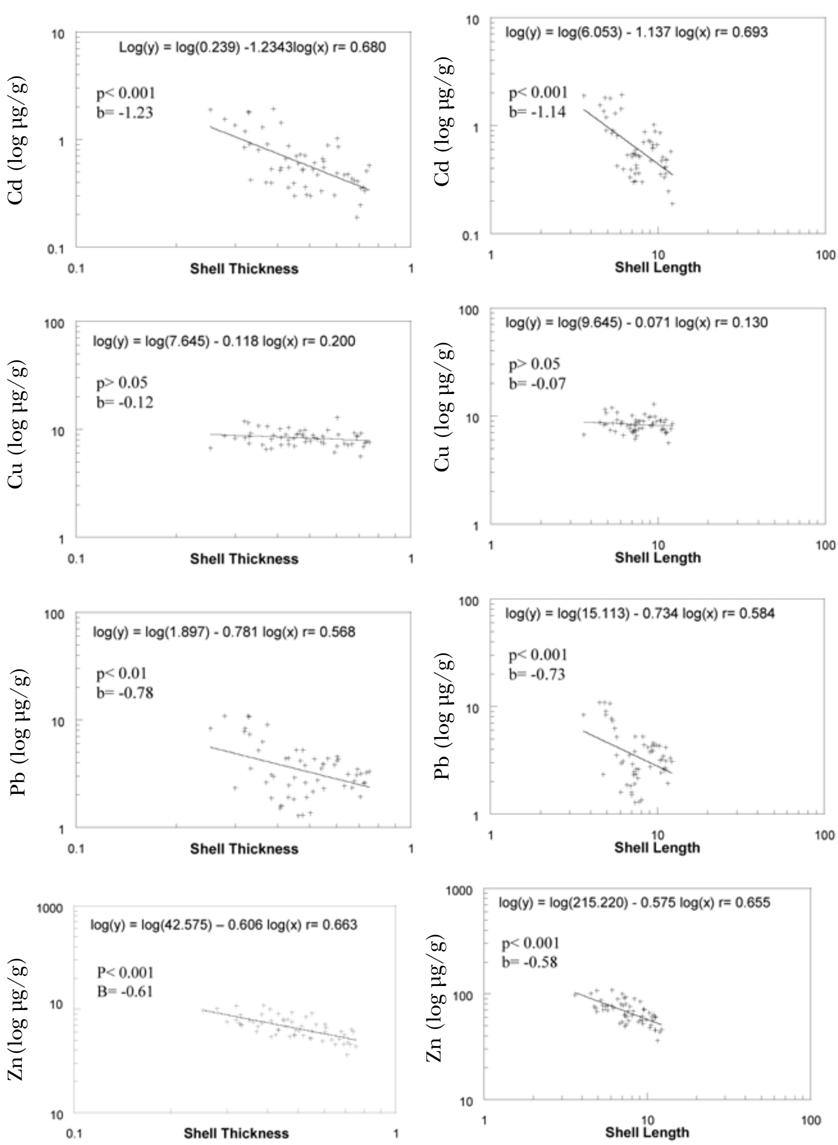

Fig. 2: The relationships between the concentrations of Cd, Cu, Pb and Zn (log µg.g^{-1}) and the thickness (log g.cm^{-2}) and length (log cm) of mussel P. viridis *from Pasir Panjang, Port Dickson (with negative regressive equation as log(Y)= log(a) - b log(X), b< 0)*

Pb showed an intermediate decrease with the increasing shell length (r= -0.58) and shell thickness (r= -0.57). The concentration of Cu illustrated an insignificant ($p > 0.05$), weak and decreasing relationship with the shell length (r= -0.13) and thickness (r= -0.20).

Boyden (1977) explained that the metal concentrations in older mussels would be lower when compared to the younger ones *(Fig. 1)*. The results could be due to the older individuals which had experienced longer terms of exposure, as compared to younger mussels. Further studies should be conducted to prove this hypothesis.

In previous studies, many authors reported negative relationships between body size and the accumulation of aquatic contaminants in suspension-feeding bivalves (Williamson, 1980; Amiard *et al.*, 1986; Martincic *et al.*, 1992). For instance, Williamson (1980) reported that higher levels of Cd, Pb and Zn were found in smaller individual snails, and suggested that this might be due to the variations in the metabolic activities at different ages of the organisms. He also suggested that the increase in metabolic rates, in relation to different body sizes, might affect the uptake and elimination of metal. The same inverse relationships between the body size and heavy metal accumulation were also observed in *Penaeus stylirostris* (Paez-Osuna and Ruiz-Fernandez, 1995) and *Corbicula fluminea* (Bilos *et al.*, 1998). Swaileh and Adelung (1994) reported that smaller individual ocean quahog *Arctica islandica* had higher concentrations of Zn than the bigger individuals. Bilos *et al.* (1998) showed significant relationships, between the body size and concentrations of Cu (positive) and Zn (negative), in clam *Corbicula fluminea.*

TABLE 1

The comparison of the correlation coefficient (r) for the metal content (μg per individual) versus the total dry soft tissue weight (g) from other studies

Species	Metals	r value	Reference
Mytilus edulis (mussel)	Cu	0.80	Boyden (1977)
Mytilus edulis (mussel)	Zn	0.85	Boyden (1977)
Mytilus edulis (mussel)	Cu	0.86	Cossa *et al.* (1980)
Mytilus edulis (mussel)	Zn	0.86	Cossa *et al.* (1980)
Mytilus edulis (mussel)	Cd	0.65	Cossa *et al.* (1980)
Cepaea hortensis (snail)	Cd	0.26	Williamson (1980)
Cepaea hortensis (snail)	Pb	0.65	Williamson (1980)
Cepaea hortensis (snail)	Zn	0.82	Williamson (1980)
Macoma balthica (clam)	Cu	0.96	Bordin *et al.* (1992)
Macoma balthica (clam)	Zn	0.98	Bordin *et al.* (1992)
Macoma balthica (clam)	Cd	no correlation	Bordin *et al.* (1992)
Arius thalassinus (fish)	Cu	0.92	Law and Singh (1991)
Arius thalassinus (fish)	Zn	0.76	Law and Singh (1991)
Arius thalassinus (fish)	Pb	0.60	Law and Singh (1991)
Perna viridis (mussel)	Cu	0.94	The present study
Perna viridis (mussel)	Zn	0.93	The present study
Perna viridis (mussel)	Pb	0.81	The present study
Perna viridis (mussel)	Cd	0.62	The present study

Since Cu and Zn are essential metals involved in several enzymatic systems, which may display partial regulation in mussels (Timmermans, 1993; Kraak *et al.*, 1994;), the relationships between body sizes (shell length and shell thickness) and concentrations of these metals observed in the mussel *P. viridis* strongly suggested different physiological requirements with size, which might be related to age.

The results gathered in the current study suggested that there might be differences in physiology between young and older mussels. Since large and aged mussels tended to pump less water, through their bodies per unit of body weight, the uptake of metals was lower than that in smaller individuals. The surface area to volume ratios decreased with size, and this affected the relative contribution of the adsorbed metal content to the total body burden of heavy metals (Cossa *et al.*, 1980; Swaileh and Adelung, 1994). Therefore, the decrease in metal concentrations with body size indicated that a significant proportion of the metal content was surface-adsorbed as smaller mussels have a larger surface area to volume ratio (Jones *et al.*, 1992). As the concentrations of heavy metal (µg per g) decreased with an increase in the body size (length and thickness of shell); this indicated that the chemical pollutants were more concentrated in young mussels due to their faster growth rate (Cossa *et al.*, 1980; Olafsson, 1986).

CONCLUSIONS

The relationships between the accumulation of heavy metal and size were observed and the correlation coefficients were found to be different between the heavy metals examined. The findings of the present study revealed that younger and smaller *P. viridis* accumulated higher concentrations of Cd, Cu, Pb and Zn. Therefore, the factor of body size should be taken into consideration in designing experiments, such as the "Mussels Watch" program in order to enhance the interpretation of ecotoxicological results, especially when these results were based on the comparisons between different mussel populations and different size groups or ages. This can be done in a number of ways; these include sampling a restricted size range of mussels at every sampling station. However, this is not a realistic approach as according to Lobel *et al.* (1991), every locality has a location-dependent maximum size. Further studies should focus on the establishment of statistical constant for each metal and the concentrations of heavy metal in different size groups, which should be normalised before valid interpretations of ecotoxicological biomonitoring data can be made.

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