

The Distribution of the Heavy Metals (Cu, Pb and Zn) in the Soft and Hard Tissues of the Green-Lipped Mussel *Perna viridis* (Linnaeus) Collected from Pasir Panjang, Peninsular Malaysia

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ABSTRACT

The distributions of Cu, Pb, and Zn in the different soft Pasir Panjang, Peninsular tissues, shell sections and layers of the green-lipped mussel *Perna viridis* from an area with unpolluted water in Malaysia, were studied. The soft tissues analysed were the byssus, mantle and gills, posterior adductor muscle, retractor byssal muscle, foot, crystalline style, gonad and the remaining visceral mass. The level of Cu in the crystalline style was significantly ($P < 0.05$) elevated when compared to the other soft tissues while elevated levels of Cu, Pb and Zn were found in the byssus. The byssus is therefore a more sensitive material for Cu, Pb and Zn. The heavy metal concentrations in the different sections of the mussel's shell layers were also differed. The level of Pb was significantly ($P < 0.05$) higher in the inner shell layer than in the periostracum layer while Cu and Zn concentrations were significantly ($P < 0.05$) higher in the periostracum layer than in the inner shell layer. Copper, Pb and Zn were evenly distributed within the different sections of the inner shell layer with no significant ($P > 0.05$) difference in the concentrations of these metals in the different sections. The periostracum shell layer was found to be a more sensitive biomonitoring material for Cu and Zn than the inner shell layer.

INTRODUCTION

In a 'Mussel Watch' approach, the determination of accumulated concentrations of heavy metals by using the total soft tissues in the mussels as integrated measures of ambient metal bioavailabilities had been a common practice (Goldberg, 1975, 1980; Goldberg *et al.*, 1978; Phillips, 1980, 1985, 1991; Phillips and Segar, 1986; Phillips and Rainbow, 1993; Rainbow and Phillips, 1993). However, the metal concentrations in the total soft tissues of the organism may not be accurately reflective for certain contaminant concentrations in individual target tissues of the organism. This argument was based on the fact that different tissues accumulate metals at different rates and that the biological half-lives of metals at each type of soft tissue also differ from one another (Lakshmanan and Nambisan, 1989). This is due to the different capacities of the cells in each type of tissue to eliminate the metals bound to the binding sites of the

metallothioneins (Viarengo *et al.*, 1980, 1985). This useful detoxification mechanism is why certain bivalves could survive with elevated levels of heavy metals accumulated in their soft tissues. The metallothioneins and granules could prevent interference by heavy metals of the basal metabolic roles and damage to the cellular structures (Viarengo *et al.*, 1985). This is because metals bound to metallothioneins represent a non-toxic form of the metal itself as shown by several researchers (Roesijadi and Robinson, 1994; Rainbow, 1997). Since each soft organ may play a different role either in its metabolic or physiological function, this may influence the distribution of metals in the different soft tissues of mussels. As a result, the metal regulation and detoxification processes could also be different. Knowledge on metal distributions in the soft tissues may help us to understand the processes involved in the uptake and excretion of metals in the different soft tissues of *P. viridis*.

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The distribution of metals in the hard tissues (periostracum and inner shell layers) of *P. viridis* was also studied. The whole shell of *P. viridis* consists mainly of the inner shell layers (prismatic and nacreous) with very superficial layer of organic periostracum layer that covers the inner shell layers (Kennedy *et al.*, 1969; Bubel, 1976). According to Phillips (1980), the adsorption of metals onto the shell surface caused difficulty in using mussel shell as a biomonitoring material for heavy metals. Several authors had investigated the suitability of mussel shell as a biomonitoring material for heavy metals (Pilkey and Goodell, 1963; Bertine and Goldberg, 1972; Goldberg, 1975, 1980; Imlay, 1982; Koide *et al.*, 1982; Szefer and Szefer, 1985; Dermott and Lum, 1986; Bourgoin, 1987). Foster and Chacko (1995) suggested that shells could accumulate a wide range of metals to varying extents. The accumulation of heavy metals in the shells had prompted some investigators (Carriker *et al.*, 1980, 1982; Koide *et al.*, 1982; Al-Dabbas *et al.*, 1984) to develop mollusks shells as a biomonitoring material for heavy metals. They believed that the chemical composition of the shells might be a good reflection of the environmental levels of heavy metals. Several researchers (Stureson, 1976, 1978; Carell *et al.*, 1987) also suggested the possible use of shell material as a permanent record of environmental changes in heavy metal contamination.

This study aimed to determine the distribution of Cu, Zn and Pb in the different soft tissues of the green-lipped mussel *P. viridis* from an unpolluted area of Malaysian coastal waters and to identify the potential biomonitoring organ for further studies. Besides that, the levels of heavy metals in the different layers and sections of the hard tissues (shell) of the mussel *P. viridis* were also studied.

MATERIALS AND METHODS

Samples of *P. viridis* were collected from an area of unpolluted water off the west coast of Peninsular Malaysia (Pasir Panjang). All samples were stored in polyethylene bags at -10°C until analysis. Before dissection, the samples were thawed at room temperature on a clean tissue paper with the posterior margins downward to drain away excess water. About 30 individuals of a similar size group (shell length 7-8 cm) were selected for metal analysis. The total soft tissues of *P. viridis* were carefully removed by deshelling

the mussels with a stainless steel knife. The soft tissues were then dissected into 8 parts namely byssal threads, mantle and gills, posterior adductor muscle, retractor byssal muscle, foot, crystalline style, gonad and remaining visceral mass. The latter fraction contained about half of the total weight of the mussel. Three parts (mantle and gills, gonad and remaining visceral mass) were analysed by sex. The sex of *P. viridis* was distinguished based on the colour of the gonads (Yap *et al.*, 1979). The different soft tissues were pooled in order to get enough samples for metal analysis. Excess water was pressed on a filter paper. The fresh tissue was then ready for further analysis.

The shells were cleaned under a jet of tap water to remove the algae, barnacles, mud and bryozoa encrusted on them. All samples were rinsed with double distilled water (ddw) and 0.5% HNO₃, and dried for 72 hours at 105°C to a constant weight (Mo and Neilson, 1994). In order to separate the outermost layers (periostracum layers) of the shells, the shells were cooled at room temperature after heating at 105°C. While the shells were cooling, most of the periostracum on the lip part of the shell cracked and fell off (Puente *et al.*, 1996). The different sections of the inner nacreous layer, as shown in Fig. 1, were analysed. These shell sections included the umbo, green-lipped part, ligament, ventral and dorsal shell parts. Pestle and agate were used to crush the hard inner nacreous shell layers into smaller pieces. All samples were weighed with an accuracy of 0.1 mg before acid digestion.

All samples were digested in concentrated HNO₃ (AnalaR grade, BDH 69%). They were placed in a hot-block digester first at low temperature for one hour and then they were fully digested at high temperature (140°C) for at least 3 hours. The digested samples were then diluted to a certain volume with ddw. After filtration, the prepared samples were determined for heavy metals by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model 4100. The data are presented in either mg/g wet or dry weight basis. To avoid possible contamination, all glassware and equipment used were acid-washed and the accuracy of the analysis was checked against the procedural blanks and standard addition testing procedure. The percentages of recoveries for the heavy metal analyses were

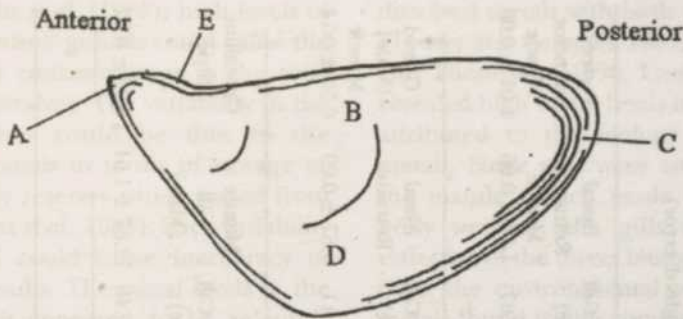


Fig. 1: Different sections of the shell of the mussel *P. viridis* used in analysis (A: the tip and the anterior shell or umbo; B: near dorsal shell part; C: green-lipped part posterior of the shell; D: near ventral shell part; E: ligament). Note: Periostracum is not shown in this drawing because this outer shell layer can only be shown in green color on the outer layer of the shell

96% for Cu, 92.5% for Pb and 92% for Zn. One-way ANOVA Student-Newman-Keuls test (Day and Quinn, 1989) was used to elucidate where differences occurred. All the comparisons were made at the 95% ($P < 0.05$) level of significance.

RESULTS AND DISCUSSION

Different Soft Tissues

The heavy metal concentrations in each soft tissue analysed were shown in Table 1. The distributions of Cu, Pb and Zn were determined from mussel samples collected from the field. 'Why did the metals distribute unevenly in the different soft tissues?' is an interesting question. Some tissues accumulated significantly higher ($p < 0.05$) levels of Cu, Pb and Zn than the other soft tissues. This could be due to each organ playing a different role in metabolism and physiological functions. The results indicated that the regulation and detoxification of metals were also different in each soft tissue.

The crystalline style accumulated significantly ($p < 0.05$) higher levels of Cu when compared to the other soft tissues (Table 1). High levels of Cu in the crystalline style may be due to a metabolic function in the digestive system of *P. viridis*. The crystalline style is the main organ of the digestive alimentary canals (Morton, 1992). It functions in the detoxification of metals and has other enzymatic activities (Phillips and Rainbow, 1993). Selvarani *et al.* (1989) revealed that the crystalline style in *P. viridis* exhibited strong amylase activity which was double that of the digestive diverticula. Since Cu is an essential element, most of the Cu was bound to metallothionein and therefore the Cu level in the crystalline style was high.

Generally, the byssus of *P. viridis* accumulated high levels of Cu, Pb and Zn when compared to the other soft tissues. This indicated that the materials available for the production of the byssal threads contained a lot of metal wastes. Cheung and Wong (1992) also recorded high metal concentrations in the byssus, particularly for Cu and Cd. This result indicated that the byssus could have been used for metal excretion (Pentreath, 1973; Goldberg *et al.*, 1978). The formation of the byssal threads requires the expenditure of a substantial amount of energy and material (Coomb and Keller, 1982). The byssus is excreted from the byssal glands at the foot and is composed of hard tanned protein (Ikuta, 1986). The byssus of *Mytilus* spp. was found to be a good biomonitoring material for Cu (Szefer *et al.*, 1997) and Hg (Szefer *et al.*, 1999).

The metal levels in the foot were lower than in the byssus and other soft tissues (Table 1). The low levels of metals in the foot are expected since this material is probably acting as a channel to transport all the waste materials to the byssal threads (Morton, 1992). Thus, the foot would hardly accumulate any metal. In addition, the foot has a lower volume of surface contact when compared to the gills.

The gonad accumulated considerable metal levels when compared to the other tissues (Table 1). The gonadal tissue is usually related to the condition of the mussel in that the physiological condition of the mussel is reflected through the amount of gonads merged with the mantle (Peddicord, 1977). In this study, the female gonad accumulated higher Pb and Zn concentrations than the other soft tissues.

TABLE 1
Student-Newman-Keuls (SNK) comparisons of Cu, Pb and Zn concentrations (means mg/g wet weight \pm standard error)
in different tissues of the mussel *P. viridis*

Organs	Crystalline Style	Byssus	Gonad (Male)	Gonad (Female)	Remaining Visceral Mass (Female)	Remaining Visceral Mass (Male)	Mantle + Gill (Male)	Foot	Mantle + Gill (Female)	Byssal Retractor Muscle	Posterior Adductor Muscle
Means \pm SE Cu	6.36 \pm 1.13	4.57 \pm 0.54	2.64 \pm 0.08	2.63 \pm 0.19	2.46 \pm 0.07	2.36 \pm 0.14	2.13 \pm 0.34	1.76 \pm 0.05	1.50 \pm 0.07	1.13 \pm 0.06	1.01 \pm 0.05
Organs	Byssus	Remaining Visceral Mass	Gonad (Female)	Remaining Visceral Mass (Female)	Foot (Male)	Posterior Adductor	Crystalline Style Muscle	Mantle + Gill (Male)	Mantle + Gill (Female)	Byssal Retractor	Gonad (Male) Muscle
Means \pm SE Pb	2.70 \pm 0.13	1.38 \pm 0.10	1.10 \pm 0.07	1.07 \pm 0.10	0.93 \pm 0.13	0.85 \pm 0.24	0.79 \pm 0.09	0.71 \pm 0.04	0.69 \pm 0.11	0.67 \pm 0.09	0.59 \pm 0.14
Organs	Remaining Visceral mass (Female)	Gonad (Female)	Byssus	Remaining Visceral Mass (Male)	Posterior Adductor Muscle	Gonad (Male)	Byssal Retractor Muscle	Foot	Mantle + Gill (Female)	Mantle + Gill (Male)	Crystalline Style
Means \pm SE Zn	23.98 \pm 1.17	21.79 \pm 0.83	19.56 \pm 2.11	17.25 \pm 0.75	16.59 \pm 0.60	16.01 \pm 1.28	15.87 \pm 0.32	14.39 \pm 0.76	14.19 \pm 0.46	12.87 \pm 1.01	3.92 \pm 0.32

Note: Means not differing significantly at $P < 0.05$ are indicated by a line under the corresponding values.

According to Wright *et al.* (1985), high levels of heavy metals in bivalves' gonads could cause the variability of metal concentrations in the total soft tissues of the bivalves. The variability in the metal accumulation could be due to the development of gonads in terms of storage or depletion of energy reserves which varied from time to time (Wright *et al.*, 1985). Such variability in levels of metal could cause inaccuracy in ecotoxicological results. The metal levels in the gonad of *P. viridis* appeared to be relatively higher than in the rest of the soft tissues analysed which corroborates the findings of Yang and Thompson's (1996). Yang and Thompson (1996) demonstrated that the distribution of Cu in the different organs of the mussel *P. viridis* was in the order gill > gonad > visceral mass > mantle > muscle whereas for Zn it was visceral mass > gonad > gill > muscle > mantle. The higher metal levels in the female's gonads compared to the male's gonads especially for Zn was perhaps due to gamete production in the female gonads that contained stored reserves with more metals (Bayne *et al.*, 1982). The metal levels in the other soft tissues were relatively similar and were not significantly ($P > 0.05$) different from one another.

Table 1 also shows that the metal concentrations in the remaining visceral mass were relatively higher than those in the other soft tissues. The relatively higher concentrations of Zn and Pb found in the remaining visceral mass may be due to the high rate of uptake and the subsequent loss of these metals in the fecal materials. This remaining visceral tissue also included the hepatopancreas and the kidneys which could contribute to the high metal concentrations. This part also contained a lot of plankton and algae which were kept in this part before further digestion. Therefore, it reflected the main food of mussels obtained through feeder-filtering activities which directly contributed to the metal uptake of the mussels.

Mantle and gills showed relatively low levels of metal concentrations when compared to other soft tissues (Table 1). This may indicate that the mussels from this population had relatively little gonads that merged with the mantle since the gonads accumulated considerable amount of metals. Since mantle and gill are in contact with large volumes of water necessary for feeding and respiration, the metals detected in these organs probably resulted from the interaction of

dissolved metals with both sediment and mussel (Tessier and Campell, 1987). Some studies (Yang and Thompson, 1996; Lares and Oriens, 1997) revealed high metal levels in the gills which were attributed to the biology and metabolism of metals. Since gills were analysed together with the mantle (which made up most of the soft body weight), the gills therefore were less reflective of the direct biological uptake of metals from the environmental seawater. As a result, metals found in the combination of mantle and gills were mainly due to the mantle since it made up the bulk the weight.

The distribution of metals in different soft tissues of *P. viridis* could also be due to the different biological half-lives of Cu, Pb and Zn. These were related to the differing capacities of the cells to eliminate the metals bound to metallothioneins (Viarengo *et al.*, 1980, 1985). The fact that the three metals, had been sequestered in different concentrations in different soft tissues, was probably due to the fact that these metals and the metallothioneins associated with them followed different biochemical pathways. Therefore, the determination of metals in the different soft tissues can be used as a method to monitor the detoxified forms of metals which had previously been accumulated in these tissues under field conditions.

Shell

For the shell of *P. viridis*, varying distributions of Cu, Pb and Zn were also found in different sections of the inner shell layer but the differences were not significant ($P > 0.05$) (Table 2). The SNK tests for the comparisons are shown in Table 2. Concentrations of Cd, Pb and Zn in the periostracum layer were significantly different ($P < 0.05$) from the concentrations in the other sections of the inner layer. Cu and Zn concentrations in the periostracum layer were significantly higher ($P < 0.05$) than in the other inner sections while Pb was the least accumulated metal in the periostracum layer being present there in a significantly lower concentration than in the inner shell layer. It is believed that there was incorporation of Cu and Zn through the surface of the periostracum. Moreover, the periostracum layer is more vulnerable and susceptible to the metal levels in the surrounding waters since it is the outermost layer of the shell. Apart from the direct adsorption pathway, the

TABLE 2
Student-Newman-Keuls (SNK) comparisons of Cu, Pb and Zn concentrations (means mg/g dry weight \pm standard error) in different sections in the shell of mussel *P. viridis*

Different sections Mean \pm SE Cu	Periostracum 12.02 \pm 0.31	Green-lipped 6.36 \pm 0.18	Ligament 6.20 \pm 0.20	Umbo 5.73 \pm 0.23	Ventral part 5.57 \pm 0.11	Dorsal part 5.55 \pm 0.19
Different sections Mean \pm SE Pb	Ligament 29.49 \pm 0.87	Green-lipped 28.65 \pm 1.44	Umbo 27.43 \pm 1.01	Ventral part 26.04 \pm 0.62	Dorsal part 25.96 \pm 0.83	Periostracum 10.87 \pm 0.75
Different sections Mean \pm SE Zn	Periostracum 13.78 \pm 1.80	Ligament 4.94 \pm 0.51	Green-lipped 3.76 \pm 0.30	Umbo 3.69 \pm 0.24	Dorsal part 2.90 \pm 0.44	Ventral part 2.68 \pm 0.10

Note: Means not differing significantly at $P < 0.05$ are indicated by a line under the corresponding values.

high concentrations of Cu and Zn in the periostracum layer could also be attributed to the biomineralization mechanism from the mantle of *P. viridis*. The periostracum layer's higher Cu and Zn levels might be due to the newly secreted extrapallial fluid which contained the components for biomineralization (Puentes *et al.*, 1996). This biomineralization composition might also contain heavy metals since the concentrations of Cu and Zn were higher in the soft tissue of *P. viridis*.

The inner shell layer exhibited insignificant differences ($p > 0.05$) in Cu, Pb and Zn concentrations amongst all sections in this shell layer although the metal distributions were not similar. The different distributions of chemical elements within the microstructure groups and mineralogical layers of the shell could be understood by looking at the chemical properties of trace elements in the shells (Carriker *et al.*, 1991). These chemical properties can provide a series of natural probes for the calcification system as well as fundamental information on the processes involved (Simkiss, 1983). Earlier, many researchers (Phillips, 1980; Wilbur and Saleuddin, 1983; Carriker *et al.*, 1991) had indicated that the heterogeneous distribution of metals in bivalve shell is a normal phenomenon. The complex polylayer of the shell structure in mussels further increased the potential for mineralogical and chemical variations.

Several authors (Wilbur and Saleuddin, 1983; Puentes *et al.*, 1996) had suggested the use of mollusc shells as biomonitoring materials. This is due to the fact that any trace metal which is actively incorporated into the shell matrix during shell growth must have been assimilated by the mussels (Wilbur and Saleuddin, 1983). According to Watson *et al.* (1995), some trace metals are incorporated into the shells of molluscs and barnacles through substitution of the calcium ion in the crystalline phase of the shell or they are associated with the organic matrix. The mineralogy and chemistry of the shell material secreted by organisms can vary with the environment of growth (Dodd, 1963). Al-Dabbas *et al.* (1984) suggested that shell composition is sufficiently sensitive to environmental variation that the environments can be distinguished within a limited water system.

CONCLUSION

The results showed tissue distribution of Cu, Pb and Zn in both the soft and hard (shell) tissues of *P. viridis*. The metal distribution in the different soft tissues could be due to different mechanisms of regulation, detoxification and physiological functions in each soft tissue. For example, specific organs such as the crystalline style, gill and byssus were identified to be potential biomonitoring organs for Cu, Pb and Zn. More ecotoxicological studies for the crystalline style, gill and byssus of *P. viridis* should be done. For the shell of *P. viridis*, the differential metal distributions found in the periostracum and in the inner layers (prismatic and nacreous) were mainly due to differences in the mineralogy and chemistry of the different shell layers. Our results indicated that the periostracum layer especially the green-lipped part of the mussel shell is a potential biomonitoring material for current metal levels in the environmental seawater. This is especially so for Cu and Zn since these metals could also adsorb onto the shell surface from the seawater. Experimental studies should be conducted for both the soft and hard tissues to confirm our hypothesis that they are suitable for use in the biomonitoring.

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