# MULTIVARIATE ANALYSIS OF HEAVY METAL CONCENTRATIONS IN THE DIFFERENT TISSUES OF FOUR INTERTIDAL CLAMS FROM PENINSULAR MALAYSIA

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**Abstract:** Four species of clams (*Macoma* sp., *Siliqua* sp., *Pharus* sp. and *Mactra* sp.) were collected from the intertidal area of Peninsular Malaysia. Their different soft tissues (siphon, muscle, foot, mantle, gill and remaining soft tissues), and shells were analyzed for the concentrations of Cd, Cu, Fe, Ni, Pb and Zn. The relationships of heavy metals in the different tissues of clams were determined using multivariate analyses including correlation analysis, cluster analysis and multiple linear stepwise regression analysis (MLSRA). Metal distribution in the clams were explained using correlation analysis, which indicated that the shell was not significantly ($P > 0.05$) correlated with other tissues and the shell is also clustered differently from the rest of soft tissues as indicated by the cluster analysis. Among the soft tissues, it was found that the gills and mantle of all clams were identified as the most influential tissues in the accumulation of heavy metals in the total soft tissues for the clams by MLSRA. The present study found that the distributions of heavy metals in the different tissues of clams were related to their differences in biological and ecological aspects. Since the multivariate analyses used in this study can reduce the cost and time involved in identifying an effective tissue to monitor the heavy metal(s) bioavailability and contamination (Yap *et al*. 2010), this preliminary finding provided an alternative for future environmental management in the intertidal area of Peninsular Malaysia.

KEYWORDS: Tropical intertidal clams, metal distribution, correlation, cluster and multiple linear stepwise regression analyses

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## Introduction

To maintain sustainable resources from intertidal area, good environmental management is of paramount importance. However, it should be noted that an effective management of a sustainable ecosystem needs multidisciplinary knowledge and understanding. Ecotoxicological knowledge is one such important field needed for good environmental management. This is because provision of environmental data on toxic chemicals in an intertidal area can potentially help environmental policy makers and managers to outline effective measures to maintain a sustainable ecosystem. In this work, we focused on the potential of selected tissues of clams in monitoring heavy metal pollution of the intertidal areas of Peninsular Malaysia, using multivariate analyses. In the literature, specific organs of selected molluscs were usually employed for the biomonitoring studies of heavy metal bioavailabilities and contamination such as the digestive gland (also termed the midgut gland or hepatopancreas), crystalline style (Yap *et al*., 2006a), gills (Raspor *et al*., 2004; Yap *et al*., 2006b; Mouneyrac *et al*., 1998; Stefano *et al*., 2008), byssus (Yap *et al*., 2003a), shells (Yap *et al*., 2003b) and adductor muscle (Stefano *et al*., 2008). These tissues are directly involved in metal uptake, storage and excretion, and they have a high capacity to synthesize metal detoxification means such as metallothioneins (Vierengo *et al*., 1985).

Another important aspect of heavy metal studies in the different tissues of clams is that they provide integrated measures of the bioavailable metals, which are of ecotoxicological significance in the study site (Rainbow *et al*., 2002; Yap *et al*., 2006a). It could be suggested that the metals accumulated in the soft tissues of clams will be a measure of the bioavailabilities of the metals originating from both natural and anthropogenic sources (Yap *et al*., 2006a). Clams are bivalves which burrow in sediment, as opposed to those which attach themselves to the substrate such as oysters and mussels. Due to their 'immobile' lifestyle (where they bury themselves in the sand or mud), they could be proposed as potential biomonitors (Phillips and Rainbow, 1993).

The application of multivariate analyses was to determine the relationships of metal distributions in the different tissues of the Malaysian clams. These statistical methods could be useful in identification of different tissues as biomonitoring tissues for future environmental management. Moreover, the application of these multivariate statistical techniques can help in the interpretation of complex data matrices and allows the identification of possible factors/ sources that influence heavy metals and offers a valuable tool for reliable management to overcome pollution problems (Vega *et al*., 1998; Adams *et al*., 2001; Reghunath *et al*., 2002; Simeonov *et al*., 2004; Habes and Nugem, 2006; Alkarkhi *et al*., 2009; Yap *et al*., 2010).

According to the Department of Environment, Malaysia (DOE, 2008), aquatic pollution in Malaysia can be categorized into point and non-point sources. The point sources include sewage treatment plants (48.3%), manufacturing industries (45.1%), animal farms (4.0%) and agro-based industries (2.5%). These point sources were found to contribute to the pollution problems in Malaysia (DOE, 2008). This is why there is a need to monitor heavy metal pollution in Malaysian coastal waters, especially by using the intertidal clams.

Therefore, the objective of this work was to study the heavy metal distributions in the different tissues of four species of Malaysian intertidal clams by using multivariate analyses namely the correlation analysis, cluster analysis and multiple linear stepwise regression analysis (MLSRA).

## Materials and Methods

All four species of clams, *Macoma* sp. (Family: Tellidae), *Siliqua* sp. (Cultellidae), *Pharus* sp. (Family: Pharidae), and *Mactra* sp. (Family: Mactridae) (Figure 1), were collected from the intertidal areas of Peninsular Malaysia (Figure 2). About 25-30 individual clams from each sampling site were used for the metal analysis. *In-situ* measurements for temperature, conductivity, total dissolved solids, salinity and dissolved oxygen were done during the sampling time. These surface water parameters were determined by using a physico-chemical meter Model YSI 556 Multi-Probe System (MPS). The descriptions of sampling sites and the surface water parameters are given in Tables 1 and 2, respectively. The identification of the four clams was done until to the genera levels, based on the book authored by Takashi (2000), and the Malaysia Fisheries Directory (2005) issued by Department of Fisheries Malaysia. The allometric parameters are shown in Table 3. The clams were dissected and pooled into several different tissues including the siphon, muscle, foot, mantle, gill and remaining soft tissues (remainder). The shells and all the different categories of soft tissues were dried at 60°C to constant dry weights. Triplicates of each dried category of the tissues were digested in concentrated $HNO_3$ (Analar grade, BDH 69%). They were initially placed in a hot-block digester first at a low temperature (40°C) for 1 hour and were then fully digested at a high temperature (140°C) for at least 3 hours. The digested samples were diluted to 40 mL with double-distilled water (DDW). After filtration, the heavy metals were determined by using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. The data were presented in μg/g dry weight.

To avoid possible contamination, all glassware and equipment used were acid-washed. Procedural blanks and quality control samples made from standard solutions for Cd, Cu, Ni, Fe, Pb and Zn were analyzed after every 5-10 samples in order to check for sample accuracy. The analytical procedures for the samples were also checked with the Certified Reference Material (CRM) for dogfish liver (DOLT-3, National Research Council Canada). The recoveries are presented in Table 4.

The relationships of heavy metals in the different tissues were examined by using correlation analysis, MLSRA, and cluster analysis. Correlation analysis and MLSRA were performed by using SPSS 12.0 while STATISTICA 99 edition was used to conduct cluster analysis. All the data were $\log_{10}$(mean + 1) transformed in order to reduce the variance (Zar, 1996). One-way ANOVA Student-Newman-Keuls test (Day and Quinn, 1989) was used to elucidate where differences occurred among the metal levels in the different tissues of oysters and sediment samples collected from all sampling sites. All the comparisons were made at the 95% ($P< 0.05$) level of significance.

**Results**

The heavy metal concentrations in the different tissues of four species of clams are given in Table 5. For Cd in the shells of *Macoma* sp., *Siliqua* sp. and *Mactra* sp., their levels were significantly ($P< 0.05$) higher than the other tissues while all the four clam species had significantly ($P< 0.05$) higher levels of Ni and Pb than other tissues. High levels of Pb and Ni were also reported in the shells of Malaysian gastropods (Edward *et al*., 2010) and bivalves (Edward *et al*., 2009). In contrast, the shells of the four species of clams had significantly ($P< 0.05$) lower levels of Fe and Zn than other tissues. For Cu, no specific tissue was identified to accumulate higher Cu levels. The highest levels of Cu were found in the mantle of *Macoma* sp. and *Pharus* sp. While the shells and remainders were found to have the highest Cu levels in *Siliqua* sp. and *Mactra* sp., respectively.

Relationships of heavy metals in the different tissues of four clam species are explained by using cluster analysis as shown in Figure 3. In general, the shell of all the four types of clams were clustered differently from the remaining soft tissues. Among the different soft tissues, it was found that the gills of the clams were clustered differently from the other soft tissues for *P. legumen* and *Siliqua* sp. As for the siphon of respective *Macoma* sp. and *Pharus* sp., it was clustered differently from other soft tissues. Siphons and muscles of *Mactra* sp. were clustered differently from other soft tissues.

The relationships of heavy metal concentrations in the different tissues of four clam species were also explained using Pearson's correlation coefficients as shown in Tables 6-9. In general, no significant correlations ($P> 0.05$) were found between the metal concentrations in the shell with different soft tissues of the four clams. These results agreed with the clustering patterns (Figure 3) in which the shells of all clams were distinctly clustered from other soft tissues.

As shown in Tables 6-9, the heavy metals in most of the different soft tissues of clams were correlated with one another ($P< 0.01$ and $P< 0.05$), which indicated that the accumulation of metal(s) in a specific organ(s) affected the accumulation of metal(s) by other soft tissues. On the other hand, strong correlation coefficients were also observed between the heavy metals in the total soft tissues and the different soft tissues. This was a clear indication that the heavy metals in the total soft tissues were influenced by the accumulation of metals by each of the soft tissues. Meanwhile, Table 10 shows the MLSRA between the total soft tissues (except for *Mactra* sp. using remainder) and the different soft tissues of the clams. From the analysis, it was found that, in general, the metals in the total soft tissues were significantly influenced by gill and mantle. Other tissues such as the siphon of *Macoma* sp. also significantly ($P< 0.05$) influenced the metals accumulation in the total soft tissues while siphon and muscle of *Mactra* sp. significantly ($P< 0.05$) influenced the metals accumulation in the remainder.

**Discussion**

The correlation analysis and cluster analysis indicated that the metal concentrations in the shells were significantly ($P< 0.05$) different from the remaining soft tissues. These findings correspond with a study conducted by Yap *et al*. (2010) for Malaysian bivalves including *Donax faba, Polymesoda erosa, Spcharca broughtonii* and *Trisidos kiyonoi*, and Yap and Edward (2009) for *Perna viridis*. This is well expected due to the differences of physiological processes between the shell and soft tissues of molluscs (Yap *et al*., 2003b, 2010).

On the other hand, the strong relationships obtained from Pearson's correlation coefficients based on the different tissues of soft tissues could be due to their similar binding characteristics which enabled them to accumulate the metals from all sources, which can offer time-integrated measures of the supply of heavy metals available to them in the intertidal areas (Phillip and Rainbow, 1993; Rainbow, 1995; Rainbow and Blackmore, 2001).

The interesting findings on the gills and mantles, which are identified by MLSRA as influential tissues in the accumulation of heavy metals in the total soft tissues of all the clams (except for *Mactra* sp.) can be interpreted as follows. Previously, Yap *et al*. (2006b) reported the gills of *P. viridis* can be used as an indicator of heavy metal levels in the ambient coastal waters. Hence, it is assumed that the gills of clams can be used for the similar purpose since the concentrations of heavy metals on the gills surfaces were due to their direct contact with seawater (Yap *et al*., 2006b). Since most of the mantles consisted of gills which were merged together with mantle, the gills were believed to be a more influential organ than the mantle in the metal accumulation of the total soft tissues of clams. Moreover, the large surface areas of the clams' gills increased their metal uptake through facilitated diffusion (Phillips and Rainbow, 1993; Yap *et al*., 2006b).

Among the different soft tissues, the differences in the affinities of the metals to the binding sites of metallothionein in the different soft tissues (Viarengo *et al*., 1985) could affect the metal distributions. On the other hand, the function or location of a specific organ in the clams could also be associated with the metal accumulation in the different tissues (Edward *et al*., 2009). Therefore, based on the multivariate analyses employed, tissues such as the gill and the mantle of the clams could be potentially used as a biomonitoring organ although further studies are still needed. According to Yap *et al*. (2010), the similarities in the roles of biochemistry (eg. metallothionein) and chemistry (eg. atomic mass) could influence the metal distributions in the different tissues of the bivalves.

On the other hand, high concentrations of Zn and Fe found in the soft tissues of the clams could be due to the metal being essential to the clams. Zn is an essential metal for animals and an important component for many enzymes (Eisler, 2000). Besides, Zn tends to accumulate in bivalves' digestive gland and stomach as excretory granules and in the kidney as concretions (Eisler, 2000). And Fe, which is also known to be essential (Rainbow, 1990), plays a variety of roles in the biochemistry of marine organisms, in particular, as enzyme cofactors (Harrison and Hoare, 1980).

Many studies on the advantages for employing the molluscs in environmental management are reported in the literature. For example, the methods developed by Salánki *et al*. (2003), allowed the molluscs studied to be applied in passive and active monitoring due to convenient installation. The use of mussels, *Mytilus edulis* facilitate the determination of sites in accordance to the requirement of the European Commission's Water Framework Directive (WFD) as was reported by Hagger *et al*. (2008) in south-west England. An ecotoxicological protocol with caged mussels, *Mytilus galloprovincialis* was developed to evaluate the potential impact of an offshore gas platform in the central Adriatic Sea (Gorbi *et al*., 2008). On the other hand, the determination of heavy metal bioavailabilities by using the different tissues of *P. viridis* was studied by Yap *et al*. (2009) to monitor the metal contamination status of Peninsular Malaysia which may allow efficient

environmental management in the future. Hence, present study on the different tissues of Malaysian clams is important in providing more options for environmental management in the future.

## Conclusions

The present study on metal distributions in the different tissues of clams using multivariate analyses indicate that clam shells have different binding affinities of metals compared to the soft tissues, while the mantle and gills were identified as more effective tissues for future biomonitoring studies. The identification of specific tissue(s) based on multivariate analyses could reduce the cost and the time needed in choosing a particular tissue to monitor heavy metal bioavailability and contamination in intertidal areas. Therefore, this preliminary finding employing multivariate statistical techniques should prompt further studies since it can be a potential alternative for future environmental management in the intertidal areas of Peninsular Malaysia.

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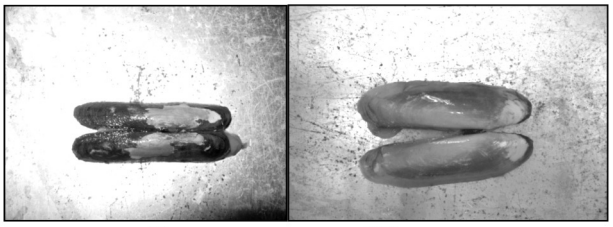
*Macoma* sp. *Mactra* sp.



*Pharus* sp. *Siliqua* sp.

Figure 1: Photographs of the four clam species collected from the intertidal area of Peninsular Malaysia.

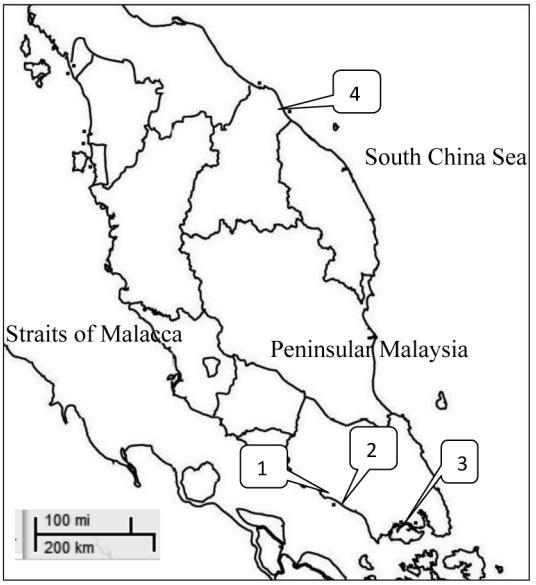



Figure 2: Sampling sites for clams in intertidal areas of Peninsular Malaysia. Note: 1= Sg. Ayam; 2= Sg. Lurus; 3= Sg. Danga; Sg. Semerak.

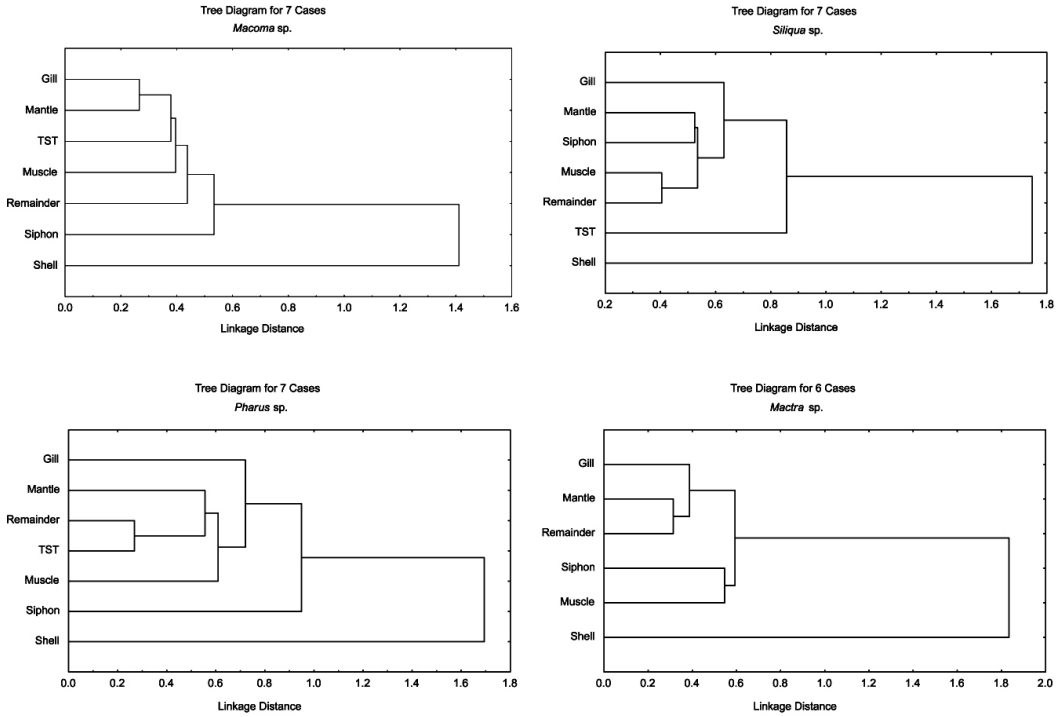



Figure 3: Hierarchical clusters based on Single-linkage Euclidean distances based on $\log_{10}$(mean + 1) transformed data of metal concentrations (Cd, Cu, Fe, Ni, Pb and Zn) in the different tissues of four clams collected from the intertidal area of Peninsular Malaysia. Note: TST= total soft tissues.

Table 1: Dates of sampling, sampling locations, and description of all sampling sites.

| No | Sampling sites | Clam collected | Sampling date | L-N | L-E | Site descriptions |
|---|---|---|---|---|---|---|
| 1. | Sg. Ayam (Johore) | *Macoma* sp. | 2 May 08 | 01° 45.165' | 102° 55.764' | A fishing village |
| 2. | Sg. Lurus (Johore) | *Siliqua* sp. | 3 May 08 | 01°45'31.5" | 102°55'48.0" | An urban and agricultural area |
| 3. | Sg. Danga (Pantai Lido, Johore) | *Pharus* sp. | 3 May 08 | 01°28'00.1" | 103°43'61.8" | An urban and aquaculture area |
| 4. | Sg. Semerak (Kelantan) | *Mactra* sp. | 13 May 08 | 05°51'56.99" | 102°30'27.67" | An aquacultural and agricultural area. |

Note: L-N=Latitude-North; L-E=Longitude-East.

Table 2: Readings (mean ± SE) of some surface water parameters recorded *in-situ* for all sampling sites.

| No. | Sampling sites | Temp (°C) | Cond. (µs/cm) | TDS (mg/L) | Salinity (ppt) | DO (mg/L) |
|---|---|---|---|---|---|---|
| 1. | Sg. Ayam | 31.1 ± 0.10 | 46773 ± 11.20 | 27.3 ± 1.00 | 26.7 ± 1.00 | 2.06 ± 0.01 |
| 2. | Sg. Lurus | 30.24 ± 0.60 | 5151 ± 160 | 3.04 ± 0.10 | 2.48 ± 0.10 | 2.97 ± 0.21 |
| 3. | Sg. Danga | 30.5 ± 1.00 | 30815 ± 4.0 | 18.1 ± 1.00 | 17.0 ± 0.50 | 2.14 ± 0.01 |
| 4. | Sg. Semerak | 30.9 ± 1.00 | 39270 ± 4.5 | 22.3 ± 1.10 | 20.9 ± 1.10 | 1.32 ± 0.01 |

Note: Temp= Temperature; Cond= Conductivity; TDS= Total dissolved solid; DO= Dissolved oxyge

Table 3: Allometric variables (mean ± SE) for the four species of clams analyzed in the present study.

| No. | Allometric parameters | *Siliqua* sp. | *Macoma* sp. | *Pharus* sp. | *Mactra* sp. |
|---|---|---|---|---|---|
| 1. | Shell length (cm) | 6.33 ± 0.09 (5.88-6.85) | 5.34 ± 0.13 (4.95-6.33) | 6.48 ± 0.13 (5.60-7.28) | 3.60 ± 0.08 (3.15-3.95) |
| 2. | Shell width (cm) | 2.14 ± 0.04 (1.93-2.30) | 2.78 ± 0.06 (2.64-3.20) | 1.46 ± 0.03 (1.34-1.63) | 2.96 ± 0.05 (2.63-3.13) |
| 3. | Shell height (cm) | 0.97 ± 0.03 (0.85-1.06) | 1.67 ± 0.04 (1.45-1.78) | 1.02 ± 0.01 (0.98-1.08) | 2.02 ± 0.06 (1.77-2.30) |
| 4. | Total soft tissue wet weight (g) | 1.52 ± 0.08 (1.16-1.91) | 2.78 ± 0.15 (2.31-3.76) | 1.99 ± 0.09 (1.62-2.47) | NA |
| 5. | Total shell wet weight (g) | 2.34 ± 0.11 1.83-2.80 | 4.78 ± 0.31 (3.37-6.30) | 2.61 ± 0.12 (2.19-3.46) | NA |
| 6. | Total soft tissue dry weight (g) | 0.34 ± 0.03 (0.22-0.55) | 0.55 ± 0.03 (0.44-0.75) | 0.39 ± 0.03 (0.27-0.55) | NA |
| 7. | Total shell dry weight (g) | 2.30 ± 0.11 (1.71-2.71) | 4.67 ± 0.30 (3.23-6.11) | 2.43 ± 0.12 (2.01-3.37) | NA |
| 8. | Water content (%) | 77.6 ± 1.99 (63.5-85.4) | 80.1 ± 0.49 (77.0-82.5) | 80.5 ± 0.87 (76.1-83.7) | NA |

NA= Data not available. Values in brackets are minimum-maximum.

Table 4: Comparisons of metal concentrations (mean, µg/g dry weight) between measured and certified values of Certified Reference Materials (CRM) (DOLT-3 Dogfish liver).

| No. | Metals | CRM values (C) | Measured values (M) | Percentage % of recovery [(M/C) ×100] |
|---|---|---|---|---|
| 1. | Cd | 19.7 | 19.4 | 98.5 |
| 2. | Cu | 32.3 | 31.2 | 96.6 |
| 3. | Fe | 1322 | 1484 | 112.3 |
| 4. | Ni | 3.95 | 2.72 | 68.9 |
| 5. | Zn | 86.7 | 86.6 | 99.9 |

Note: CRM for Pb was not available.

Table 5: Heavy metal concentrations (mean ± SE, µg/g dry weight) in the different tissues of clams collected from intertidal areas of Peninsular Malaysia.

| Site | Species | Tissues | Cd | Cu | Fe | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|---|
| Sg. Ayam | *Macoma* sp. | Gills | 0.08 ± 0.01 A | 34.5 ± 0.10 DE | 1437 ± 50 G | 1.27 ± 0.30 A | 48.2 ± 2.00 C | 168 ± 5.0 D |
| | | Mantle | 0.07 ± 0.01 A | 48.2 ± 1.12 F | 1109 ± 26 F | 1.17 ± 0.07 A | 34.9 ± 1.71 B | 122 ± 10 C |
| | | Siphon | 0.08 ± 0.01 A | 10.4 ± 0.24 A | 325 ± 16 A | 1.57 ± 0.11 A | 29.2 ± 0.49 B | 87.3 ± 2.0 B |
| | | Shell | 3.22 ± 0.11 B | 25.6 ± 0.37 C | 200 ±13 B | 26.6 ± 1.35 C | 88.9 ± 3.42 D | 12.4 ± 1.14 A |
| | | Muscle | 0.14 ± 0.02 A | 17.3 ± 0.49 B | 691 ± 17 C | 3.93 ± 0.13 B | 50.5 ± 1.21 C | 95.6 ± 1.97 B |
| | | Remainder | 0.08 ± 0.01 A | 36.3 ± 0.40 E | 807 ± 19 D | 5.21 ± 0.10 B | 11.5 ± 0.59 A | 186 ± 14.0 D |
| | | TST | 0.07 ± 0.01 A | 32.6 ± 2.51 D | 854 ± 23 E | 3.43 ± 0.31 B | 29.6 ± 1.83 B | 138 ± 2.65 C |
| Sg. Lurus | *Siliqua* sp. | Gills | 1.67 ± 0.03 D | 7.35 ± 0.05 A | 2771 ± 20 D | 9.12 ± 0.06 D | 11.8 ± 0.05 A | 582 ± 10.0 G |
| | | Mantle | 1.04 ± 0.02 C | 10.2 ± 0.05 B | 2078 ± 10 B | 2.95 ± 0.05 C | 19.2 ± 0.05 A | 241 ± 5.0 D |
| | | Siphon | 0.08 ± 0.01 A | 7.64 ± 0.05 A | 2755 ± 50 C | 1.03 ± 0.01 A | 37.6 ± 0.07 B | 196 ± 2.0 B |
| | | Shell | 3.97 ± 0.09 E | 15.7 ± 1.25 D | 178 ± 5.0 A | 26.6 ± 1.26 E | 52.4 ± 1.24 C | 12.0 ± 0.85 A |
| | | Muscle | 0.66 ± 0.05 A | 12.7 ± 0.78 C | 1913 ± 84 C | 7.53 ± 0.87 C | 49.1 ± 15.0 C | 217 ± 2.18 C |
| | | Remainder | 0.18 ± 0.02 A | 11.6 ± 0.07 BC | 2565 ± 39 C | 4.32 ± 0.36 B | 52.0 ± 0.24 C | 420 ± 13 F |
| | | TST | 0.08 ± 0.01 B | 11.4 ± 0.16 BC | 374 ± 42 A | 1.32 ± 0.11 A | 12.6 ± 0.48 A | 337 ± 4.95 E |
| Pantai Lido | *Pharus* sp. | Gills | 0.37 ± 0.01 D | 45.7 ± 1.56 E | 2526 ± 32 F | 15.0 ± 0.52 E | 39.3 ± 0.43 C | 219 ± 10 E |
| | | Mantle | 1.94 ± 0.09 C | 54.1 ± 1.45 D | 761 ± 37 D | 2.97 ± 0.04 D | 48.8 ± 3.12 B | 110 ± 6 B |
| | | Siphon | 0.64 ± 0.02 A | 9.47 ± 0.36 B | 574 ± 7 B | 5.27 ± 0.14 C | 6.32 ± 0.62 A | 81.1 ± 3 B |
| | | Shell | 0.33 ± 0.03 B | 7.81 ± 0.31 F | 134 ± 5 C | 30.8 ± 0.54 A | 68.8 ± 2.97 D | 6.90 ± 0.16 D |
| | | Muscle | 4.49 ± 0.19 E | 31.1 ± 0.90 A | 1100 ± 50 A | 8.54 ± 0.00 F | 26.5 ± 0.08 E | 91.5 ± 6 A |
| | | Remainder | 1.00 ± 0.02 B | 16.9 ± 0.42 C | 1146 ± 45 D | 3.26 ± 0.11 A | 50.7 ± 0.49 D | 87.5 ± 3.1 B |
| | | TST | 0.57 ± 0.02 B | 16.9 ± 0.61 C | 1791 ± 31 E | 4.56 ± 0.18 B | 42.4 ± 2.26 C | 102 ± 2.0 C |
| Sg. Semerak | *Mactra* sp. | Gills | 1.15 ± 0.02 E | 7.50 ± 0.05 D | 2265 ± 30 A | 9.81 ± 0.05 C | 26.8 ± 0.05 D | 146 ± 5.0 D |
| | | Mantle | 0.72 ± 0.01 D | 6.14 ± 0.05 B | 1819 ± 50 A | 3.79 ± 0.02 B | 24.6 ± 0.08 B | 146 ± 5.0 D |
| | | Siphon | 0.11 ± 0.01 A | 6.74 ± 0.05 C | 643 ± 10 A | 3.20 ± 0.04 B | 24.7 ± 1.00 C | 67.9 ± 3.0 A |
| | | Shell | 4.35 ± 0.13 B | 8.18 ± 0.22 A | 62 ± 2.5 A | 29.0 ± 1.62 A | 35.7 ± 1.34 B | 5.03 ± 0.31 B |
| | | Muscle | 0.28 ± 0.01 F | 4.64 ± 0.05 E | 284 ± 10 A | 0.74 ± 0.01 D | 25.9 ±0.10 B | 78.9 ± 2.0 A |
| | | Remainder | 0.44 ± 0.04 C | 12.6 ± 0.14 F | 1930 ± 60 A | 3.96 ± 0.23 B | 23.5 ± 0.16 A | 112 ± 3.8 C |

Note: TST= total soft tissues. Student-Newman-Keuls (SNK) comparisons of metal concentrations in the different tissues of clams. Metal concentration of different tissues sharing a common letter are not significantly different ($P>0.05$) while those with a different letter are significantly different ($P< 0.05$).

Table 6: Pearson's correlation coefficients of metal concentrations (Cu, Cd, Fe, Ni, Pb and Zn) in the different tissues of *Macoma* sp. collected from Peninsular Malaysia. N= 21.

| Tissues | TST | Shell | Mantle | Siphon | Muscle | Remainder | Gills |
|---|---|---|---|---|---|---|---|
| TST | 1.000 | 0.728 | 0.991** | 0.985** | 0.987** | 0.982** | 0.993** |
| Shell | | 1.000 | 0.716 | 0.717 | 0.792 | 0.641 | 0.722 |
| Mantle | | | 1.000 | 0.976** | 0.974** | 0.961** | 0.996** |
| Siphon | | | | 1.000 | 0.993** | 0.947** | 0.990** |
| Muscle | | | | | 1.000 | 0.946** | 0.986** |
| Remainder | | | | | | 1.000 | 0.958** |
| Gills | | | | | | | 1.000 |

Note: **= Correlation is significant at the 0.01 level (2-tailed). TST= total soft tissues.

Table 7: Pearson's correlation coefficients of metal concentrations (Cu, Cd, Fe, Ni, Pb and Zn) in the different tissues of *Siliqua* sp. collected from Peninsular Malaysia. N= 21.

| Tissues | TST | Shell | Mantle | Siphon | Muscle | Remainder | Gills |
|---|---|---|---|---|---|---|---|
| TST | 1.000 | 0.522 | 0.965** | 0.950** | 0.935** | 0.962** | 0.949** |
| Shell | | 1.000 | 0.688 | 0.736 | 0.787 | 0.729 | 0.621 |
| Mantle | | | 1.000 | 0.990** | 0.977** | 0.985** | 0.978** |
| Siphon | | | | 1.000 | 0.985** | 0.991** | 0.944** |
| Muscle | | | | | 1.000 | 0.995** | 0.948** |
| Remainder | | | | | | 1.000 | 0.956** |
| Gills | | | | | | | 1.000 |

** Correlation is significant at the 0.01 level (2-tailed). TST= total soft tissues.

Table 8: Pearson's correlation coefficients of metal concentrations (Cu, Cd, Fe, Ni, Pb and Zn) in the different tissues of *Pharus* sp. collected from Peninsular Malaysia. N= 21.

| Tissues | TST | Shell | Gills | Mantle | Siphon | Muscle | Remainder |
|---|---|---|---|---|---|---|---|
| TST | 1.000 | 0.654 | 0.986** | 0.965** | 0.960** | 0.988** | 0.997** |
| Shell | | 1.000 | 0.589 | 0.533 | 0.525 | 0.612 | 0.650 |
| Gills | . | | 1.000 | 0.957** | 0.988** | 0.998** | 0.974** |
| Mantle | | | | 1.000 | 0.912* | 0.968** | 0.975** |
| Siphon | | | | | 1.000 | 0.977** | 0.940** |
| Muscle | | | | | | 1.000 | 0.979** |
| Remainder | | | | | | | 1.000 |

Note: ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed). TST= total soft tissues.

Table 9: Pearson's correlation coefficients of metal concentrations (Cu, Cd, Fe, Ni, Pb and Zn) in the different tissues of *Mactra* sp. collected from Peninsular Malaysia. N= 18.

| Tissues | Shell | Gills | Mantle | Siphon | Muscle | Remainder |
|---|---|---|---|---|---|---|
| Shell | 1.000 | 0.561 | 0.505 | 0.554 | 0.425 | 0.522 |
| Gills | | 1.000 | 0.994** | 0.985** | 0.945** | 0.987** |
| Mantle | | | 1.000 | 0.991** | 0.972** | 0.992** |
| Siphon | | | | 1.000 | 0.978** | 0.993** |
| Muscle | | | | | 1.000 | 0.963** |
| Remainder | | | | | | 1.000 |

** Correlation is significant at the 0.01 level (2-tailed).

Table 10: Multiple linear stepwise regression analysis (MLSRA) between the soft tissues (and remainder) and the different soft tissues of the selected clams based on the concentrations of Cu, Cd, Fe, Ni, Pb and Zn.

| No. | Species | MLSRA equation | R | $R^2$ |
|---|---|---|---|---|
| 1. | *Macoma* sp. | TST= -0.002 -3.298 (gill) + 2.706 (mantle) + 1.994 (siphon) | 0.997 | 0.994 |
| 2. | *Mactra* sp. | Remainder = -0.328 -2.146 (gill) + 2.746 (mantle) + 1.689 (siphon)-1.433 (muscle) | 0.999 | 0.999 |
| 3. | *Pharus* sp. | TST= -0.222 + 0.607 (gill) + 0.438 (mantle) | 0.986 | 0.972 |
| 4. | *Siliqua* sp. | TST= -0.057 + 0.083 (gill) + 0.811 (mantle) | 0.963 | 0.928 |

Note: For *Mactra corallina*, its total soft tissue was not analyzed for metals and therefore, remaining soft tissues were used as the dependent variable in the MLSRA. TST= total soft tissues.