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Heavy metal distribution in the different parts of *Cerithidea obtusa* by using multivariate analysis

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ABSTRACT The intertidal gastropod, *Cerithidea obtusa* were obtained from Bako and Sematan (Sarawak) and Deralik (Perak) in February and December 2006. Besides the shells, the snails were dissected into six different soft tissues. The soft tissues and the shell were used for detection of heavy metals. It was found that the highest concentrations of Cu (112 - 178 μg/g dw) and Zn (117 – 161 μg/g dw) were found in the tentacle; the highest concentrations of Cd (4.41 - 5.37 μg/g dw), Pb (53.2 – 63.8 μg/g dw) and Ni (26.1 - 27.9 μg/g dw) were found in the shell. On the other hand, the highest Fe concentrations (910 - 2921 μg/g dw) were found in the operculum. The cluster analysis revealed that the accumulation of heavy metals were clustered into a few groups, where metals were found in the shell are significantly different from the other soft tissues. The multivariate statistical analyses revealed that the accumulation by the different parts were inter-related with one another. Based on the multiple linear stepwise regression analysis, it was also found that the caecum was the most influential organ in accumulation of the studied heavy metals in the total soft tissues. The results indicate the ability of *C. obtusa* to accumulate heavy metals in the different tissues, hence fulfilling the important criteria as a good biomonitor.

Cerithidea obtusa, gastropod, heavy metal, different parts, multivariate analysis.

INTRODUCTION

The determination of heavy metal concentrations in whole soft tissues of molluscs presents little interest, since the main metal accumulating organs such as gills, digestive gland and kidney are constituting only a small part of a total soft tissue [1]. Besides, the spawning season of the gastropods and environmental factors may contribute to the wide variability of heavy metal concentrations in the total soft tissues of gastropods. Thus, the above points strongly evidenced that the disadvantages of the use of the total tissue in monitoring of metal bioavailability in marine environment.

The present study focuses on the different parts of *Cerithidea obtusa* (Family: Potamididae) as a biomonitor of heavy metal contamination in tropical coastal waters. The gastropods which are found abundantly in the coastal areas and their ability to accumulate metals fulfilled one of the criteria as biomonitors of heavy metal contamination. In the literature, the reliability of gastropods as biomonitors of heavy metal contamination had been studied by many researchers [2-4]. Liang et al. [2], found that *Rapana venosa* accumualated high levels of Cd in the Bohai Sea, China. The ability of *Patella caerulea* to accumulate metals was revealed in a study conducted by Hamed and Emara [3] in the Gulf of Suez, Red Sea. The study of heavy metals in *Patella caerulea* and *Mullus barbatus* was done by Storelli and Marcotrigiano [4] in the Ionian Sea, Italy. These authors supported the application of gastropods as biomonitors of heavy metal pollution in the marine environment.

On the other hand, correlation and cluster analysis were applied in this study to observe the differences of metal distribution in the different tissues of the *C. obtusa*. Correlation and cluster analysis (CA) are the most usual multivariate statistical methods used in environmental studies [5-7] especially in the studies of heavy metals in sediment [7-11]. However, the application of multivariate statistical methods to determine the distribution of heavy metals in marine molluscs especially *C. obtusa* are scarce. A few studies reported on the use of cluster analysis in the determination of heavy metals in the marine molluscs were found in the literature. Conti and Cecchetti [12] reported the use of CA in classifying heavy metals in *Monodonta turbinata*, *Patella cerulea* and *Mytilus galloprovincialis* in accordance to sites with wide ranges of human activities. Besides, Pourang et al. [13] and Pourang and Dennis [14] used CA to study the distribution of heavy metals in five sturgeon species and two shrimp species, respectively.

The use of multivariate statistical techniques, such as cluster analysis (CA) is useful in the interpretation of complex data matrices to investigate the heavy metals and ecological status of the systems studied, allowing the identification of possible factors/ sources that might influence heavy metals and can offer a valuable tool for reliable management as well as rapid solution to pollution problems [15-19]. In addition to the use of multivariate analysis, statistically, could assist in determining the potential biomonitor accurately, by referring to cluster groups of their different parts. Moreover, multivariate methods are recommended for the use in monitoring studies since they can help reduce the costs of carrying out further environmental surveys [20-21].

Therefore, present study aims to determine the distribution of heavy metals in the different parts of *C. obtusa* and to determine the possible significant relationships between the concentrations of the different parts by using correlation, cluster and multiple stepwise linear regression analyses.

MATERIALS AND METHODS

Sampling was carried out conducted in Deralik (Perak) to collect the samples. Samples from Bako and Sematan (Sarawak) were obtained from the local villagers, as shown in Figure 1 and Table 1. The identification of the species followed the descriptions by Lim et al. [22]. All the samples were stored in an ice compartment < 10 °C until transportation to Universiti Putra Malaysia laboratory. In the laboratory, the samples were kept at -10 °C until further analysis. For the metal analysis, 30 individual of snails with almost similar sized were randomly taken from the main sample and thawed at room temperature (26 - 29 °C) on a clean tissue paper. These snails were measured for their shell heights and shell width (Table 1). The soft tissues were then separated from the shell by crunching (using a clean pestle) the shell carefully. The soft tissues of the snails were then dissected and pooled into six different components namely caecum, foot, muscle, operculum, remainder, and tentacle besides the shell. The dissected and pooled tissues and the shells were dried for 72 hours at 60 °C in an oven to constant dry weights [23-24].

About 0.5 gram of *C. obtusa* tissues were digested in 10ml of concentrated nitric acid (AnalaR Grade; 69 %). They were placed in a hot block digester first at low temperature (40 °C) for 1 hour and were then fully digested at high temperature (140 °C) for at least 3 hours. The digested samples were then diluted to a volume of 40 ml with double distilled water (DDW). The sample was then filtered through Whatman No.1 filter paper (Dia: 110mm; Schleicher & Schuell, Whatman International Ltd Maidstone England) and they were determined for Cd, Cu, Fe, Ni, Pb and Zn by using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Model AAnalyst 800. The detection limits of the AAS for Cd, Cu, Fe, Ni and Zn were 0.009, 0.010, 0.010, 0.009 and 0.010, respecetively. From the pooled tissues, the samples were analyzed in one and three replicates (Based on the availability of the pooled tissues). The data were presented in µg/g dry weight (dw) basis. Multi-level calibration standards were analysed to generate calibration curves against which sample concentrations were calculated. Standard solutions were prepared from 1000 mg/L stock solutions of each metal (Merck Titrisol).

All the glassware and plastic materials used were acid-washed in 10% concentrations of concentrated acid in order to minimize external contamination. Quality control samples made from standard solutions of Cd, Cu, Fe, Ni, Pb and Zn were analyzed once in every ten samples to check for the metal recoveries. The analytical procedures for the snail samples were checked with the Certified Reference Material (CRM) for dogfish liver (DOLT-3, National Research Council Canada) and the recoveries of all metals were satisfactory (Table 2).

For the statistical analysis, the distributions of heavy metals in the different parts were determined by using cluster analysis. The relationships between the heavy metals in the different parts were analyzed using Spearman's correlation coefficient. Multiple stepwise linear regression analysis was used to determine the influential tissues in affecting the accumulation and concentration contributing to the metals of total soft tissues. All data were log10 (X + 1) [25] transformed prior to the statistical analysis. SPSS 12.0 was used to conduct the correlation and multiple stepwise linear regression analyses while STATISTICA 99 edition was used to conduct the cluster analysis. Cluster analysis was conducted by determining the similarity of a set of variable (eg. Different tissues by heavy metal concentrations) as were described by Simeonov et al. [26] and Spanos et al. [27].

RESULTS AND DISCUSSION

Heavy metal concentrations in the different parts of the *C. obtusa* collected from the three sampling sites are shown in Table 3. In general, it was found that the tentacle of the gastropods were highly accumulative of Cu and Zn as shown by the gastropods from all the sites, where they ranged from 112 - 178 µg/g dw and 117 - 161 µg/g dw, respectively. Meanwhile, the operculum was mostly accumulative of Fe, in samples of gastropods collected from Bako and Sematan, and its range was 638 - 2921 µg/g dw. On the other hand, the shell was highly accumulative of Cd (4.41 - 5.37 µg/g dw), Pb (53.2 - 63.8 µg/g dw) and Ni (24.4 - 27.9 µg/g dw).

Distribution of heavy metals in the different parts of the *C. obtusa* are illustrated in cluster analysis (Figure 2). Generally, the accumulations of Cd, Cu, Fe, Ni, Pb and Zn in the shell were significantly different from the other tissues whereas they were solely clustered into one group. This could be due to the fact that some trace metals are incorporated into the shells of the *C. obtusa* through substitution of the calcium ion in the crystalline phase of the shell or are associated with the organic matrix of the shell [28-29] instead of induction of metallothionein as being found in the soft tissues [23]. However, for the accumulation of Ni, the shell and tentacle were significantly different ($P< 0.05$) from the other soft tissues. Furthermore, most of the soft tissues were found to be clustered into two distinct groups (by ignoring the shell). For the accumulation of Cu, the caecum, muscle and operculum were clustered as one group while another group consisted of the remainder, tentacle and feet were clustered in the other group. Cd, the first group consisted of the caecum and concerning operculum while the second group consisted of the remainder, foot, muscle and tentacle. Accumulation of Zn by the operculum was significantly different from the remainder ($P< 0.05$) while the caecum, remainder, muscle, foot and tentacle were clustered in one group.

For Pb, the caecum, remainder, muscle and foot were clustered into one group while the operculum and tentacle were clustered into another group. Meanwhile for Ni, the first group of the soft tissues consisted of the caecum and remainder and the second group consisted of the operculum, muscle and foot. Two distinct cluster groups were also observed in the accumulation of Fe by the soft tissues, where the first group consisted of the tentacle, muscle and foot while the second group consisted of the caecum, remainder and operculum. Generally, the cluster analyses indicated the differences of heavy metal accumulation by the different parts, in other words, each tissue accumulate different concentrations of metals.

The relationships between heavy metal concentrations in the different parts of the *C. obtusa* were carried out by the Spearman's correlation coefficients in Table 4. It was found that accumulation of the heavy metal concentrations in the different parts were inter-related with one another. Interestingly, from Table 4, it was observed that the concentrations found in the different parts were correlated with those metal concentrations found in the total soft tissues. The concentrations of Cu by the total soft tissues was negatively and significantly correlated with those found in the caecum ($R= -0.683$; $P< 0.05$) and tentacle ($R= -0.667$; $P< 0.05$) but positively and significantly correlated with the operculum ($R = 0.967$; $P< 0.05$). On the other hand, the concentrations of Cd in the total soft tissues were significantly correlated with the concentrations found in shell ($R= - 0.933$; $P< 0.05$).

Concentrations of Zn and Pb in the total soft tissues was correlated with the concentrations in two different parts namely the caecum and tentacle: For Zn, the concentrations found in the total soft tissues were significantly correlated with the concentrations found in the caecum ($R= 0.700$; $P< 0.05$) and tentacle ($R= 0.677$; $P< 0.05$).

While for the concentrations of Pb, the total soft tissues were significantly correlated with operculum (R= 0.820; P< 0.05) and tentacle (R= 0.854; P< 0.05). On the other hand, concentrations of Ni and Fe in the total soft tissues were correlated with the metal concentrations of four parts. In case of Ni, the concentrations in the total soft tissues were significantly correlated with the concentrations found in the caecum (R= -0.683; P< 0.05), operculum (R= 0.883; P< 0.05), muscle (R= 0.917; P< 0.05) and foot (R= 0.900; P< 0.05); and for Fe, the concentrations found in the total soft tissues were significantly correlated with the Fe concentrations found in the remainder (R= 0.833; P< 0.05), tentacle (R=0.700; P< 0.05), muscle (R= 0.683; P< 0.05) and foot (R= 0.767; P< 005).

The correlation analysis revealed that the metal concentrations found in the caecum, operculum and tentacle were significantly correlated (P< 0.05) with the concentrations found in the total soft tissues for most of the metals studied. The function and location of the particular organ(s) could contribute to the relationships. The caecum is an organ that plays a crucial role in the snail's nutritional physiology [29]. Besides, the tentacle, operculum and the foot are considered as external organs and they are in contact with the surrounding environment, are responsible for the adsorption of metals to the snail. This confirmed that the differences in the surface of contact of the different soft tissues could affect the accumulation of metals by the tissues as reported by Yap et al. [30] in *Perna viridis*. On the other hand, the highest correlation of Ni and Fe, were found between the different parts and total soft tissues. This phenomenon could be caused by the metal (Example: Ni) bioavailability in the sampling sites in addition to the ability of the *C. obtusa* to regulate Ni in the different parts of their body. The habitat of the *C. obtusa* which is mangrove area could also contribute to the high number of correlations found between the different parts and total soft tissues for the accumulation of Fe. According to Defew et al. [31], mangrove sediments are anaerobic, reduced and rich in sulphide and organic matter. Therefore, this allows the retention of water-borne heavy metals [31-33] especially Fe and the subsequent oxidation of sulphide between tides which could prompt the Fe mobilization and bioavailability [34].

The relationships between the different parts and the total soft tissues are further explained in the multiple stepwise linear regression analysis (Table 5). The caecum was found to be the influential tissues in the accumulation of heavy metals besides the remainder and operculum. The importance of the caecum which was being discussed in the previous paragraph could be the main tissues of metals uptake into the snail's body before the metals are distributed in other tissues of the *C. obtusa*.

CONCLUSION

It can be concluded that, i) the ability of *C. obtusa* to accumulate and regulate heavy metal concentrations in their body as revealed by the multivariate analysis; ii) the accumulation of metals by the shell was significantly different from the remaining soft tissues and iii) Multiple stepwise linear regressions also revealed that the caecum was the most influential organ in the accumulation of heavy metals by the total soft tissues of *C. obtusa*.

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