# USE OF DIFFERENT SOFT TISSUES OF FLAT-TREE OYSTER *Isognomon alatus* AS BIOMONITORS OF BIOAVAILABILITIES AND CONTAMINATION BY Zn IN THE MANGROVE AREAS OF PENINSULAR MALAYSIA

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**Abstract:** Oyster is a net accumulator of metals, especially Zn, which can be used as a biomonitor of time-integrated records of bioavailable Zn fractions over its life time. Total soft tissues of oysters have been frequently used in metal bio-monitoring study. However, a lot of environmental and biological effects could influence the metal data. In order to reduce such confounding factors, the use of different soft tissues in oysters is proposed in this study. The muscle, mantle plus gills, byssus and remaining soft tissues of flat-tree oyster *Isognomon alatus*, collected from the estuaries of Lukut (5 sites), Sepang Besar (2 sites) and one polluted site at Kg. Pasir Puteh, were analysed for Zn. Significant spatial differences in the accumulated Zn concentrations of the oysters (and hence bioavailabilities) were found between sampling sites and estuaries, and these could be attributed to anthropogenic inputs, including discharges of shrimp ponds (Lukut), animal husbandry (Sepang Besar), sewage, shipping and industrial effluents (Kg. Pasir Puteh). The use of different soft tissues of *I. alatus* as biomonitors of bioavailability and contamination by Zn in the mangrove area of Peninsular Malaysia is proposed. The erroneous results due to spawning and the problem of defaecation before dissection could be potentially reduced by using the oyster muscle. Hence, a more accurate interpretation of the bioavailability and contamination by heavy metals in coastal waters could be obtained. Overall, the present baseline data based on different soft tissues of the oysters can be used for regular biomonitoring, considering the rapid land-based development in the coastal area of Peninsular Malaysia.

KEYWORDS: Biomonitoring, *Isognomon alatus*, Zn bioavailabilities, mangrove area of Peninsular Malaysia

## Introduction

Oysters are known to accumulate trace metals in their soft tissues to potentially high concentrations (Lauenstein *et al.*, 1990; Phillips and Rainbow, 1994). They have been widely employed as biomonitors of trace metal pollution as a result of their ecological characteristics such as their abundance, size, wide distribution and easy collection (Presley *et al.*, 1990; Phillips and Rainbow, 1994; Rainbow, 1995). Furthermore, the accumulated metal concentrations of biomonitors such as oysters can provide a time-integrated measure of the amounts of bioavailable trace metals.

The bioavailable fraction is the only portion that is ecologically relevant, and biomonitors, therefore, give a better indication of the trace metal pollutant status of an aquatic habitat than do measurements of metal concentrations in the local waters or sediments (Phillips and Rainbow, 1994; Rainbow, 1995). Hence, high metal concentrations found in the tissues of oysters are high ambient metal bioavailabilities to that biomonitor. The flat-tree oyster *Isognomon alatus* (Family: Isognomonidae), found on the prop roots of mangrove trees along the estuaries of the Sepang and Lukut areas in the west coast of Peninsular Malaysia, was focussed upon in this study. However, it is not an important seafood delicacy due to its limited flesh content.

In the literature, only limited biomonitoring studies on heavy metals were reported by using *I. alatus* except for those reported by Saed *et al.* (2001, 2004) and Sbriz *et al.* (1998).

However, most of the reported studies using marine oysters for the metal biomonitoring studies employed total soft tissues which may be dependent on lots of biological and environmental factors. The biological factors such as the spawning conditions, gut contents and sizes could potentially influence the data while such environmental factors as water salinity and temperature could affect the metal speciation and thus metal bioavailability to the biomonitors. The use of different soft tissues of molluscs is believed to reduce erroneous results due to spawning and the problem of defaecation before dissection could be overcome. Hence, a more accurate interpretation of the bioavailability and contamination by heavy metals in coastal waters could be obtained (Yap *et al.*, 2006a). Recently, Yap *et al.* (2011) suggested the use of different soft tissues of flat-tree oyster *I. alatus* for the biomonitoring of Fe, Cu and Ni.

In this paper, Zn was focussed upon because marine oysters are known to be super-accumulators of Zn (Phillips and Rainbow, 1994). Earlier, Saed *et al.* (2001) studied metal concentrations in oysters after exposure to pig-farm effluents and Saed *et al.* (2004) reported heavy-metal depuration using *I. alatus* under field and laboratory conditions. However, there has been no detailed information on the Zn contamination and bioavailability based on the distribution of metals in the different soft tissues of *I. alatus* collected from the mangrove area of Peninsular Malaysia. Therefore, the objective of the present study is to determine the bioavailabilities and contamination by Zn in four different soft tissues (mantle plus gill, byssus, muscle, and remaining soft tissues.) of *I. alatus* collected from eight geographical populations at Sepang (Selangor), Lukut

(Negeri Sembilan) and Johore Straits (Johore).

## Materials and Methods

All the sampling sites are shown in Figure 1. About 30 individuals of flat-tree oysters were collected from each sampling site at the estuaries of the Sepang Besar River (2 sites, Selangor) and Lukut River (5 sites, Negeri Sembilan). They were collected on 1 December 2007, intertidally from prop roots of mangrove trees and littoral rock. Oyster samples from a metal-contaminated site at Kg. Pasir Puteh (Johore) (Yap *et al.*, 2002, 2003) were collected on 3 May 2008. *In-situ* measurements for temperature, conductivity, total dissolved solids (TDS) and salinity were done during the sampling. The descriptions for all the sampling sites are given in Table 1. At each sampling site, surface sediments (1-5 cm) were also collected. Reason why water samples were not collected although oysters are filter-feeders is because water samples are easily
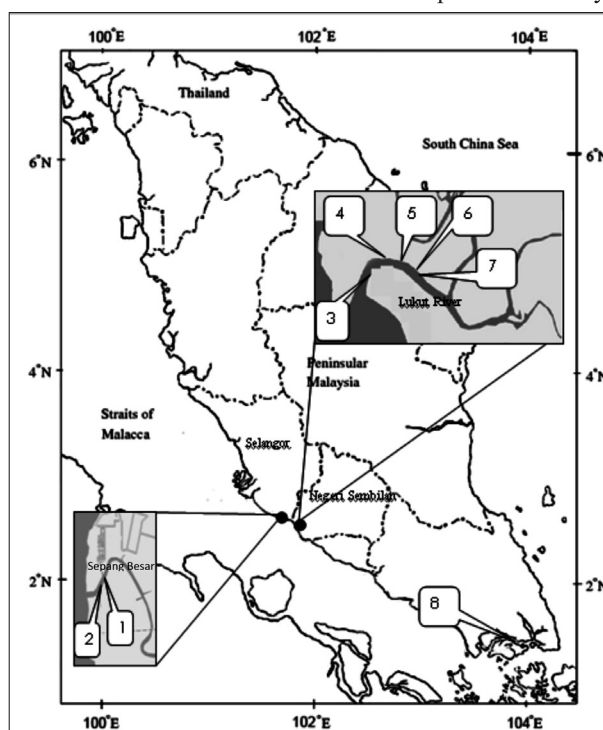


Figure 1: Map showing sampling sites where *Isognomon alatus* were collected. (1= Sepang-1; 2= Sepang-2; 3= Lukut-1; 4= Lukut-2; 5= Lukut-3; 6= Lukut-4; 7= Lukut-5; 8= Kg. Pasir Puteh)

Table 1: Site descriptions, GPS, mean shell lengths of the *Isognomon alatus* populations collected from Lukut River, Sepang Besar River and Kg. Pasir Puteh.

| No | Sites | Sampling date | Longitude | Latitude | Shell length (mm) | Description of sampling site |
|---|---|---|---|---|---|---|
| 1 | Sg.Sepang Besar-1 (Sepang-1) | 1 Dec 2007 | N 02° 36.102' | E 101° 42.337' | 51.02 (47.17-52.96) | A mangrove area and estuary. No observable human activities. |
| 2 | Sg. Sepang Besar-2 (Sepang-2) | 1 Dec 2007 | N 02° 36.042' | E 101° 42.461' | 52.52 (49.15-55.93) | A mangrove area and estuary. Near a prawn aquaculture site. |
| 3 | Lukut-1 | 1 Dec 2007 | N 02° 34.978' | E 101° 47.607' | 46.02 (23.48-56.88) | Boating activities. |
| 4 | Lukut-2 | 1 Dec 2007 | N 02° 34.979' | E 101° 47.668' | 55.22 (46.11-66.02) | A fishery and recreational area. |
| 5 | Lukut-3 | 1 Dec 2007 | N 02° 34.847' | E 101° 47.795' | 49.57 (40.06-57.88) | In the vicinity of a construction area. |
| 6 | Lukut-4 | 1 Dec 2007 | N 02° 34.853' | E 101° 47.882' | 53.83 (48.40-59.08) | A residential area with some aquaculture activities. |
| 7 | Lukut-5 | 1 Dec 2007 | N 02° 34.737' | E 101° 48.033' | 57.55 (51.50-60.45) | A residential area. |
| 8 | Kg. Pasir Puteh (KPPuteh) | 3 May 2008 | N 01°26.125' | E 103°56.128' | 50.11 (42.90-58.58) | Industrial and mooring activities and urban areas. |

Note: Shell lengths in brackets are the ranges (minimum to maximum values).

contaminated and it just provides the water quality during time of sampling while sediment is a storage and transport of metals (Yap *et al.*, 2002). Thus, sediment can provide an estimate of metal contamination of the sampling sites.

All the oyster and sediment samples were kept in an ice-compartment during transportation to laboratory. Upon reaching laboratory, all the samples were stored at -10ºC in a freezer until analysis. At each sampling site, 30 to 40 oysters of relatively similar sizes (shell lengths of 46-48 mm) were analysed. In the laboratory, the oysters were dissected and pooled into four different soft parts namely, mantle plus gill, byssus, muscle, and remaining soft tissues.

The different soft parts were then dried in the oven for 72 hours at 105º C to constant dry weights (Mo and Neilson, 1994). The dried tissues were then ground to homogenized samples by using a pestle and mortar. About 0.5 g of dried tissue for each part were weighed and placed into a digestion tube. Then, the dried tissue was digested in concentrated nitric acid (AnalaR grade, BDH 69%). They were placed in a hot-block digester at low temperature (40º C) for one hour, and then they were fully digested at high temperature (140º C) for at least three hours (Yap *et al.*, 2005a). Following that, the digested samples were diluted to a certain volume (20 ml) with double-distilled water. The digested samples were then filtered through Whatman No. 1 filter papers into acid-washed pill boxes.

For the sediment samples, the total Zn concentrations were analysed by the method described by Yap *et al.* (2002) while sequential extraction technique was used which resulted in four geochemical fractions (easily, freely, leacheable or exchangeable [EFLE], acid-reducible, oxidisable-organic and resistant) followed the methods described by Badri and Aston (1983) and slightly modified by Yap *et al.* (2002). Triplicates were analysed for each sampling site.

Lastly, the prepared samples were analysed for Zn by using a flame Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. After conversion, the data were presented in $\mu g/g$ dry weight. All glassware and equipments used were acid-washed. Procedural blanks and quality control samples made from the standard solution for Zn were analysed once for every five samples in order to check for accuracy. The percentages of recoveries for the Zn analyses were acceptable at 80-110%. The quality of the methods used were checked with Certified Reference Materials for Soil-5

(IAEA) [certified Zn value: 368 µg/g dry weight, measured Zn value: 326 µg/g dry weight; recovery= 88.6%], while the tissues of oysters were verified using Dogfish Liver (DOLT-3, National Research Council Canada) [certified Zn value: 86.6 µg/g dry weight, measured Zn value: 87.6 µg/g dry weight; recovery= 101.2%].

The relationships of Zn concentrations between the different tissues in the oysters and geochemical factions in the sediments were elucidated using cluster analysis. The cluster analysis was based on Single Linkage Euclidean distances, the Zn concentrations in the four geochemical fractions, total summation of four fractions and total Zn concentrations was based on direct aqua-regia method. The statistical analysis of surface sediments, collected from eight sampling sites, was done by using STATISTICA 99" Edition (Version 5.5). Pior to cluster analysis, all the data were $\log_{10}(\text{mean} + 1)$ transformed in order to reduce the variance (Zar, 1996). In order to elucidate where differences occurred among the metal levels in the different tissues of oysters and sediment samples collected from all sampling sites, one-way ANOVA Student–Newman–Keuls test was used (Day and Quinn 1989). All the comparisons were made at the 95% (P<0.05) level of significance.

## Results and Discussion

Table 2 shows the physico-chemical parameters in the water samples of *I. alatus* habitats were 27.91-30.81°C for water temperature, 28093-44721 µs/cm for conductivity, 16.63-26.16 g/L for TDS, 15.50 – 25.56 ppt for water salinity. The wide range of water salinity indicated that the oysters were tolerant of high tidal changes.

The results for the Zn concentrations in the sediment samples are given in Table 3. It is found that Kg. Pasir Puteh had the highest concentration of Zn for total Zn concentration, oxidisable-organic and resistant fractions. The highest Zn EFLE fraction was found in Lukut1, followed by Kg. Pasir Puteh and other sites. The highest level of Zn acid-reducible fraction was found at Lukut-5. However, in general, all these sediment data indicated Kg. Pasir Puteh showed

the highest Zn contamination when compared to the Lukut River and the Sepang Besar River. However, ratios of nonresistant to resistant fraction of Zn showed that majority of total Zn in the sediment for all the eight sampling sites were dominated by nonresistant with ratios > 1.0.

The concentrations of Zn in the different tissues of all oyster populations are presented in Table 4. All the tissues including muscle, byssus, mantle plus gill and remainder had the highest Zn levels in Kg. Pasir Puteh population compared to sampling sites at Lukut and Sepang Besar. This phenomenal raised Zn levels in all the tissues of Kg. Pasir Puteh indicated the highest Zn bioavailability to the biomonitor *I. alatus* at the sampling site. Hence, the highest Zn bioavailability at Kg. Pasir Puteh compared to Lukut and Sepang Besar sampling sites can be assumed (Phillips and Rainbow, 1994). According to Langston *et al.* (1998), the accumulation of Zn in oysters was found in detoxified granules and the bioavailability of these detoxified granules is relatively low to subsequent predators (Nott, 1998). This indicated that the raised Zn concentrations in the soft tissues of *I. alatus* may not be of ecotoxicological significance in the local diet (Silva *et al.*, 2003).

The wide ranges of Zn levels (Byssus: 94.6-678 µg/g dry weight; mantle plus gills: 414-1767 µg/g dry weight; muscle: 413-1662 µg/g dry weight; remainder: 374-1663 µg/g dry weight) indicated significant differences between sampling sites in the accumulated concentrations of Zn in the oyster populations. Given that the accumulated concentrations are time-integrated records of the bioavailability of each metal to the oysters (Phillips and Rainbow, 1994; Rainbow, 1995), the bioavailability of Zn varies geographically in all the eight oyster populations. The reason for the observed geographical variation in the bioavailabilities of Zn is most probably due to the input of Zn in dissolved and/or particulate form through anthropogenic activities such as shrimp ponds in Lukut, animal husbandry in Sepang and sewage, shipping and industrial effluents in Kg. Pasir Puteh. It is also inevitable that industrial

Table 2: Some physico-chemical parameters of the surface-water samples recorded during sampling time.

| No. | Sampling sites | Temp (C) | Cond (µs/cm) | TDS (mg/L) | Sali (ppt) |
|---|---|---|---|---|---|
| 1. | Sepang-1 | 28.01 ± 0.01 | 36619 ± 31.0 | 22.51 ± 0.01 | 21.69 ± 0.01 |
| 2. | Sepang-2 | 27.91 ± 0.03 | 36686 ± 144 | 22.59 ± 0.08 | 21.78 ± 0.08 |
| 3. | Lukut-1 | 29.89 ± 0.03 | 33343 ± 134 | 19.82 ± 0.09 | 18.81 ± 0.09 |
| 4. | Lukut-2 | 29.83 ± 0.05 | 28452 ± 255 | 16.93 ± 0.17 | 15.82 ± 0.17 |
| 5. | Lukut-3 | 29.40 ± 0.07 | 29706 ± 391 | 17.81 ± 0.21 | 16.73 ± 0.22 |
| 6. | Lukut-4 | 29.73 ± 0.01 | 33140 ± 184 | 19.76 ± 0.12 | 18.75 ± 0.12 |
| 7. | Lukut-5 | 30.14 ± 0.00 | 28093 ± 35.0 | 16.63 ± 0.02 | 15.50 ± 0.02 |
| 8. | KPPuteh | 30.81 ± 0.20 | 44721 ± 298 | 26.16 ± 0.10 | 25.56 ± 0.10 |

Note: Temp= temperature; Cond= conductivity; TDS= total dissolve solids; sal= salinity.

Table 3: Concentrations (mean ± standard error, µg/g dry weight) of Zn in the geochemical fractions of the surface sediments.

| Sampling sites | Total AR | Sum of SET | F1 | F2 | F3 | F4 | NR/R |
|---|---|---|---|---|---|---|---|
| Sepang-1 | 74.83 ± 1.91 C | 66.50 ± 0.69 E | 1.53 ± 0.03 E | 15.81 ± 0.56 C | 22.52 ± 0.32 D | 26.64 ± 0.42 D | 1.50 C |
| Sepang-2 | 88.25 ± 4.91 B | 106.15 ± 2.45 B | 1.55 ± 0.08 E | 19.08 ± 2.00 AB | 43.83 ± 2.61 B | 41.68 ± 1.76 B | 1.55 C |
| Lukut-1 | 45.52 ± 1.00 | 54.34 ± 2.55 F | 4.84 ± 0.22 A | 14.99 ± 0.17 C | 18.80 ± 2.13 E | 15.71 ± 0.36 E | 2.46 A |
| Lukut-2 | 79.34 ± 0.91 BC | 101.71 ± 0.16 B | 2.54 ± 0.14 C | 26.15 ± 0.18 A | 37.33 ± 0.64 C | 35.69 ± 0.47 C | 1.85 B |
| Lukut-3 | 74.29 ± 0.74 C | 97.15 ± 2.24 B | 2.04 ± 0.02 D | 22.25 ± 0.02 A | 33.97 ± 0.50 C | 38.88 ± 2.94 B | 1.50 B |
| Lukut-4 | 61.96 ± 2.82 D | 78.09 ± 0.40 D | 2.03 ± 0.04 D | 19.88 ± 0.08 AB | 23.47 ± 1.31 D | 32.70 ± 0.87 C | 1.39 C |
| Lukut-5 | 74.40 ± 1.21 C | 88.48 ± 0.46 C | 2.71 ± 0.03 C | 24.06 ± 1.19 A | 33.34 ± 1.19 C | 28.38 ± 0.50 D | 2.12 A |
| KPPuteh | 98.56 ± 0.49 A | 129.23 ± 0.24 A | 4.02 ± 0.02 B | 12.92 ± 0.05 D | 58.45 ± 0.17 A | 53.84 ± 0.34 A | 1.40 C |

Note: AR= total Zn concentrations obtained using direct aqua-regia method.
F1= easily, freely, leacheable or exchangeable (EFLE); F2= acid-reducible; F3= oxidisable-organic; F4= resistant; SUM of SET= summation of F1, F2, F3 and F4 based on sequential extraction technique; NR/R= ratio of nonresistant fractions (summation of F1, F2 and F3) to resistant fraction (F4). Student–Newman–Keuls (SNK) comparisons of Zn concentrations in geochemical fraction of the sediments from all sampling sites; same letters indicated they are not significantly different (P> 0.05).

From Table 4, an overall pattern is found, namely byssus (358 µg/g dry weight) < remainder (mean: 844 µg/g dry weight) ≤ muscle (mean: 851 µg/g dry weight) ≤ mantle plus gill (mean: 859 µg/g dry weight). This indicated that mantle plus gill, muscle and remainder were the main storage sites for Zn while byssus could be an excretion route for Zn as proposed in *Perna viridis* (Yap *et al.*, 2005b). This accumulation pattern for Zn pointed to the fact that marine oysters such as *I. alatus* are an excellent accumulator

waste from the Pasir Gudang industrial area had entered the Kg. Pasir Puteh coastal water via drainages which appeared then to be the major sources of the high availabilities of Zn in the Kg. Pasir Puteh oyster population and the sediments collected therein. This source of sewage and other domestic wastes most probably had increased the local bioavailabilities of Zn to the oyster populations (Bowen, 1979).

of Zn since the lowest Zn found in the byssus indicated low rate constants of loss (Luoma and Rainbow, 2005). High concentrations of Zn are accumulated in the mantle plus gill, muscle and remainder before the rate of excretion matches that of uptake. Mantle plus gill had higher concentrations of Zn when compared to remainder and byssus. This indicated that both the gills were good accumulators of Zn.

Table 4: Concentrations (mean ± standard error, µg/g dry weight) of Zn in different soft tissues of *Isognomon alatus* collected from all sampling sites.

| No. | Sampling sites | Tissues | N | Mean | SE | SNK |
|-----|----------------|---------|---|------|-----|-----|
| 1. | Sepang-1 | Mantle plus gill | 2 | 871.00 | 4.58 | A |
| | | Remainder | 3 | 883.00 | 9.26 | A |
| | | Byssus | 3 | 323.00 | 26.40 | B |
| | | Muscle | 2 | 878.00 | 2.11 | A |
| 2. | Sepang-2 | Mantle plus gill | 3 | 898.00 | 3.35 | A |
| | | Remainder | 2 | 890.00 | 9.08 | A |
| | | Byssus | 3 | 621.00 | 244.00 | B |
| | | Muscle | 2 | 900.00 | 23.70 | A |
| 3. | Lukut-1 | Mantle plus gill | 3 | 414.00 | 3.79 | A |
| | | Remainder | 3 | 374.00 | 33.60 | C |
| | | Byssus | 3 | 393.00 | 2.02 | B |
| | | Muscle | 3 | 413.00 | 3.94 | A |
| 4. | Lukut-2 | Mantle plus gill | 3 | 660.00 | 70.30 | B |
| | | Remainder | 3 | 722.00 | 3.30 | A |
| | | Byssus | 2 | 261.00 | 27.20 | C |
| | | Muscle | 3 | 726.00 | 3.24 | A |
| 5. | Lukut-3 | Mantle plus gill | 3 | 973.00 | 19.9 | A |
| | | Remainder | 3 | 940.00 | 8.81 | A |
| | | Byssus | 3 | 94.60 | 14.10 | B |
| | | Muscle | 3 | 943.00 | 6.73 | A |
| 6. | Lukut-4 | Mantle plus gill | 3 | 647.00 | 1.42 | A |
| | | Remainder | 3 | 640.00 | 1.27 | A |
| | | Byssus | 3 | 320.00 | 133.00 | B |
| | | Muscle | 3 | 646.00 | 1.75 | A |
| 7. | Lukut-5 | Mantle plus gill | 3 | 645.00 | 2.33 | A |
| | | Remainder | 3 | 637.00 | 1.33 | A |
| | | Byssus | 3 | 177.00 | 76.20 | B |
| | | Muscle | 3 | 637.00 | 0.56 | A |
| 8. | KPPuteh | Mantle plus gill | 3 | 1767.00 | 5.81 | A |
| | | Remainder | 3 | 1663.00 | 9.340 | A |
| | | Byssus | 3 | 678.00 | 26.80 | B |
| | | Muscle | 3 | 1662.00 | 17.40 | A |
| | Overall | Mantle plus gill | 8 | 859 (414-1767) | 144 | A |
| | | Remainder | 8 | 844 (374-1663) | 134 | A |
| | | Byssus | 8 | 358 (95-678) | 72 | B |
| | | Muscle | 8 | 851 (413-1662) | 131 | A |

Note: Values in brackets are ranges or minimum to maximum levels. Student–Newman–Keuls (SNK) comparisons of Zn concentrations in different tissues of the oysters from all sampling sites; same letters indicated they are not significantly different (P> 0.05).

This was due to the high surface area of the mantle and gill and the chemical nature of the mucus on the gills (Cunningham, 1979). Oysters are typically filter-feeders, therefore, all small particles and food in the water are filtered in the gills. The particles trapped are absorbed by the mucus in the gill, and the large surface area of the gill tissue enhances this process. Their close contact with the surrounding water resulted in greater and faster accumulation of metals (Yap *et al.*, 2006b).

Based on Figure 2, byssus was clustered differently from mantle plus gills, remainder and muscles. This indicated that byssus had different mechanisms of Zn accumulation when compared to other soft tissues. Based on Figure 3, it is found that Kg. Pasir Puteh had distinctly a higher Zn bioavailability among all the populations since it is clustered differently from the other populations of the Lukut River and the Sepang Besar River. This is basically due to the fact that the four soft tissues of Kg. Pasir Puteh had the highest Zn levels when compared to those of the other populations from Sepang and Lukut.

Figure 4 shows that the EFLE is clustered differently from other geochemical fractions. This indicated that the EFLE fraction had a different source of Zn input from the others. From Figure 5, based on all the geochemical fractions of the Zn in the surface sediments, it is
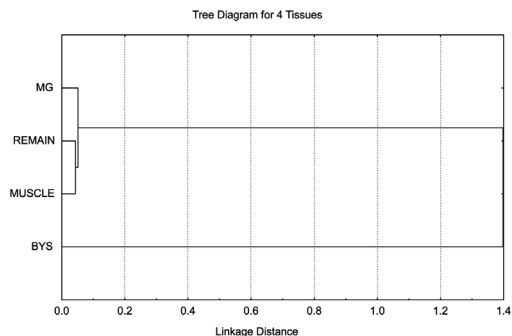
Figure 2: Hierachical cluster analysis based on Zn concentrations (log $_{10}$ mean +1) in the different parts of *Isognomon alatus* from 8 populations. Note: Mantle plus gill; Remain= remaining soft tissues/remainder; BYS= byssus.
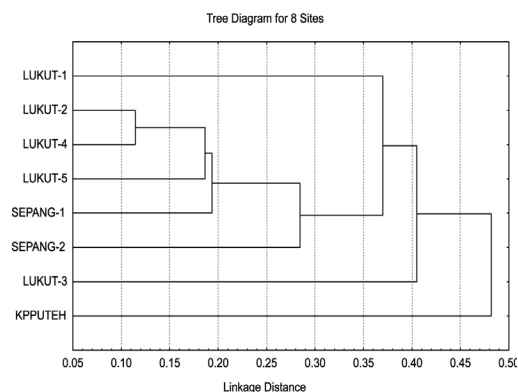


Figure 4: Hierachical cluster analysis based on Zn concentrations (log$_{10}$ [mean +1]) in the geochemical fraction of surface sediments of 8 sampling sites. Note: AR= total Zn concentrations using direct aqua-regia method; F1= easily, freely, leacheable or exchangeable (EFLE); F2= acid-reducible; F3= oxidisable-organic; F4= resistant; SUM= summation of F1, F2, F3 an F4.



Figure 3: Hierachical cluster analysis based on Zn concentrations (log $_{10}$ mean +1) of four different tissues of 8 *Isognomon alatus* oyster populations.



Figure 5: Hierachical cluster analysis based on Zn concentrations (log $_{10}$ mean +1) of 4 geochemical fractions in the 8 sampling sites.

found that Kg. Pasir Puteh is not really clustered differently from the other sampling sites. Hence, this clustering pattern is not in agreement with that found for the oyster population clustering pattern (Figure 3). This could be due to the fact that the highest Zn levels in Kg. Pasir Puteh were only found for total summation of all geochemical fractions, oxidisable-organic fraction and resistant fraction. However, since the highest levels of EFLE and acid-reducible fractions were found in Lukut-1 and Lukut-2, respectively, this could be the reason why Kg. Pasir Puteh was not clustered differently from other sites. Furthermore, the highest ratio of NR/R (1.46) was found at Lukut-1 (Table 3),
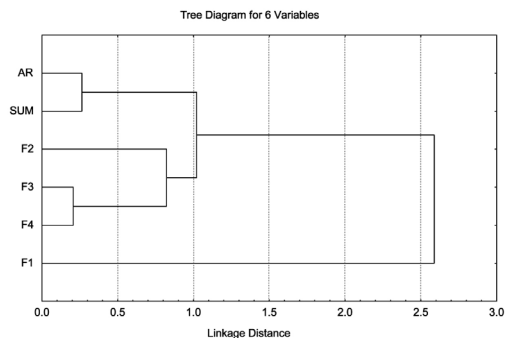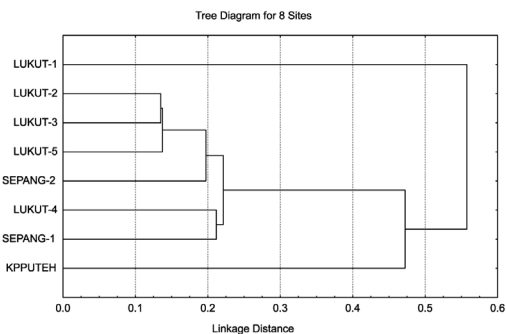
thus, explaining why Lukut-1 was clustered differently form other sampling sites.

When the oyster Zn concentrations are compared to those found in their surface sediments (Table 3), Zn was highly bioaccumulated in oyster when compared to the sediment. It has been well reported in the literature that Zn is easily accumulated in oysters, by far surpassing the maximum limit of tolerance (Frias-Espericueta *et al.*, 1999). The present results confirm this trend showing the capacity of oysters to metabolically control high Zn concentrations in the different soft tissues.

It is well known that living organisms require trace amounts of Zn. Many metals

exist naturally, at background levels in the environment. Zn is biologically essential and plays an important role as cofactors in enzymatic processes (Singh and Steinnes, 1994) and in the physiology of bivalves (Sajwan *et al.*, 2008). This suggests that physiologically, Zn should have higher assimilation efficiency as compared to those of other metals.

Table 5 compares the data collected in this study with other reported data for the genus *Isognomon* elsewhere in the world. However, it is difficult to compare the reported Zn levels in the total soft tissues by Sbriz *et al.* (1998) and Saed *et al.* (2001), with mantle plus gill, muscle and remainder as presented in this study. Therefore, it is incomparable. It must be noted that the comparison is based on similar oyster species and therefore, the comparison does provide us a general picture on Zn levels in the different tissues and total soft tissues of *I. alatus*. The Zn concentrations in muscle (1662 µg/g dry weight), mantle plus gill (1768 µg/g dry weight) and remainder (1663 µg/g dry weight) of *I. alatus* from Kg. Pasir Puteh can be interpreted to be high when compared to Zn levels in populations from Lukut and Sepang Besar. These Zn levels were also higher than mantle (302-315 µg/g dry weight), gill (317-326 µg/g dry weight), remainder (304-311 µg/g dry weight) and muscle (300-305 µg/g dry weight) of rock oyster *Saccostrea cucullata* collected from Penang Bridge (Yap *et al.*, 2010). Thefore, present Zn levels in oyster population from Kg. Pasir Puteh indicated an atypically raised bioavailability of Zn. Even though other potential influences of size, age, sex reproductive stage, and physiological condition on the concentrations of Zn in oysters cannot be ruled out (Phelps *et al.*, 1985; Presley *et al.*, 1990), the present results indicated that anthropogenic sources are the major cause of raised Zn levels in both the oysters and the surface sediments at the sampling site in Kg. Pasir Puteh.

Finally, we propose the use of different soft tissues of *I. alatus* as biomonitors of bioavailabilities and contamination by Zn in the mangrove areas of Peninsular Malaysia due to following positive points. First, plentiful literature documenting heavy metals using marine oysters (although usually based on total soft tissues) can be easily found and its usefulness has been widely accepted for the biomonitoring purpose. Second, the use of different soft tissues of molluccscs can reduce erroneous results due to spawning and the problem of defaecation before dissection could be overcome (Yap *et al.*,

Table 5: Comparisons of concentrations (µg/g dry weight) of Zn in *Isognomon alatus* from this study with other reported studies.

| No | Locations | Part(s) | Concentrations | References |
|---|---|---|---|---|
| 1. | Rio San Juan, Dominican Republic | Total soft tissue | 4000 | Sbriz *et al.* (1998) |
| 2. | Sg. Sepang Besar, Selangor, Malaysia. | Total soft tissue | 148-564 | Saed *et al.* (2001) |
| 3. | Sg. Sepang Kecil, Selangor, Malaysia. | Total soft tissue | 23-24 | Saed *et al.* (2001) |
| 4. | Sg Sepang Besar, Selangor (2 sites) | Remainder | 884-1299 | This study |
| | | Mantle plus gill | 871-1262 | |
| | | Muscle | 879-1291 | |
| | | Byssus | 323-901 | |
| 5. | Sg. Lukut, Negeri Sembilan (5 sites) | Remainder | 374-940 | This study |
| | | Mantle plus gill | 414-973 | |
| | | Muscle | 413-943 | |
| | | Byssus | 94.6-393 | |
| 6. | Kg. Pasir Puteh, Johore (1 site) | Remainder | 1663 | This study |
| | | Mantle plus gill | 1768 | |
| | | Muscle | 1662 | |
| | | Byssus | 495 | |

2006a). For example, muscle is least affected by spawning condition since it did not merge with gonadal tissues like mantle and gill. Byssus can also be used for the same purpose since it is an excretion route for metals. Interpreting the metal data in the different soft tissues of *I. alatus* and surface sediment, a more accurate interpretation of the bioavailability and contamination by Zn in coastal waters could be obtained.

## Conclusions

The oyster *I. alatus* clearly has great potential for further biomonitoring of trace metal availabilities in the mangrove-lined estuaries of the west coast of Peninsular Malaysia that are coming under increasing pressure from land-based development. It is of paramount importance to use *I. alatus* to continually trace the current metal levels in the mangrove areas of Peninsular Malaysia since marine oysters have been widely used as biomonitors for Zn. The use of different soft tissues of *I. alatus* as biomonitors of bioavailability and contamination by Zn in the mangrove area of Peninsular Malaysia is proposed, for future biomonitoring purpose. The use of muscle for a more accurate interpretation of the bioavailability and contamination by Zn in coastal waters is also suggested.

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