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Concentrations of Heavy Metals in Different Tissues of the Bivalve *Polymesoda erosa*: Its Potentials as a Biomonitor and Food Safety Concern

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ABSTRACT

Three populations of *Polymesoda erosa* collected from the west coast of Peninsular Malaysia were analyzed for heavy metals. Their soft tissues were dissected into muscle, foot, mantle, gill and remaining soft tissues (remainder). Overall metal concentrations (µg/g dry weight) in five soft tissues of the three clam populations were Cd (0.25-2.86), Cu (1.80-21.0), Ni (0.66-30.0), Pb (0.94-7.09), and Zn (79.2-365), and these were Cd (3.64-7.07), Cu (2.37-3.29), Ni (26.2-30.0), Pb (58.8-61.6), and Zn (3.84-8.78) for the shell ranges. Among the three *Polymesoda* populations, gill was found to have accumulated higher Cu and Zn concentrations compared to other soft tissues, whereas shell was found to have high levels of non-essential Cd, Pb and Ni. Information on heavy metals obtained in this study could serve as baseline data for this particular species since the information is lacking in the literature. The present study has evidently shown that different soft tissues of *P. erosa* are potential biomonitoring for Cd, Cu, Ni and Zn, whereas the clam shell as a potential biomonitoring material for Pb based on: (1) positive results based on biota-sediment accumulation factors (BSAF) (being macroconcentrators), (2) positive and significant correlations of metals between all five soft tissues (foot, gill, mantle, muscle and remainder) and the sedimentary geochemical fractions and total metal concentrations, and (3) comparisons to two similar burrowing bivalves (*Donax faba* and *Gelonia expansa*). Regardless of some metals in edible soft tissues having exceeded the food safety permissible limits, the concentrations of Cd, Cu, Ni and Zn in the soluble fractions (which is more bioavailable to consumers) of all the five edible soft tissues of *P. erosa* are below all the permissible metal limits. Therefore, these estimations clearly showed that the consumption of *P. erosa* could pose no toxicological risks to consumers.

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INTRODUCTION

Elevated concentrations of heavy metals have been recorded in intertidal areas all over the world, which often reflect long-term pollution caused by human activities (Hedouin *et al.*, 2009; Sakellari *et al.*, 2013; Zhao *et al.*, 2013). High degrees of industrialization and urbanization have led to strong risks of heavy metal contamination in the intertidal environment, particularly mangrove ecosystems in tropical countries (Lewis *et al.*, 2011; Wang *et al.*, 2013).

As a part of our continual biomonitoring effort following Mussel Watch in Malaysia (Yap, 2012) to assure that the pollution levels are controlled, this study focused on *Polymesoda erosa* (Corbicilidea family) which is a burrowing bivalve compared to the well-studied suspension-feeder green-lipped mussel *Perna viridis*. From the literature, this clam species is known to be hardy since they experience low pH, as indicated by the shape of the shells, which are badly eroded by acid mangal sediment (Morton, 1976). This clam species inhabits mangrove mudflats, which are under the constant influence of varying environmental stresses such as broad salinity range (7-22 ppt) (Ingole *et al.*, 1994) and it has been reported to possess high bioaccumulation capacity of heavy metals from ambient water (Modassir, 2000). Economically, it is an important, large and fleshy bivalve which can usually grow to marketable shell sizes ranging from 6 to 11 cm in length (Gimin *et al.*, 2004). In general, clams are good biomonitor of metal pollution (see Tarique *et al.*, 2013). Therefore, the above review generally supports the use of *P. erosa* as a potential biomonitor, besides being sedentary, long-lived, widely distributed in the mangrove area and the metal concentrations found in the soft tissues of bivalves can provide a time-integrated measurement of bioavailability of metals in the coastal waters (Phillips & Rainbow, 1993; Rainbow, 1995; Yap *et al.*, 2006).

The objectives of the present study were to: 1) provide concentrations of heavy metals in the different tissues of *P. erosa* from Malaysia; 2) assess its potential as a biomonitor for heavy metals; and 3) check whether the metal levels in the edible tissues of *P. erosa* exceeded some of the food safety permissible limits based on the total metals (total fractions) and soluble fractions in the edible soft tissues of the clams.

MATERIALS AND METHODS

About 25-30 of the bivalve *P. erosa* individuals from Telok Mas (Malacca), Sg. Sepang Kecil (Selangor) and Parit Jawa (Johore) (Fig.1), Peninsular Malaysia, were used for the metal analysis. However, surface sediment was only collected from the sampling site at Sg. Sepang Kecil due to the fact that the other two sites were actually bought from roadside stalls. In order to compare with the same burrowing bivalves, two different bivalves species, namely, *Donax faba* and *Gelonia expansa*, were collected from Pasir Panjang (Negeri Sembilan) and Kg. Pasir Puteh (Johore)

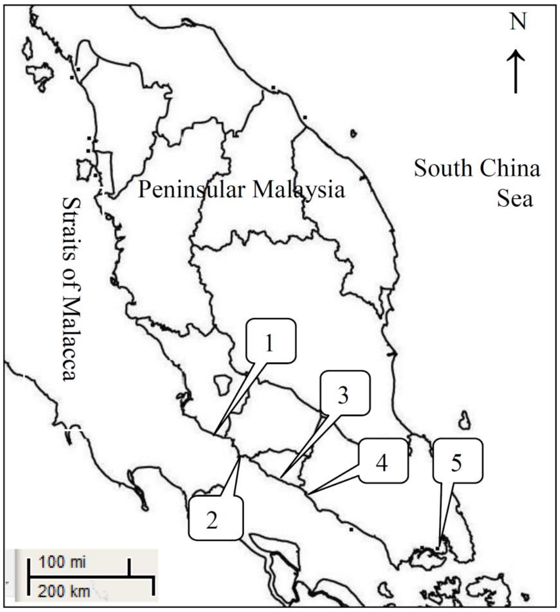

Fig.1: Map showing all the sampling sites of bivalves in Peninsular Malaysia. Note: 1= Sg. Sepang Kecil; 2= Pasir Panjang; 3= Telok Mas; 4= Parit Jawa; 5= Kg. Pasir Puteh.

(Fig.1), respectively, together with their habitat surface sediments. All the samples were collected between 28 and 30 April 2006. The identification of the bivalves was based on the book by Malaysia Fisheries Directory (2005) issued by the Department of Fisheries Malaysia. All bivalves were measured for shell lengths, shell heights, shell widths, total soft tissues wet weight, total soft tissues dry weight, condition index (CI) and water contents, and their values are presented in Table 1.

All the bivalve populations were dissected, divided and pooled into muscle, foot, mantle, gill and remaining soft tissues (remainder). The sediments, shells, and all the five different pooled categories of bivalve soft tissues were dried at 60°C in an oven to constant dry weights. Later, the dried sediments were grinded by using pestle and mortar, and sifted through a 63 µm stainless steel aperture.

Geochemical fractions of Cd, Cu, Ni, Pb and Zn in the sediments were obtained by using the modified sequential extraction technique (SET), as described by Badri and Aston (1983). Two replicates (N= 2) were obtained from the sieved main sample. The four fractions used in the present study were easily, freely, leachable or exchangeable (EFLE), acid-reducible (AR), oxidisable-organic (OO) and resistant fractions.

Triplicates of each dried category of the bivalve tissues were digested in concentrated HNO_3 (Analar grade, BDH 69%). The resistant fractions of the SET were digested in a combination of concentrated nitric acid (69%) and perchloric acid (60%) (ratio 4:1), as in the direct aqua-regia method. They were first placed in a hot-block digester first at a low temperature for 1 hour and then fully digested at a high temperature (140°C) for at least 3 hours (Yap *et al.*, 2003a).

The digested samples were diluted to 40 mL with double-distilled water. After filtration, the concentrations of Cd, Cu, Ni, Pb and Zn were determined by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. All data are presented in µg/g dry weight.

In order to see the difference of metal bioaccumulation between two different size groups of *P. erosa*, the clams of a single population was divided into two groups of shell lengths: 60.0-66.1mm and 50.0-57.8mm. Total soft tissues of the two size groups were analyzed similarly for the five metals described previously.

In order to estimate the metal portion soluble or to make it more available to consumers, the soluble and insoluble metal fractions in the different soft tissues of the three *P. erosa* populations were determined, whereby the two fractions were separated by using a modified method as described by Bragigand *et al.* (2004). Ground dried soft tissues (0.5 g) were homogenized with 2mL of Ultra-turrax in a TRIS buffer solution 20nM, NaCl 150 mM, pH 8.6, at 4°C, based on 4 mL/g fresh weight, as suggested by Bragigand *et al.* (2004). The soluble and insoluble fractions were separated by centrifugation (25 rpm for 55 min). After centrifugation, S fraction was analyzed using AAS. The insoluble fraction was further digested with 2.5 mL of nitric acid (HNO_3) (1 mL of HNO_3 per 0.2 g of I). After digestion, acid solutions were made up to 10 mL with double distilled water (DDW), before being analyzed similarly for the five metals described previously.

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, Pb and Zn were analyzed after every 5-10 samples in order to check for sample accuracy. Besides, the analytical procedures for the bivalves and soils were checked with the Certified Reference Material (CRM) for dogfish liver (DOLT-3, National Research Council Canada) and soil (International Atomic Energy Agency, Soil-5, Vienna, Austria). The recoveries of all the metals were satisfactory (see Table 2).

In this study, CI of the bivalves was determined because it is an indicator of the bivalve's physiological state and also as a guide to the bivalve's metabolic response to environmental stress (Yap *et al.*, 2002a). The CI (g/cm^3) value was calculated as the quotient of the total soft tissue dry weight for each bivalve and the shell volume (shell width [cm] × shell length [cm] × shell height [cm]) for each bivalve, multiplied by a constant, namely, 1000 (Lares & Orian, 1997; Yap *et al.*, 2003b). The formula is as follows:

$$\frac{\text{total soft tissue dry weight (g)}}{\text{Shell volume (cm}^3)} \times 1000$$

In order to estimate the distribution of metals occurred between the different tissues of bivalves and in associated

TABLE 1
Measurements (mean ± standard error) of shell lengths (SL; cm), shell heights (SH; cm), shell widths (SW; cm), total soft tissues wet weight (WW; g), total soft tissues dry weight (DW; g) condition index (CI; g/cm^3) and water contents (WC; %) of the three species of bivalves in the present study

Species	N	SL	SH	SW	WW	DW	CI	WC
Polymesoda								
Telok Mas	10	5.29 ± 0.06	4.71 ± 0.07	2.81 ± 0.04	6.83 ± 0.23	0.95 ± 0.04	13.6 ± 0.50	86.0 ± 0.62
SSK	10	6.28 ± 0.06	5.84 ± 0.05	3.35 ± 0.05	5.62 ± 0.28	0.81 ±0.04	6.57 ± 0.26	85.6 ± 0.39
Parit Jawa	5	5.50 ± 0.09	4.90 ± 0.12	3.19 ± 0.10	8.52 ± 0.89	1.32 ± 0.15	15.3 ± 1.14	84.4 ± 1.11
Donax faba	19	3.68 ± 0.15	2.64 ± 0.13	1.83 ± 0.11	2.59 ± 0.34	0.51 ± 0.07	26.0 ± 0.82	80.6 ± 0.36
Gelonia expansa	10	7.16 ± 0.33	6.71 ± 0.29	3.92 ± 0.18	12.5 ± 0.73	1.94 ± 0.18	9.32 ± 0.31	84.5 ± 0.59

Note: N= number of samples analyzed. SSK= Sungai Sepang Kecil

TABLE 2
Comparison of heavy metal concentrations between measured and certified values of certified reference material for Dogfish liver and soil. All values are presented in µg/g dry weight

Metal	Sample	Certified values (C)	Measured values (M)	Recovery (M/C × 100%)
Cd	Dogfish-liver	19.4 ± 0.600	20.5 ± 0.439	106 ± 2.26
	Soil-5	1.5	1.41	94.60
Cu	Dogfish-liver	31.2 ± 1.00	26.5 ± 2.58	85.0 ± 8.28
	Soil-5	77.1	72.9	94.4
Pb	Dogfish-liver	NA	NA	NA
	Soil-5	129	144.7	112.2
Ni	Dogfish-liver	2.72 ± 0.350	2.77 ± 0.741	102 ± 27.2
	Soil-5	13.0	12.3	94.6
Zn	Dogfish-liver	86.6 ± 2.40	80.9 ± 1.94	93.4 ± 2.24
	Soil-5	368	326	88.6

Note: CRM value is not available.

sediment, biota-sediment accumulation factors (BSAF) was calculated (based on a formula by Szefer *et al*., 1999; see below) for the five metals in the *P. erosa* from Sg. Sepang Kecil, *D. faba* and *G. expansa,* where their habitat surface sediments were collected and these three bivalves species are burrowers.

$$\mathrm{BSAF} = \frac{\mathrm{C}x}{\mathrm{C}s}$$

Where Cx and Cs are the mean metal concentrations in the different tissues of bivalves and non-resistant fractions in the habitat surface sediment, respectively. In the present study, the non-resistant fractions (summation of EFLE, AR and OO) were used as sediment metal value in the BSAF calculation because the non-resistant is more related to its bioavailability characteristic to the living organisms and of anthropogenic sources (Yap & Wong, 2011).

For statistical analyses, comparison of metal levels of any two different size groups was performed by using t-test. On the other hand, Analysis of Variance (ANOVA) was applied to test the differences between the mean values of the metal concentrations with the subsequent comparison between individual means using Student-Newman-Kuels (S-N-K) multiple comparison test (Zar, 1999). The relationships between the different parts and sediment were tested using Pearson's correlation coefficient based on the concentrations of Cd, Cu, Ni, Pb and Zn. The correlation analysis was carried out by using log transformed data (Leung *et al*., 2005). All the above statistical analyses were performed by using SPSS version 12.

RESULTS AND DISCUSSION

The mean values of condition index (CI), water contents and other allometric parameters of *P. erosa* are shown in Table 1. The CI of the clams collected from Sg. Sepang Kecil, Parit Jawa and Telok Mas, was 6.57 ± 0.261 g/cm^3, 15.3 ± 1.14 g/cm^3 and 13.6 ± 0.50 g/cm^3, respectively. The percentages of water content were 85.6 ± 0.39%, 84.4 ± 1.11% and 86.0 ± 0.62% for Sg. Sepang Kecil, Parit Jawa and Telok Mas, respectively, and these are within the ranges reported for the oyster species [72.5-90.0%] (Watling & Watling, 1976), the blue mussel *Mytilus edulis* [85.8%] and *Perna viridis* [81.8-85.4%] (Yap *et al*., 2003b).

i) Metal concentrations in the different tissues of bivalves

Heavy metal concentrations in the different tissues of *P. erosa* are shown in Table 3. The overall metal concentrations (µg/g dry weight) in the five soft tissues (foot, gill, mantle, muscle and remaining soft tissues) of three *Polymesoda* populations are Cd (0.25-2.86), Cu (1.80-21.0), Ni (0.66-30.0), Pb (0.94-7.09), and Zn (79.2-365), while for the shell ranges are Cd (3.64-7.07), Cu (2.37-3.29), Ni (26.2-30.0), Pb (58.8-61.6), and Zn (3.84-8.78).

When compared to soft tissues of *P. erosa*, the metal ranges (µg/g dry weight) for the two other burrowing bivalve species (*D. faba* and *G. expansa*) are broader and higher in Cd (0.30-3.92), Cu (4.71-79.8), Pb (0.95-39.7), and Zn (28.3-379), but these are narrower and lower in Ni (1.58-15.4). When compared to shells of *P. erosa*, the metal

TABLE 3
Concentrations (mean ± standard error, µg/g dry weight) of heavy metals in the different soft tissues of three populations of *Polymesoda erosa*, in comparison to those of *Donax faba* and *Gelonia expansa* populations

Species	Tissues	Cd	Cu	Ni	Pb	Zn
Polymesoda erosa						
Parit Jawa	Foot	1.47 ± 0.14^{ab}	3.77 ± 0.28^{a}	7.83 ± 0.45^{a}	2.18 ± 0.66^{a}	92.1 ± 11.4^{b}
	Gill	1.46 ± 0.21^{ab}	12.8 ± 0.19^{c}	11.1 ± 0.36^{b}	2.56 ± 0.29^{a}	263 ± 0.75^{c}
	Mantle	2.40 ± 0.11^{c}	9.34 ± 0.60^{b}	15.1 ± 0.67^{c}	6.44 ± 0.35^{b}	270 ± 1.14^{c}
	Muscle	1.79 ± 0.08^{b}	3.58 ± 0.23^{a}	7.76 ± 0.20^{a}	3.11 ± 0.72^{a}	104 ± 9.88^{b}
	Remainder	1.17 ± 0.09^{a}	11.8 ± 0.49^{c}	6.22 ± 0.36^{a}	2.07 ± 0.61^{a}	79.2 ± 4.17^{b}
	Shell	7.07 ± 0.22^{d}	3.17 ± 0.36^{a}	28.9 ± 0.40^{d}	61.6 ± 1.67^{c}	6.47 ± 0.16^{a}
Sg. Sepang Kecil	Foot	0.48 ± 0.11^{a}	3.36 ± 0.53^{a}	3.98 ± 0.24^{a}	2.10 ± 0.40^{a}	87.5 ± 14.1^{b}
	Gill	1.22 ± 0.00^{bc}	21.0 ± 0.00^{e}	23.5 ± 0.00^{c}	4.61 ± 0.00^{a}	349 ± 0.00^{c}
	Mantle	1.36 ± 0.00^{c}	13.2 ± 0.00^{c}	12.6 ± 0.00^{b}	4.79 ± 0.00^{a}	365 ± 0.00^{c}
	Muscle	0.78 ± 0.10^{ab}	5.76 ± 0.13^{b}	5.28 ± 0.07^{a}	3.41 ± 0.16^{a}	150 ± 9.14^{b}
	Remainder	0.73 ± 0.10^{ab}	14.9 ± 0.69^{d}	5.26 ± 0.32^{a}	4.35 ± 0.34^{a}	154 ± 32.4^{b}
	Shell	3.64 ± 0.12^{d}	2.37 ± 0.30^{a}	30.0 ± 0.44^{d}	58.8 ± 1.47^{b}	3.84 ± 0.77^{a}
Telok Mas	Foot	0.25 ± 0.02^{a}	1.80 ± 0.23^{a}	0.66 ± 0.15^{a}	0.94 ± 0.25^{a}	105 ± 0.53^{b}
	Gill	2.86 ± 0.00^{c}	8.81 ± 0.00^{c}	10.7 ± 0.00^{c}	6.51 ± 0.00^{c}	263 ± 0.00^{d}
	Mantle	1.64 ± 0.30^{b}	5.82 ± 0.23^{b}	11.8 ± 0.04^{d}	7.09 ± 0.25^{c}	264 ± 2.30^{d}
	Muscle	0.70 ± 0.08^{a}	1.86 ± 0.12^{a}	2.61 ± 0.34^{b}	1.89 ± 0.53^{ab}	129 ± 2.27^{c}
	Remainder	0.61 ± 0.11^{a}	8.60 ± 0.87^{c}	1.87 ± 0.49^{ab}	3.59 ± 0.62^{b}	138 ± 8.28^{c}
	Shell	6.60 ± 0.18^{d}	3.29 ± 0.40^{a}	26.2 ± 0.36^{e}	59.6 ± 0.72^{d}	8.78 ± 1.27^{a}
Donax faba						
Pantai Pasir Panjang	Foot	3.68 ± 0.14^{a}	5.98 ± 0.39^{a}	1.58 ± 0.09^{a}	2.36 ± 0.08^{c}	38.8 ± 0.66^{c}
	Gill	3.92 ± 0.18^{a}	12.9 ± 0.54^{c}	4.76 ± 0.15^{c}	2.09 ± 0.08^{c}	93.4 ± 1.16^{e}
	Mantle	2.95 ± 0.14^{a}	6.86 ± 0.10^{a}	3.25 ± 0.58^{b}	1.47 ± 0.10^{b}	46.8 ± 2.29^{d}
	Muscle	3.74 ± 0.35^{a}	4.71 ± 0.20^{a}	2.03 ± 0.44^{ab}	2.51 ± 0.13^{c}	28.3 ± 0.81^{b}
	Remainder	2.74 ± 0.17^{a}	15.1 ± 1.32^{d}	2.70 ± 0.33^{ab}	0.95 ± 0.16^{a}	42.6 ± 0.45^{cd}
	Shell	7.20 ± 0.43^{b}	9.88 ± 0.16^{b}	28.5 ± 0.19^{d}	5.27 ± 0.17^{d}	4.56 ± 0.01^{a}
Gelonia expansa						
Kg. Pasir Puteh	Foot	0.36 ± 0.09^{a}	79.8 ± 32.6^{a}	9.47 ± 0.18^{a}	19.4 ± 2.53^{b}	124 ± 3.56^{b}
	Gill	1.21 ± 0.00^{b}	26.2 ± 0.00^{a}	15.4 ± 0.00^{c}	19.5 ± 0.00^{b}	379 ± 0.00^{e}
	Mantle	0.71 ± 0.17^{ab}	61.6 ± 1.92^{a}	11.4 ± 0.05^{b}	39.7 ± 0.93^{c}	275 ± 1.42^{d}
	Muscle	0.30 ± 0.03^{a}	7.73 ± 2.67^{a}	10.4 ± 0.65^{ab}	4.53 ± 0.91^{a}	214 ± 7.01^{c}
	Remainder	0.59 ± 0.16^{ab}	19.7 ± 0.71^{a}	8.58 ± 0.40^{a}	4.72 ± 1.07^{a}	180 ± 34.6^{c}
	Shell	6.99 ± 0.29^{c}	2.80 ± 0.26^{a}	25.0 ± 0.48^{d}	56.9 ± 0.65^{d}	6.67 ± 0.30^{a}

Note: Values sharing similar alphabet are not significant different with others, $P > 0.05$.

ranges (µg/g dry weight) for the other two burrowing bivalve species are narrower but slightly higher in Cd (6.99-7.20), broader, and also higher in Cu (2.80-9.88), almost within the Ni range (25.0-28.5), broader but slightly lower in Pb (5.27-56.6), and within the Zn range (4.56-6.67). However, it is difficult to assess whether *P. erosa* is a good biomonitor for heavy metals based on the above comparisons. The following section (ii) on the correlation analysis and BSAF values could better assess the potential of *P. erosa* as a biomonitor.

Higher metal concentrations found in the different tissues of *P. erosa* from Sg. Sepang Kecil indicated a high metal contamination at the sampling site. This indication was complemented by the lower condition index (CI) values found in the Sg. Sepang Kecil samples than two other locations. This also suggested that *P. erosa* from Sg. Sepang Kecil could have suffered from metal pollution stress, since the different tissues of these clam exhibited the highest concentrations (µg/g dry weight) of Cu in the remainder (14.9 ± 0.69) and gill (21.0); and Zn in the mantle (365) and gill (349). The negative relationships between the bivalves' CI values and metal concentrations in the mussel soft tissues have been reported by Yap *et al.* (2002a). Therefore, it is assumed that the higher metal levels and lower CI values in *P. erosa* collected from Sg. Sepang Kecil have a higher metal bioavailabilities and contamination at the sampling site. The higher Cu and Zn levels found in some tissues of *P. erosa* collected from Sg. Sepang Kecil could be explained by the domination of non-resistant fractions of Cu (63.9%; Yap *et al.*, 2013) and Zn (55.1%; Yap *et al.*, 2011) in the surface sediments, indicating anthropogenic sources (Yap & Pang, 2011).

Furthermore, the high metal concentrations in the clams indicated high metal bioavailabilities of metals at the sampling site. This is because analysis of bivalves can provide measurement of the integrated bioavailability of metals in coastal environment (Phillips & Rainbow, 1993; Rainbow, 1995; Silva *et al.*, 2001; Rainbow *et al.*, 2002; Yap *et al.*, 2006).

The shells of *P. erosa* are the best accumulators of non-essential Cd, Ni and Pb. The shells of *P. erosa* from Parit Jawa exhibited the highest concentrations of Cd (7.07 ± 0.216 µg/g dry weight) and Pb (61.6 ± 1.67 µg/g dry weight), indicating Cd and Pb of water and sediment as sources of these metals in Parit Jawa. The assumption could be made because bivalve shells have been used quite frequently as biomonitoring materials for heavy metals (see Yap *et al.*, 2003c; Lazaretha *et al.*, 2003; Gillikin *et al.*, 2005; Protasowicki *et al.*, 2008).

Fig.2 shows the heavy metal concentrations in the two different size groups of bivalve *P. erosa*. Smaller sized groups (50.0-57.8mm) accumulated higher concentrations of nonessential Cd, Ni and Pb (although not significantly different for Cd and Ni; $p>0.05$) when compared with the larger sized groups (60.0-66.1mm). This is in accordance with the results reported by Gilek *et al.* (1996) and Yap *et al.* (2003d) that smaller bivalves accumulate greater

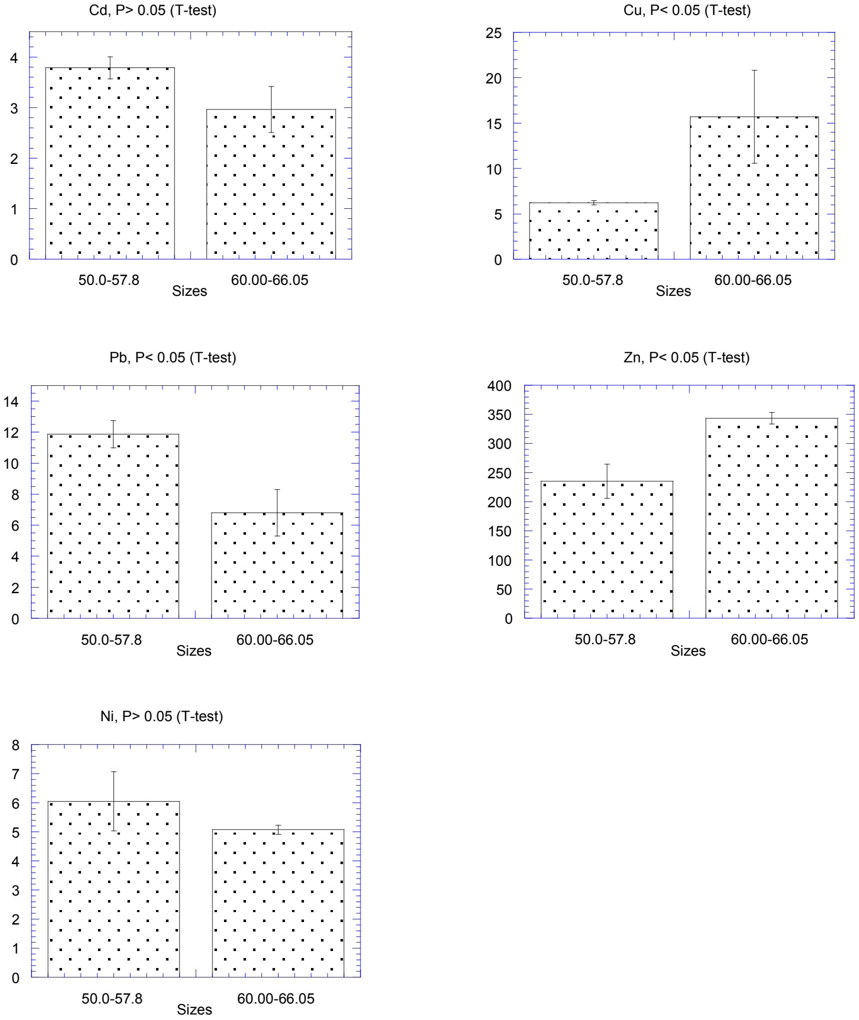

Fig.2: Comparisons of concentrations (mean ± standard error, µg/g dry weight) of heavy metals [nonessential (Cd, Ni and Pb) and essential (Cu and Zn)] between two significant different sized groups (shell lengths: 60.0-66.1mm and 50.0-57.8mm) of *Polymesoda erosa.*

concentrations of heavy metal. According to Gilek *et al.* (1996), tissue concentrations decreased with increasing body size, and the size-dependent dissimilarities in bioaccumulation were caused primarily by size-related differences in uptake rate. However, larger individual accumulated more concentrations of Cu and Zn when compared with the smaller individuals ($p<0.05$). This could be due to higher metabolic rates probably possessed by the larger individual may be resulting in higher accumulation rates of Cu and Zn, since these two metals are essential to the living organisms. Also, this could be explained by the differences in the accumulation pathways and the processes affecting the bioavailability of the nonessential metals to *P. erosa* are dissimilar to essential metals (Yap *et al.*, 2003d).

The gill and the remainder of *P. erosa* accumulated higher concentrations of Cu when compared to mantle and the other soft tissues. Meanwhile, the mantle and the gill accumulated higher concentrations of Zn than other tissues (Fig.3). The metal distribution in the different tissues of bivalves could be related to cellular processes participated in metal metabolisms (Marigómez *et al.*, 2002). For example, the higher metal levels found the gills could be explained by the gill being a key interface for dissolved metal uptake (Marigómez *et al.*, 2002). Other explanations are differences in affinities of metals to bind to metallothionein in the different tissues where they incorporated into lysosomes and later being eliminated towards the circulating hemocytes and blood plasma of bivalves (Roesijadi, 1980). The reason for this was the high (or low) concentrations of metal found in the different tissues could be related to the different rates of accumulation and excretion of metals due to internal metal handling (Gundacker, 1999).

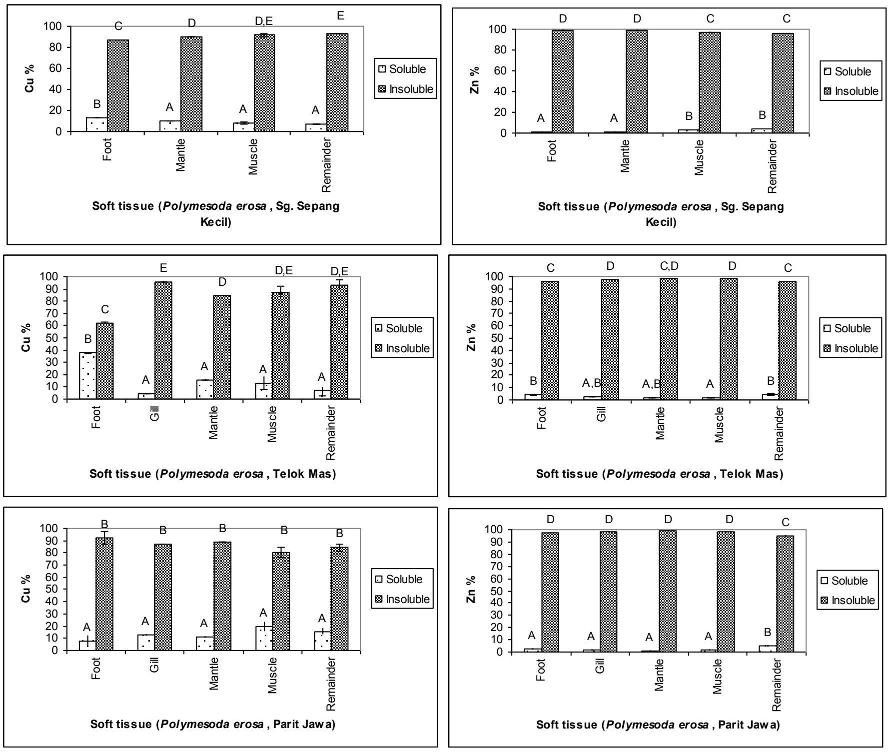

Fig.3: The soluble and insoluble fractions (%) of essential metals (Cu and Zn) in the different soft tissues of the *Polymesoda erosa*.

Note: Values sharing similar alphabet are not significant different with others, P > 0.05.

ii) Assessment of the potential of Polymesoda as a biomonitor for heavy metals

The potential of *P. erosa* as a biomonitor of the five metals are based on: (1) correlation coefficients of heavy metal concentrations between the different tissues of three burrowing bivalves species and their geochemical fractions of surface sediment (see Table 4); (2) BSAFs based on the different parts of the three bivalves species (see Table 5), and (3) comparison to two similar burrowing bivalve species (*D. faba* and *G. expansa*) based on the ground of points 1 and 2 above.

BSAF was calculated for all the different tissues of bivalves with the assumption that the distribution of a metal in the environment is controlled by a continuous exchange among phrases such sediment and molluscs (Hsu *et al.*, 2006). BSAF is used to classify the different tissues of bivalves as macroconcentrators (BSAF> 2), microconcentrators (1< BSAF< 2) and deconcentrators (BSAF < 1), as proposed by Dallinger (1993).

For *Polymesoda* clams (Table 4), all the five soft tissues (foot, gill, mantle, muscle, and remainder) positively and significantly correlated (R= 0.752-0.997; P< 0.001) with AR, OO and SUM, while foot, muscle and shell only weakly and positively correlated (P< 0.05) with EFLE. From Table 5, all the six soft tissues of *P. erosa* appear to be macroconcentrators (> 2.0) for Cd, five soft tissues (foot, gill, mantle, muscle and remainder) as macroconcentrators for Zn, three tissues (gill, mantle and remainder) as macroconcentrators for Cu, and three tissues (gill, mantle and shell) as macroconcentrators for Ni. Only shell appears to be macroconcentrator for Pb. Since these tissues are also significantly correlated with the sedimentary geochemical fractions and total metal concentrations, therefore, *P. erosa* is a potential biomonitor of Cd, Zn, Cu and Ni.

For *D. faba* (Table 4), four tissues (foot, gill, mantle and muscle) only weakly and positively correlated (P< 0.05) with OO, resistant and SUM. Only shell strongly corrected (R= 0.779; P< 0.001) with EFLE. From Table 5, only shells of *D. faba* appear to be macroconcentrator for Cd and Ni, three tissues (gill, remainder and shell) as macroconcentrators for Cu and four tissues (foot, gill, mantle and remainder) for Zn. Three tissues are found to be microconcentrators (1<BCF<2) for Cd and Cu, while only two tissues as microconcentrator. Others are found to be deconcentrators (BCF < 1). Therefore, together with the correlation results in Table 4, only the shells of *D. faba* could be evidently shown as potential biomonitoring materials for Cd and Ni, while soft tissues for Zn and Cu require further studies.

For *G. expansa* (Table 4), all the five soft tissues positively and significantly correlated (R= 0.599-0.983; at least P< 0.05) with all the four fractions (EFLE, AR, OO and resistant) and SUM. From Table 5, only the shells of *G. expansa* appear to be macroconcentrator for Cd and Pb, while two tissues as macroconcentrators for Cu and Zn. Gill is found to be microconcentrators

TABLE 4
Pearson's correlation of five metals (Cd, Cu, Ni, Pb and Zn) concentrations between the different tissues of three burrowing bivalves species and their geochemical fractions of surface sediment. N= 15.

Species	Different parts	EFLE	AR	OO	Resistant	SUM
Polymesoda erosa	Foot	0.524*	0.962**	0.974**	0.431	0.779**
	Gill	0.414	0.868**	0.997**	0.406	0.752**
	Mantle	0.451	0.929**	0.994**	0.403	0.759**
	Muscle	0.529*	0.964**	0.976**	0.457	0.797**
	Remainder	0.456	0.908**	0.967**	0.477	0.794**
	Shell	0.586*	-0.225	-0.312	0.567*	0.272
Donax faba	Foot	-0.580*	-0.246	0.535*	0.577*	0.546*
	Gill	-0.380	-0.235	0.616*	0.572*	0.558*
	Mantle	-0.392	-0.206	0.627*	0.564*	0.555*
	Muscle	-0.553*	-0.180	0.573*	0.577*	0.558*
	Remainder	-0.417	-0.444	0.449	0.463	0.426
	Shell	0.779**	0.169	-0.044	-0.389	-0.310
Gelonia expansa	Foot	0.639*	0.599*	0.938**	0.877**	0.927**
	Gill	0.901**	0.869**	0.868**	0.909**	0.963**
	Mantle	0.743**	0.695**	0.957**	0.954**	0.983**
	Muscle	0.939**	0.924**	0.744**	0.799**	0.875**
	Remainder	0.861**	0.853**	0.795**	0.802**	0.888**
	Shell	-0.078	-0.174	0.094	0.142	0.049

Note: ** Correlation is significant at the 0.01 level (2-tailed);* Correlation is significant at the 0.05 level (2-tailed); EFLE= Easily, freely, leachable. extractable; AR=Acid-reducible; OO=Organic oxidisable; SUM= Summation of EFLE, AR and OO.
The *Polymesoda* clams were collected from Sg. Sepang Kecil.

(1<BCF<2) for Cu, and only two tissues (gill and shell) as microconcentrator for Ni. Only mantle was found to be microconcentrator for Pb while three tissues (foot, muscle and remainder) were found to be microconcentrator for Zn. Others were shown to be deconcentrators. Therefore, together with the correlation results in Table 4, only the shells of *G. expansa* could be evidently shown as potential biomonitors for Cd and Pb, while soft tissues for Zn and Cu; however, further studies are still needed.

Therefore, *P. erosa* appears to be the best biomonitor of heavy metals when compared to *D. faba* and *G. expansa*. Although the findings of the present study indicate potentials of *P. erosa* as a good biomonitor of heavy metal pollution, other recommended criteria for a good biomonitor should be tested, including genetic variations of different geographical populations (Yap *et al.*, 2002b) and its metal accumulation capacity under laboratory experimental studies (Yap *et al.*, 2004), before it can be used as a valid biomonitor.

TABLE 5
Biota-sediment accumulation factors (BSAF) based on the different parts of bivalves. Calculation of BSAFs based on metal concentrations in the different dissected tissues of bivalves and non-resistant fractions in the sediment.

Species	Different parts	Cd	Cu	Ni	Pb	Zn
Polymesoda erosa	Foot	2.78	1.14	1.21	0.55	3.12
	Gill	6.61	6.59	6.56	1.17	12.15
	Mantle	7.37	4.42	3.47	1.21	12.85
	Muscle	4.33	1.96	1.61	0.89	5.38
	Remainder	4.00	5.06	1.60	1.13	5.54
	Shell	20.67	0.81	9.10	15.28	0.14
Donax faba	Foot	1.23	1.71	0.13	0.25	2.46
	Gill	1.16	3.69	0.29	0.24	4.39
	Mantle	0.98	1.96	0.27	0.16	2.90
	Muscle	1.25	1.35	0.17	0.27	1.80
	Remainder	0.91	4.32	0.22	0.10	2.78
	Shell	2.40	2.83	2.35	0.56	0.29
Gelonia expansa	Foot	0.32	3.36	0.71	0.78	1.01
	Gill	0.95	1.07	1.05	0.76	3.05
	Mantle	0.62	2.59	0.86	1.59	2.24
	Muscle	0.25	0.33	0.78	0.18	1.74
	Remainder	0.48	0.83	0.64	0.19	1.47
	Shell	5.92	0.12	1.87	2.28	0.05

Note: *Polymesoda* clams were collected from Sg. Sepang Kecil.

iii) Estimation of metal soluble fractions and comparisons with the food permissible limits

The soluble and insoluble fractions (%) of essential metals (Cu and Zn) in the different edible soft tissues of *P. erosa* are presented in Fig.3, while those of nonessential metals (Cd, Ni and Pb) are given in Fig.4. In general, the insoluble fractions dominated (> 50%) in the five metals investigated. This could be due to the fact that the metals were generally and strictly bound to metallothionein (Viarengo *et al*., 1985; Roesijadi, 1992). In addition, the formation of a metal-thiolate complex with the cysteine residues inside the lysosomes has caused the slower release of heavy metals found in the different edible soft tissues (Yap *et al*., 2003e), which could result in high percentages of insoluble fractions. Also, the high percentages in the insoluble fractions could be due to the soft tissues being linked to mineral granules (Geffard *et al*., 2002), which could limit the elimination of heavy metals from the edible soft tissues.

For the essential Cu and Zn (Fig.3), the soluble fractions in all the edible soft tissues of *P. erosa* ranged from 4.40-38.20% (13.11 ± 2.24%) and 0.60-5.10% (2.45 ± 0.36%), respectively, whereas for the nonessential

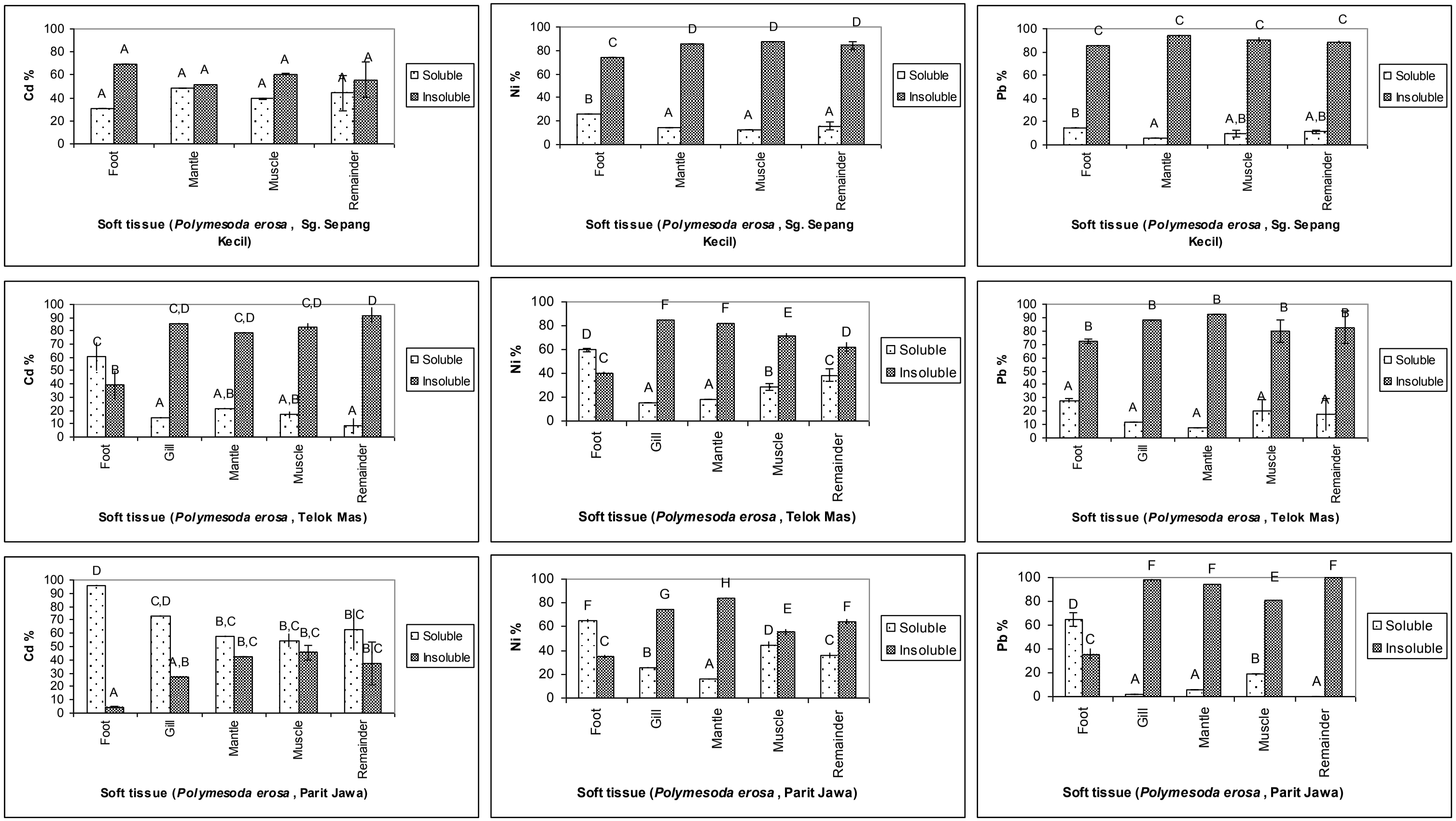

Fig.4: The soluble and insoluble fractions (%) of nonessential metals (Cd, Ni and Pb) in the different soft tissues of the *Polymesoda erosa*. Note: Values sharing similar alphabet are not significant different with others, $P > 0.05$.

Cd, Ni and Pb (Fig.4), the soluble fractions ranged from 8.40-95.40% (44.76 ± 6.62%), 12.40-65.30% (29.68 ± 4.58%) and 4.40-64.40% (15.57 ± 4.25%), respectively. This clearly indicated that the essential metals have lower percentages of soluble fractions when compared to the nonessential metals. This indicated that the essential metals could be possibly and tightly bound to metallothionein and therefore significantly higher percentages of insoluble fractions of Cu and Zn were found. However, this interesting discovery disagrees with the finding by Yap *et al.* (2012) in which they found the essential Zn was not tightly bound to metallothionein and thus easily remobilized (Yap *et al.*, 2012) after boiling. Since the finding by Yap *et al.* (2012) was highly supported by previous studies (see Phillips, 1985; Yap *et al.*, 2002c), and the differences of metal fractionations in both studies, the present results need further studies in the future.

The estimation of the total quantities of metals (total fraction) present in edible soft tissues of bivalves will lead to an overestimation of the quantities likely to be bioavailable to a consumer (Bragigand *et al.*, 2004). Therefore, the determination of the soluble and insoluble fractions employed in the present study was significant. This is because the information on the soluble fractions in the bivalves is important in order to estimate the toxicological risks of the bivalves to their consumers. It is assumed that the metals present in the soluble fractions are more bioavailable to consumers than metals bound to insoluble fractions (Wallace *et al.*, 2003). Nevertheless, according to Bragigand *et al.* (2004), the physic-chemical characteristics of a heavy metal in the soft tissues also play a significant role in determining the bioavailability of dietary metal in the digestive tract of the consumer. As suggested by the finding of Geffard *et al.* (2002) in the *Crassostrea gigas*, the higher the level of contamination, the more important metal-binding to cytosolic ligands for Cd will be. This could explain the elevated metal percentages found in the soluble fractions of the foot and mantle of the bivalves, in which the locations of the tissues are more exposed to the contaminated external environment.

The levels of Cd, Cu, Pb and Zn found in the total and soluble fractions of the edible tissues of *P. erosa* were compared with some established food safety guidelines or maximum permissible limits set by some countries or agencies (Table 6). No permissible limits for Ni could be found, and therefore, no comparison was made for the levels of Ni in the bivalves.

From Table 6, the total fractions of Cd and Pb in gill and mantle of *P. erosa* are both below all the permissible limits, except for the metal limits set by USEPA (2003) for Median International Standards for metals in mollusks. However, the total fraction of Pb in mantle is slightly higher than that by the Ministry of Public Health, Thailand (MPHT, 1986). All total fractions of Zn in all the five edible soft tissues exceeded the metal limits by USEPA (2003). In addition, total fractions of gill and mantle also exceeded the Maximum permissible levels

TABLE 6
Comparisons of heavy metal concentrations (µg/g dry weight) in the different edible soft tissues of *Polymesoda erosa* based on total metal concentrations (total fraction) and soluble fractions, with some selected food permissible limits (also presented in µg/g dry weight).

		Cd	Cu	Pb	Zn
Foot	Total fraction	0.25-1.47	1.80-3.77	0.94-2.18	87.5-105
	Soluble fraction	0.30-0.49	0.15-1.05	0.87-1.56	0.99-3.16
Gill	Total fraction	1.22-2.86	8.81-21.0	2.56-6.51	263-349
	Soluble fraction	0.41-0.89	0.35-1.29	0.16-0.88	3.59-3.81
Mantle	Total fraction	1.36-2.40	5.82-13.2	4.79-7.09	264-365
	Soluble fraction	0.33-0.75	0.72-1.05	0.51-0.86	1.83-3.32
Muscle	Total fraction	0.70-1.79	1.86-5.76	1.89-3.41	104-150
	Soluble fraction	0.17-0.35	0.33-0.68	0.82-1.01	1.54-3.91
Remainder	Total fraction	0.61-1.17	8.60-14.9	2.07-4.35	79.2-154
	Soluble fraction	0.08-0.44	0.40-1.27	0.01-1.13	4.23-5.8
1. Permissible limit set by the ministry of Public Health, Thailand (MPHT, 1986)		NA	133	6.67	667
2. Australian Legal Requirement (NHMRC, 1987)		10.0	350	NA	750
3. Maximum permissible levels established by Brazilian Ministry of Health (ABIA, 1991)		5.0	150	10	250
4. Food and Drug Administration of the USA (USFDA, 1990)		25.0	NA	11.5	NA
5. Median International Standards for metals in mollusks compiled by the Food and Agricultural Organization of the United Nations (USEPA, 2003)		2.00	10-30	1.00-6.00	40-100

Note: NA= Not available.
All the ranges for total and soluble fractions above are based on three populations of *P. erosa* as shown in Table 3 except for soluble fraction of gill which is only based on two populations from Telok Mas and Parit Jawa.

established by Brazilian Ministry of Health (ABIA, 1991). It is good to see that all the total fractions of Cu in the different edible soft tissues are below all the permissible limits. Regardless of some metals in the edible soft tissues having exceeded the food safety permissible limits, all the four metal levels in the soluble fractions of all the five edible soft tissues of *P. erosa* are below all the permissible metal limits. Therefore, this estimation clearly shows that the consumption of *P. erosa* could pose no toxicological risks to the consumers.

CONCLUSION

From the present study, the metal levels found in the different soft tissues and shells of three populations of *P. erosa* are important for future reference. Being macroconcentrators based on BSAFs, and positively and significantly correlations of metals between all the five soft tissues (foot, gill, mantle, muscle and remainder) and the sedimentary geochemical fractions and total metal concentrations, the different soft tissues of *P. erosa* are potential biomonitoring tissues for Cd, Zn, Cu and Ni, while the clam shell is a potential biomonitoring material for Pb. In comparison to two similar burrowing bivalves (*D. faba* and *G. expansa*), *P. erosa* was shown to be the best biomonitor of heavy metals. Regardless of some metals in the edible soft tissues having exceeded the food safety permissible limits, all the four metal levels in the soluble fractions of all the five edible soft tissues of *P. erosa* are below all the permissible metal limits. Therefore, these estimations clearly showed that the consumption of *P. erosa* could pose no toxicological risks to the predators (consumers).

REFERENCES

Badri, M. A., & Aston, S. R. (1983). Observation on heavy metal geochemical associations in polluted and non-polluted estuarine sediments. *Environ. Poll., Ser. B 6*, 181-93.

Bragigand, V., Berthet, B., Amiard, J. C., & Rainbow, P.S. (2004). Estimates of trace metal bioavailabilty to humans ingesting contaminated oysters. *Food Chem. Toxicol., 42,* 1893-1902.

Dallinger, R. (1993). Strategies of metal detoxification in terrestrial invertebrates. In R. Dallinger and P.S. Rainbow (Eds.), *Ecotoxicology of metals in invertebrates* (pp. 245-289) Boca Raton: Lewis Publishers.

Department of Fisheries Malaysia. (2005). *Malaysia Fisheries Directory 2005-06*. Asia Medialine (M) Sdn. Bhd.

Geffard, A., Amiard, J. C., & Amiard-Triquet, C. (2002). Kinetic of metal elimination in oyster from a contaminated estuary. *Comp. Biochem. Physiol. (Part C), 131,* 281-293.

Gilek, M., Biork, M., & Naf, C. (1996). Influence of body size on the polychlorinated biphenyl congeners by Baltic Sea Blue Mussel, *Mytilus edulis*. *Mar.Biol.*, *125,* 499-510.

Gillikin, D. P., Dehairs, F., Baeyens, W., Navez, J., Lorrain, A., & Andre, L. (2005). Inter- and intra-annual variations of Pb/Ca ratios in clam shells (*Mercenaria mercenaria*): A record of anthropogenic lead pollution? *Mar. Pollut. Bull., 50,* 1530–1540.

Gimin, R., Mohan, R., Thinh, L. V., & Griffiths, A. D. (2004). The relationship of shell dimension and shell volume to live weight and soft tissue weight in the mangrove clam, *Polymesoda erosa* (Solander, 1786) from northern Australia. *NAGA Worldfish Cent. Quart.*, *27*(3 & 4), 32-35.

Gundacker, C. (1999). Tissues-specific heavy metal (Cd, Pb, Cu & Zn) deposition in a natural population of the zebra mussel *Dreissena polymorpha* Pallas. *Chemosphere, 38,* 3339-3356.

Hedouin, L., Bustamante, P., Churlaud, C., Pringault, O., Fichez, R., & Warnau, M. (2009). Trends in concentrations of selected metalloid and metals in two bivalves from the coral reefs in the SW lagoon of New Caledonia. *Ecotox. Environ. Safety, 2,* 372– 381.

Hong Kong Government. (1989). *Public Health and Municipal Services Ordinance*, Chapter 132. Government Printer, Hong Kong.

Hong Kong Government. (1989). Public Health and Municipal Services Ordinance, Chapter 132. Government Printer, Hong Kong.

Hsu, M. J., Selvaraj, K., & Agoramoorthy, G. (2006). Taiwan's industrial heavy metal pollution threatens terrestrial biota. *Environ. Poll., 143(2),* 327-334.

Ingole, B. S., Krishnakumari, L., & Ansari, Z. A. (1994). New record of mangrove clam *Geloina erosa* from the west coast of India. *J. Bombay Natural Hist. Soc.*, *91,* 338-339.

Lares, M. L., & Orians, K. J. (1997). Natural Cd and Pb variations in *Mytilus californianus* during the upwelling season. *Sci. Tot. Environ., 197,* 177-195.

Lazaretha, C. E., Vander Puttena, E., Andre′b, L., & Dehairs, F. (2003). High-resolution trace element profiles in shells of the mangrove bivalve *Isognomon ephippium*: a record of environmental spatio-temporal variations? *Estuar. Coast. Shelf Sci., 57,* 1103–1114.

Leung, K. M. Y., Dewhurst, R. E., Halldorsson, H., & Svavarsson, J. (2005). Metallothioneins and trace metals in the dogwhelk *Nucella lapillus* (L.) collected from Icelandic coasts. *Mar. Poll. Bull., 51,* 729-737.

Lewis, M., Pryor, R., & Wilking, L. (2011). Fate and effects of anthropogenic chemicals in mangrove ecosystems: A review. *Environ. Poll., 159,* 2328-2346.

Marigómez, I., Soto, M., Cajaraville, M. P., Angulo, E., & Giamberini, L. (2002). Cellular and subcellular distribution of metals in molluscs. *Microsc. Res. Tech., 56*(5), 358–392.

Modassir, Y. (2000). Effect of salinity on the toxicity of mercury in mangrove clam, *Polymesoda erosa* (Lightfoot 1786). *Asian Fish. Sci.*, *13,* 335-341.

Morton, B. (1976). The biology and functional morphology of Southeast Asian mangrove bivalve, *Polymesoda* (Gelonia) *erosa* (Solander 1786) (Bivalvia: Corbiculidae). *Can. J. Zool*, *54,* 482-500.

MPHT (Ministry of Public Health, Thailand). (1986). *Residues in foods*, Part 23, vol.103 (pp. 1123-1124). Special Issue, 16 February, 1986. The Government Gazette, Bangkok, Thailand.

NHMRC (National Health and Medical Research Council) (1987). *National food standard A 12: metal and contaminants in food*. Canberra: Australia: Australia Government Publishing Services.

Phillips, D. J. H. (1985). Organochlorines and trace metals in green-lipped mussels *Perna viridis* from Hong Kong waters: a test of indicator ability. *Mar. Ecol. Progr. Ser., 21*, 251-8.

Phillips, D. J. H., & Rainbow, P. S. (1993). *Biomonitoring of trace aquatic contaminants* (2nd ed.) London: Chapman and Hall.

Protasowicki, M., Dural, M., & Jaremek, J. (2008). Trace metals in the shells of blue mussels (*Mytilus edulis*) from the Poland coast of Baltic sea. *Environ. Monit. Assess., 141,* 329-337.

Rainbow, P. S. (1995). Biomonitoring of heavy metal availability in the marine environment. *Mar. Poll. Bull*, *31,* 183-192.

Rainbow, P. S., Smith, B. D., & Lau, S. S. (2002) Biomonitoring of trace metal availabilities in the Thames estuary using a suite of littoral biomonitors. *J. Mar. Biol. Asso. UK*, *82,* 793-799.

Roesijadi, G. (1980). The significance of low molecular weight, metallothionein-like protein in marine invertebrates: Current status. *Mar. Environ. Res.*, *4,* 167-179.

Roesijadi, G. (1992). Metallothionein in metal regulation and toxicity in aquatic animals. *Aquat. Toxicol., 22,* 81–113.

Sakellari, A., Karavoltsos, S., Theodorou, D., Dassenakis, M., & Scoullos, M. (2013). Bioaccumulation of metals (Cd, Cu, Zn) by the marine bivalves *M. galloprovincialis*, *P. radiata*, *V. verrucosa* and *C. chione* in Mediterranean coastal microenvironments: association with metal bioavailability. *Environ. Monit. Assess., 185,* 3383–3395.

Silva, C. A. R., Rainbow, P. S., Smith, B. D., & Santos, Z. L. (2001). Biomonitoring of trace metal contamination in the Potengi Estuary, Natal (Brazil), using the oyster *Crassostrea Rhizophorae*, a local food source. *Wat. Res., 35*(17)*,* 4072–4078,

Szefer, P., Ali, A. A., Ba-Haroon, A. A., Rajeh, A. A., Geldon, J., & Nabrzyski, M. (1999). Distribution and relationships of selected trace metals in molluscs and associated sediment from the Gulf of Aden, Yemen. *Environ. Poll., 106,* 299-314.

Tarique, Q., Burger, J., & Reinfelder, J.R. (2013). Relative importance of burrow sediment and porewater to the accumulation of trace metals in the clam *Amiantis umbonella*. Arch *Environ. Contam. Toxicol., 65,* 89–97.

USEPA. (2003). *Food and Agricultural Organization of the United Nations*. California, US.

USFDA. (1990). *Food and Drug Administration of the United States*. US Food and Drug Administration Shellfish Sanitation Branch, Washington, D.C.

Viarengo, A., Palmero, S., Zanicchi, G., Capelli, R., Vaissiere, R., & Orunesu, M. (1985). Role of metallothioneins in Cu and Cd accumulation and elimination in the gill and digestive gland cells of *Mytilus galloprovincialis* (Lam.). *Mar. Environ. Res.*, *16,* 23-36.

Wallace, W. G., Lee, B. G., & Luoma, S .N. (2003). Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). *Mar. Ecol. Progr. Ser., 249,* 183-197.

Wang, Y., Qiu, Q., Xin, G., Yang, Z., Zheng, J., Ye, Z., & Li, S. (2013). Heavy metal contamination in a vulnerable mangrove swamp in South China. *Environ. Monit. Assess., 185,* 5775–5787.

Watling, H. R., & Watling, R. J. (1976). Trace metals in oysters from the Knysna Estuary. *Mar. Poll. Bull.*, *7,* 45-48.

Yap, C. K. (2012). *Mussel Watch in Malaysia: Past. Present and Future*. Universiti Putra Malaysia Press. Serdang. p. 137.

Yap, C. K., & Pang, B. H. (2011). Assessment of Cu, Pb and Zn contamination in sediment of north western Peninsular Malaysia by using sediment quality values and different geochemical indices. *Environ. Monit. Assess., 183*(1-4), 23–39.

Yap, C. K., & Wong, C. H. (2011). Assessment Cu, Ni and Zn pollution in the surface sediments in the southern Peninsular Malaysia using cluster analysis, ratios of geochemical nonresistant to resistant fractions, and geochemical indices. *Environ. Asia, 4*(1)*,* 53-61.

Yap, C. K., Ismail, A., & Tan, S. G. (2002a). Condition index of green-lipped mussel *Perna viridis* (Linnaeus) as a potential physiological indicator of ecotoxicological effects of heavy metals (Cd and Pb). *Malays. Appl. Biol., 31*(2), 37-45.

Yap, C. K., Tan, S. G., Ismail, A., & Omar, H. (2002b). Genetic variation of green-lipped mussel *Perna viridis* (Linnaeus) from the west coast of Peninsular Malaysia. *Zool. Stud., 41*(4), 376-387.

Yap, C. K., Ismail, A., Tan, S. G., & Omar, H. (2002c). Correlations between speciation of Cd, Cu, Pb and Zn in sediment and their concentrations in total soft tissue of green-lipped mussel *Perna viridis* from the west coast of Peninsular Malaysia. *Environ. Int., 28*(1-2), 117-126.

Yap, C. K., Ismail, A., & Tan, S. G. (2003a). Background concentrations of Cd, Cu, Pb and Zn in the green-lipped mussel *Perna viridis* (Linnaeus) from Peninsular Malaysia. *Mar. Poll. Bull*, *46,* 1035-1048.

Yap, C. K., Ismail, A., & Tan, S. G. (2003b). Water content, shell thickness, conversion factor and condition index of green-lipped mussel *Perna viridis* (Linnaeus) from Malaysian Coastal Water. *Malay. Nature J.*, *56*(4), 387-396.

Yap, C. K., Ismail, A., Tan, S. G., & Abdul Rahim, I. (2003c). Can the shell of the green-lipped mussel *Perna viridis* (Linnaeus) from the west coast of Peninsular Malaysia be a potential biomonitoring material for Cd, Pb and Zn ? *Estuar. Coast. Shelf Sci., 57,* 623-630.

Yap, C. K., Ismail, A., & Tan, S. G. (2003d). Effects of total soft tissue and shell thickness on the accumulation of heavy metals (Cd, Cu, Pb and Zn) in the green-lipped mussel *Perna viridis*. *Russian J. of Mar. Biol*, *29*(5), 323-327.

Yap, C. K., Ismail, A., Omar, H., & Tan, S. G. (2003e). Accumulation, depuration and distribution of cadmium and zinc in the green-lipped mussel *Perna viridis* (Linnaeus) under laboratory conditions. *Hydrobiologia*, *498,* 151-160.

Yap, C. K, Ismail, A., Tan, S. G., & Rahim Ismail, A. (2004). Assessment of different soft tissues of the green-lipped mussel *Perna viridis* (Linnaeus) as biomonitoring agents of Pb: Field and laboratory studies. *Wat Air Soil Poll., 153,* 253-268.

Yap, C. K., Ismail, A., Edward, F. B., Tan, S. G., & Siraj, S. S. (2006). Use of different soft tissues of *Perna viridis* as biomonitors of bioavailability and contamination by heavy metals (Cd, Cu, Fe, Pb, Ni and Zn) in a semi-enclosed intertidal water, the Johore Straits. *Toxicol. Environ. Chem.*, *88*(1-4), 683-695.

Yap, C. K., Noorhaidah, A., & Tan, S. G. (2011). Zn concentrations in the different soft tissues of *Telescopium telescopium* and their relationships with Zn speciation by sequential extraction in surface sediments: A statistical multiple linear stepwise regression analysis. In A.M. Bianchi and J.N. Fields (Eds.), *Gastropods: Diversity, Habitat and Genetics*. New York: Nova Science Publishers.