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IDENTIFICATION OF POTENTIAL INTERTIDAL BIVALVES AS BIOMONITORS OF HEAVY-METAL CONTAMINATION BY USING BIVALVE-SEDIMENT ACCUMULATION FACTORS (BSAFS)

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Abstract: This study provides important information on the biota-sediment accumulation factor (BSAF) of three species of tropical bivalves from Peninsular Malaysia and focussed on the accumulation factors of the different parts of the bivalves. This study found that the different parts of the bivalves could be classified into a few groups, such as macroconcentrator (BSAF> 2), microconcentrator (1< BSAF< 2) or deconcentrators (BSAF< 1) as proposed by Dallinger (1993). From the results obtained, it was found that *Polymesoda erosa* is an overall good macroconcentrator (therefore the most potential biomonitor among the three species) for Cd (in all parts, namely muscle, foot, mantle, gill, shell and remainder), followed by Zn (except for shell), Ni, Cu and Pb. For *Donax faba*, it is a good macroconcentrator for Zn (foot, mantle, gill and remainder), followed by Cu, Cd and Ni. For *Gelonia expansa*, only 1-2 parts were considered as macroconcentrators for the accumulations of Cu, Zn, Pb and Cd. Based on the BSAF values, it is suggested that the macroconcentrators found in some parts of the bivalves, especially in *P. erosa,* can be particularly useful as potential biomonitors of heavy-metal pollution. Therefore, the present finding should merit further studies using the BSAF values of bivalves as indicators of metal bioavailabilities and contamination in coastal areas of Peninsular Malaysia receiving anthropogenic inputs.

KEYWORDS: Biota-sediment accumulation factor, metal distribution, bivalves

Introduction

The ability of an organism to reflect the pollutant levels in its environment is one of the important criteria as a biomonitor (Phillips and Segar, 1986; Rainbow, 1997). In the study of ecotoxicology, the main priority is to predict the effects and to investigate the causes of ecological or biological effects that resulted from exposure to chemicals (heavy metals) and environmental stresses (Widdows and Donkin, 1992). Therefore, it is necessary to establish the relationship between the concentration of a pollutant present in the tissues of a proposed biomonitor with those in its environment and the cause-effect relationships between metal concentrations in the soft tissues of the biomonitor and the resultant biological effects.

Bioaccumulation is an important process through which chemicals can affect living organisms. An increase in the concentration of a chemical in a biological organism over time may occur when compared to the chemical concentration in the environment (Zhou *et al.*, 2008). According to Zhou *et al.* (2008), bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is lost. Several processes, including uptake, storage and elimination, are involved during bioaccumulation. Bioaccumulation results from a dynamic equilibrium between exposure to the outside environment and uptake, excretion, storage and degradation within an organism (Zhou *et al.,* 2008).

Many factors are known to affect the accumulation of heavy metals by marine organisms.

Metal concentrations in the total soft tissues of marine organisms are many times greater than the concentrations of metal in the surrounding sea water (Lobel *et al.*, 1982). Often, the metal level in the organism is proportional to the metal level in sea water so that the organism can be used as a biological indicator of metal pollution (Phillips, 1980; Lobel *et al.*, 1982). In addition, since biomonitoring programmes employ various species to monitor environmental metal pollution, there is a need to better understand the process and significance of metal bioaccumulation in order to interpret the data generated from such programmes (Wang and Rainbow, 2008).

In order to characterise metal bioavailability more quantitatively, the biota-sediment accumulation factors (BSAFs) are estimated on the basis of both experimentally-and environmentally-derived data, though, whenever possible, field data are preferred (Szefer *et al.*, 1999). BSAFs have been proposed as a simple model for predicting the bioaccumulation of sediment-associated neutral organic contaminants by infaunal invertebrates (Di Toro *et al.*, 1991; Boese *et al.*, 1996; Kannan, 1999). According to Szefer *et al.* (1999), knowledge of concentration factors allow the relative ability of molluscs to bioaccumulate selected metals from the medium in which they live to be determined. Therefore, the objective of this paper was to determine the bivalves-sediment accumulation factors based on the different parts of three species of intertidal tropical bivalves from Peninsular Malaysia.

Materials and Methods

Sampling, storage and sample preparation

Three species of bivalves, namely *Polymesoda erosa*, *Donax faba* and *Gelonia expansa,* were collected from Sepang Kecil, Pantai Pasir Panjang and Kg. Pasir Puteh, respectively, in Peninsular Malaysia (Figure 1). The Global Positioning System (GPS) of the sampling locations and allometric information of the bivalves are shown in Table 1. To avoid differences in metal concentrations because of size or reproductive stage, only the commercial-sized individuals of each species were collected and analysed (Saavedra *et al.* 2004). About 20-25 individuals of similar-sized bivalve species were used for the analysis of heavy metals in the different tissues. The bivalves were dissected and pooled into foot, gill, gonad, mantle, muscle and remainder. All the different categories of pooled tissues were dried at 60°C to constant dry weights. Besides these different soft tissues, all the shells were also pooled and analysed.

Sample digestion

About 0.5-0.7g of dried tissues from each category were weighed and placed in acid-washed digestion tubes. A total of 10 mL of concentrated nitric acid (AnalaR grade, BDH 69%) was added to the digestion tube to digest the tissues. The tubes were placed in a digestion block at 40˚C for 1 hour and the tissues were then fully digested at 140˚C for 3 hours (Yap *et al.* 2003). After being cooled, the content of each tube was diluted to 40 mL with double de-ionised water. The digested samples were then filtered through Whatman No.1 (filter speed: medium) filter papers in funnels into acid-washed pill boxes.

For sediment samples, the direct aqua-regia method was applied. About 1 g of each dried sample was digested in a combination of concentrated nitric acid (69%) and perchloric acid (60%) in the ratio of 4:1, first at low temperature (40˚C) for one hour and then the temperature was increased to 140˚C for three hours (Yap *et al.*, 2002).

The content of each tube was then diluted to 40 mL with double de-ionised water. The digested samples were then filtered through Whatman No.1 (filter speed: medium) filter papers in funnels into acid-washed pill boxes.

Speciation of Cd, Cu, Ni, Pb and Zn in sediments

The 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) and Tessier and Campbell (1987). The four fractions considered, the extraction solutions and the conditions employed were as follows:-

(1) Easily, freely, leacheable or exchangeable (EFLE): About 10g of sample was continuously shaken for 3 hours with 50 ml of 1.0 M ammonium acetate (NH_4CH_3COO), pH 7.0, at room temperature.

(2) 'Acid-reducible': The residue was continuously shaken for 3 hours with 50 ml of 0.25 M hydroxylammonium chloride ($NH_2OH.HCL$) acidified to pH 2 with HCL, at room temperature.

(3) 'Oxidisable-organic': The residue was first oxidised with 30% H_20_2 in a water bath at 90-95˚C. After cooling, the metal released from the organic complexes was continuously shaken for 3 hours with 1.0 ammonium acetate (NH_4CH_3COO) acidified to pH 2.0 with HCL, at room temperature.

(4) Resistant: The residue from (3) was digested in a combination of concentrated nitric acid (69%) and perchloric acid (60%) as in the direct aqua-regia method.

The residue used for each fraction was weighed before the next fractionation was carried out. The residue was washed with 20 ml of DDW. It was then filtered through Whatman No.1 filter paper and the filtrate was stored until metal determination. For each fraction of the sequential extraction procedure, a blank was employed using the same procedure to ensure that the samples were free from contaminants.

Determination of Cd, Cu, Ni, Pb and Zn

The concentrations of all the pill boxes were then analysed for heavy metals by using an air-acetylene Perkin-Elmer^{TM} flame Atomic Absorption Spectrophotometer (AAS) model AAnalyst 800. Blank determination was carried out for calibration of the instrument. Standard solutions were prepared from 1000 ppm stock solutions provided by MERCK Titrisol for Cd, Cu, Ni, Pb and Zn and the data obtained from the AAS were presented in µg/g dry weight basis.

Quality control

To avoid possible contamination, all glassware and equipment used were acid-washed. Recoveries were done by using prepared standard solutions for each metal. In addition, the analytical procedures for the bivalves and sediment were checked with the Certified Reference Material (CRM) for Dogfish Liver (DOLT-3, National Research Council Canada) and soil (Soil-5, International Atomic Energy Agency, Vienna, Austria). The recoveries of all the metals were satisfactory as shown in Table 2.

Statistical analyses

The relationships among the different tissues and the geochemical fractions of the sediment were tested using Pearson's correlation based on the concentrations of Cd, Cu, Ni, Pb and Zn. The correlation analyses were carried out by using log-transformed data (Leung *et al.*, 2005). The existence of a multivariate relationship was determined by the Pearson's correlation coefficient (R) with a significance level of $P< 0.05$. Pearson's correlations were analysed using SPSS version 12.

In order to estimate the proportion in which metal occurs in the organism and in associated sediment, BSAFs were calculated for selected metals in the bivalves studied according to a formula (Szefer *et al.*, 1999) below:-

$$\text{BSAF} = \frac{Cx}{Cs}$$

where Cx and Cs are the mean metal concentrations in the bivalve and in associated sediment, respectively. In the present study, the mean metal sediments were the summation of non-resistant geochemical fractions, namely the EFLE, acid-reducible and oxidisable-organic, in which they were applied to the BSAF formula due to its bioavailabilities characteristic to the living organisms. Based on the BSAF, the different parts of the bivalves can be classified into macroconcentrator (BSAF > 2), microconcentrator (1 < BSAF <2) or deconcentrators (BSAF < 1), as proposed by Dallinger (1993).

Results

The relationships of the metal concentrations between the bivalves and their environment were conducted based on the data given in Tables 3 and 4. The Pearson's correlations of the metal concentrations between the bivalves: *G. expansa*, *P. erosa* and *D. faba,* and the metal concentrations in their environment are shown in Table 5.

The metal concentrations in the soft tissues of the bivalves were significantly ($P< 0.05$) correlated with the concentrations found in the geochemical fractions which showed the ability of the bivalves to accumulate heavy metals from their surroundings. The most number of significant correlations were found in all of the different soft tissues of *G. expansa* while the least number of significant correlations were found in *D. faba*. There was also a correlation observed between the metal concentrations in the shell of *D. faba* with the EFLE fraction. As for the metal concentrations in the shell of the bivalves, there were correlations found between the shell of *P. erosa* with the EFLE and resistant fractions.

The BSAF values of the different parts of the selected bivalves are shown in Table 6. Most obviously, it was found that *P. erosa* is a good macroconcentrator for Cd in all parts, namely muscle, foot, mantle, gill, shell and remainder, followed by Zn (except for shell), Ni, Cu and Pb.

For *D. faba*, it is a good macroconcentrator for Zn (foot, mantle, gill and remainder), followed by Cu, Cd and Ni. Relatively lower BSAF values (0.10-0.56 except for Ni shell with 2.35) were obtained for the accumulations of Pb and Ni while higher BSAF values (1.16-2.40) for Cd were obtained from the muscle, foot, gill and shell, which indicated that the tissues of *D. faba* were good accumulator of non-essential Cd.

For *G. expansa*, only 1-2 parts were considered as macroconcentrators for the accumulations of Cu, Zn, Pb and Cd. Greater BSAF values were obtained from the foot, mantle and gill of *G. expansa* for Cu. As for Zn, high BSAF values were obtained from most of the different tissues except for the shell. However, for Pb, Ni and Cd, high BSAF values were only obtained from a few tissues, namely the mantle and shell for Pb; the gill and shell for Ni; and the shell for Cd.

Discussion

In general, for *P. erosa*, most parts could be classified as macroconcentrators due to the high BSAF values for the accumulation of Cu (mantle, gill and remainder), Zn (muscle, foot, mantle, gill and remainder), Pb (shell), Ni (mantle, gill and shell) and Cd (all parts). Therefore, *P. erosa* is the most potential biomonitor since most parts are macroconcentrator of metals based on BSAF values when compared with *G. expansa* and *D. faba*.

On the other hand, the different parts of *G. expansa* could be classified as microconcentrators and deconcentrators for certain metals. Interestingly, some tissues of *G. expansa* could be classified as macroconcentrators since high BSAF values were found for the accumulation of Cu by the foot; Cu and Zn by the mantle; Zn by the gill; and Pb and Cd by the shell. For *D. faba*, the gill, shell and remainder were classified as macroconcentrators of Cu accumulation, while for the accumulation of Zn, the foot, mantle, gill and remainder of *D. faba* could be classified as macroconcentrators. As for the accumulation of Pb, Ni and Cd, most of the different parts of *D. faba* could be classified as microconcentrators and deconcentrators with $1<$ BSAF<2 and BSAF<1, respectively. It should be noted that more future studies are needed since different bivalve species have different capabilities in the uptake of heavy metals and different sampling sites have different concentrations of heavy metals.

Li *et al.* (2009) explained that metal bioconcentration (or BSAF) is likely to vary within tissues as observed in the crabs *Ucides cordatus* and *Callinectes danae* that showed significantly-elevated Cd, Hg, Pb and Zn concentrations in the hepatopancreas when compared to other tissues. The results could be linked to metal sequestration and detoxification by metallothioneins in the hepatopancreas. In the present study, the remainder of *P. erosa,* which contained hepatopancreas, had recorded high BSAF values for Cu and Zn which could be due to similar metal sequestration and detoxification processes. Besides, the levels and bioconcentration of heavy metals in marine organisms usually fluctuate, perhaps due to the changes of some seasonal factors such as diet and temperature (Stewarts *et al.*, 1994; Li *et al.*, 2009). Variations in BSAF values recorded by the bivalves studied suggest that their feeding habits might influence the BSAF in the different tissues.

Among the heavy metals determined in the present study, Cu is a cofactor for regulating the activities of Cu-dependent enzymes (Lehtonen and Leiniö, 2003) and also it is an essential component for the synthesis of hemocyanin (Méndez *et al.*, 2001). As for Zn, the metal is an essential element for animals, being an important component of many enzymes, and its levels in molluscs were elevated in animals collected near anthropogenic point sources of Zn (Eisler, 2000). Cd and Pb are considered as toxicants, being able to cause some toxic effects on marine organisms if excessively concentrated (ATSDR, 2005).

Various studies reported that the BSAF or bioconcentration of heavy metal in molluscs were related to anthropogenic activities. Blackmore (1998) found that heavy metal BSAF in mussel *Perna viridis* reflected the degree of industrialisation and population density in coastal areas of Hong Kong. Liang *et al.* (2004) also found that the bivalve, the oyster *Crassostrea,* possessed high BSAF for heavy metals mainly due to the wastewater and waste-residue drainage from the industries nearby. Therefore, the macroconcentrators in some tissues of *G. expansa* collected from a metal-polluted site at Kg. Pasir Puteh (Yap *et al.*, 2002), could be due to the anthropogenic sources.

Therefore, it is useful to use BSAF values to recognise whether any specific bivalve species is a potential biomonitor based on the relationship between the concentrations of a given metal in the different tissues of bivalves to its metal concentration in the associated sediment.

Conclusion

The BSAF of selected bivalves obtained from the present study revealed that most of the different parts of the bivalves were macroconcentrators of heavy metals. In conclusion, it was found that *P. erosa* is an overall good macroconcentrator for Cd (in all parts, namely muscle, foot, mantle, gill, shell and remainder), followed by Zn (except for shell), Ni, Cu and Pb. *D. faba* is a good macroconcentrator for Zn (foot, mantle, gill and remainder), followed by Cu, Cd and Ni. For *G.*

expansa, only 1-2 parts were considered as macroconcentrators for the accumulations of Cu, Zn, Pb and Cd. Therefore, *P. erosa* is the most potential biomonitor since most macroconcentrators in the different parts were found and they could be suggested to be biomonitors of metal bioavailabilities resulting from metal contamination.

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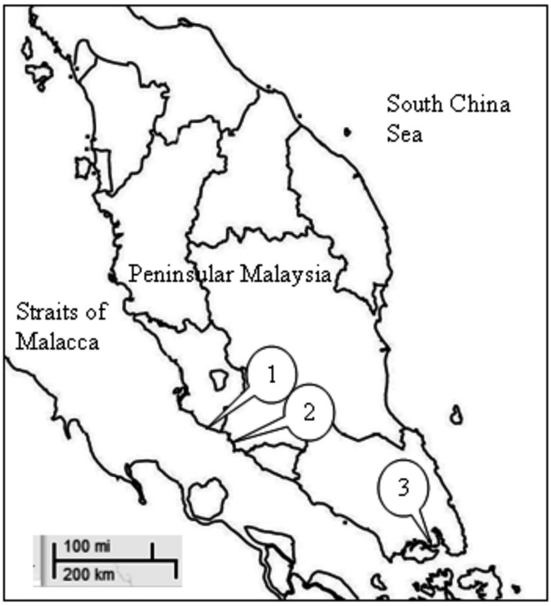

Figure 1: The sampling locations of the bivalves in Peninsular Malaysia. Note: 1= Sungai Sepang Kecil (Selangor); 2= Pantai Pasir Panjang (Negeri Sembilan); 3= Kampung Pasir Puteh (Johore)

Table 1: The descriptions of the sampling locations and information on the bivalves collected.

No	Location	Sampling date	GPS	Site description	Samples collected
1.	Sungai (Sg.) Sepang Kecil, Selangor	30 April 2006	N 02° 36'50.62" E 101°40'59.83"	1. Mangrove area 2. Prawn breeding sites 3. Fish culture farm. 4. Recreational area.	1. *Polymesoda erosa* 2. Surface sediment
2.	Pantai Pasir Panjang, Negri Sembilan	28 April 2006	N 02° 24' 54.9 E 101° 56' 31.1	1. A few restaurants by the beautiful seaside. 2. Recreational areas.	1. *Donax faba* 2. Surface sediment
3.	Kg. Pasir Puteh, Johore	30 April 2006	N 01 °26' 05.8" E 101°56' 02.4"	1. A fishing village. 2. Mangrove forest. 3. Close to one of the important industrial area in Malaysia; Pasir Gudang industrial area.	1. *Gelonia (Polymesoda) expansa* 2. Surface sediment

Table 2: Measured and certified concentrations (µg/g dry weight) of Certified Reference Materials (CRM) of Dogfish Liver and Soil-5.

Metal	Sample	Certified values in CRMs	Measured values in CRMs	% of recovery
Cd	Dogfish Liver (DOLT-3)	19.4	20	103
	Soil-5 (IAEA)	1.50	2.16	144
Cu	Dogfish Liver (DOLT-3)	31.2	32.0	103
	Soil-5 (IAEA)	77 ± 4.7	72.8	94.4
Ni	Dogfish Liver (DOLT-3)	2.7 ± 0.4	2.77 ± 0.74	102 ± 27
	Soil-5 (IAEA)	13.0	17.9 ± 0.53	138
Zn	Dogfish Liver (DOLT-3)	86.6	100	116
	Soil-5 (IAEA)	368 ± 8.00	326 ± 1.01	88.6

Note: CRM certified value for Pb is not available.

Table 3: Ranges of heavy-metal concentrations (mg/g dry weight) in the different parts of bivalves.

Metal	Species	Sampling sites	Muscle	Foot	Mantle	Gill	Shell	Remainder
Cu	*Gelonia expansa*	Kg Pasir Puteh	3.97-12.9	34.9-143	60.0-63.5	24.6-26.2	2.30-3.14	18.4-20.9
	Polymesoda erosa	Sg. Sepang Kecil	5.54-5.98	2.83-3.89	12.8-13.2	17.8-21.0	1.92-2.93	13.8-16.2
	Donax faba	Pantai Pasir Panjang	4.51-4.92	5.58-6.37	6.76-6.97	12.4-13.4	9.73-10.0	13.8-16.4
Zn	*Gelonia expansa*	Kg Pasir Puteh	201-225	120-131	274-277	368-379	6.13-7.15	113-228
	Polymesoda erosa	Sg. Sepang Kecil	135-167	73.5-102	353-365	330-349	2.96-5.38	104-215
	Donax faba	Pantai Pasir Panjang	27.5-29.1	38.1-39.4	42.2-49.1	44.6-94.5	4.55-4.57	43.1-44.6
Pb	*Gelonia expansa*	Kg Pasir Puteh	3.12-6.22	15.3-24.0	38.7-40.6	18.4-19.5	56.2-58.2	2.84-6.54
	Polymesoda erosa	Sg. Sepang Kecil-	3.13-3.68	1.70-2.50	4.56-4.79	4.37-4.61	56.9-61.7	3.69-4.80
	Donax faba	Pantai Pasir Panjang-	2.38-2.63	2.28-2.43	1.37-1.57	2.01-2.46	5.10-5.43	0.79-1.12
Ni	*Gelonia expansa*	Kg Pasir Puteh	9.63-11.7	9.16-9.79	11.4-11.5	12.7-15.5	24.4-25.9	7.79-9.12
	Polymesoda erosa	Sg. Sepang Kecil	5.17-5.40	3.74-4.22	10.3-12.6	19.7-23.5	29.4-30.8	4.72-5.82
	Donax faba	Pantai Pasir Panjang	1.59-2.47	1.49-1.67	2.66-3.83	2.10-4.91	28.3-28.7	2.37-3.03
Cd	*Gelonia expansa*	Kg Pasir Puteh	0.26-0.34	0.20-0.51	0.54-0.87	1.04-1.21	6.41-7.35	0.28-0.80
	Polymesoda erosa	Sg. Sepang Kecil	0.57-0.88	0.37-0.58	1.25-1.36	1.12-1.22	3.44-3.84	0.57-0.91
	Donax faba	Pantai Pasir Panjang	3.39-4.08	3.54-3.83	2.81-3.09	3.21-3.75	6.77-7.63	2.57-2.91

Table 4: Ranges of heavy-metal concentrations (mg/g dry weight) in the geochemical fractions and total concentration of the surface sediments.

Metals	Sampling sites	EFLE	AR	OO	Resistant	Total concentrations
Cu	Kg Pasir Puteh	0.62-0.65	0.35-0.37	22.7-22.9	8.16-11.1	28.7-28.8
	Sg. Sepang Kecil	0.11-0.15	0.34-0.43	2.39-2.44	3.86-4.79	6.14-6.54
	Pantai Pasir Panjang	0.55-0.66	0.77-0.78	2.08-2.16	15.0-15.4	12.0-12.7
Zn	Kg Pasir Puteh	45.3-46.4	39.5-40.6	36.1-38.3	34.5-36.0	142-143
	Sg. Sepang Kecil	1.92-2.01	9.54-10.5	15.7-15.9	7.95-9.06	31.7-33.1
	Pantai Pasir Panjang	0.37-0.47	2.52-2.87	12.5-12.9	82.8-86.0	65.1-69.9
Pb	Kg Pasir Puteh	1.60-1.88	0.63-0.64	22.0-22.9	18.3-21.1	34.3-39.0
	Sg. Sepang Kecil	2.52	0.72-0.78	0.57	14.3-17.4	17.9-19.4
	Pantai Pasir Panjang	0.83-0.87	3.87-4.75	4.27-4.39	40.2-41.4	27.4-29.9
Ni	Kg Pasir Puteh	1.64-1.89	1.09-1.12	10.0-10.7	4.87-6.45	15.5-16.3
	Sg. Sepang Kecil	0.69-0.73	0.25-0.35	2.26-2.42	4.02-4.87	5.98-6.74
	Pantai Pasir Panjang	1.55-1.59	4.27-4.48	6.11-6.41	10.9-14.5	20.0-21.0
Cd	Kg Pasir Puteh	0.23-0.33	0.10-0.63	0.62-0.68	1.35-1.40	2.11-2.15
	Sg. Sepang Kecil	0.04-0.06	0.03-0.07	0.08-0.10	0.83-0.91	1.15-1.24
	Pantai Pasir Panjang	0.27-0.30	1.53-1.67	1.08-1.15	2.23-2.47	2.19-2.48

Note: EFLE= Easily, freely, leacheable or exchangeable; AR=Acid-reducible; OO=Organic oxidisable; Total concentrations were based on direct aqua-regia method.

Table 5: Pearson's correlation coefficients between the heavy-metal concentrations (based on Cd, Cu, Ni, Pb and Zn) in the different parts of bivalves and the metal concentrations in the four geochemical fractions of the surface sediments.

Species/ Site						
	GE	EFLE	AR	OO	Resistant	SumSET
Gelonia expansa	Foot	0.639*	0.599*	0.938**	0.877**	0.927**
Kp. Pasir Puteh	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
	Total tissue	0.862**	0.851**	0.753**	0.849**	0.877**
Polymesoda erosa	Foot	0.524*	0.962**	0.974**	0.431	0.779**
Sg. Sepang Kecil	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
	Total tissue	0.464	0.963**	0.932**	0.400	0.738**
Donax faba	Foot	-0.580*	-0.246	0.535*	0.577*	0.546*
Pantai Pasir Panjang	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
	Siphon	-0.484	-0.159	0.624*	0.617*	0.601*
	Total tissue	-0.353	0.140	0.725**	0.860**	0.838**

Note: **= P< 0.01; *= P< 0.05; N=15; EFLE= Easily, freely, leacheable or exchangeable; AR=Acid-reducible; OO=Organic oxidisable; Sum SET=Summation of the 4 geochemical fractions based on SET.

Table 6: Biota-sediment (non-resistant fractions) accumulation factors (BSAF) based on the different parts of bivalves.

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

Note: Values in bold are the macroconcentrator (BSAF> 2).