

DISTRIBUTION OF HEAVY METAL CONCENTRATIONS IN DIFFERENT SOFT TISSUES AND SHELLS OF THE BIVALVE *Psammotaea elongata* AND GASTROPOD *Faunus ater* COLLECTED FROM PANTAI SRI TUJUH, KELANTAN

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Abstract: Snails (*Psammotaea elongata*) and clams (*Faunus ater*) collected from Pantai Sri Tujuh, Tumpat (Kelantan) were dissected into operculum, foot, gill, muscle, and digestive caecum. The clams were dissected into siphon, foot, gill, muscle, mantle and remainder parts. The different soft tissues and shells were pooled and determined for Cd, Cu, Pb and Zn. Both species were found to have higher concentrations of non-essential Cd and Pb in their shells than those in the different soft tissues. On the other hand, the soft tissues (especially remainder parts) had consistently higher concentrations of essential Zn and Cu than those in the shells in both molluscs. The ecological distribution and metal concentrations for the two molluscs species found at Pantai Sri Tujuh can serve as a baseline information.

KEYWORDS: *Psammotaea elongata*, *Faunus ater*, snails, clams, soft tissues and shells

Introduction

Ecological distribution of clam *Psammotaea elongata* (Family: Psammobiidae) and gastropod *Faunus ater* (Family: Potamididae) are reported to occur in habitats particularly in the tropical intertidal areas such as Peninsular Malaysia and Phillipines (Valtat, 2006; Anon, 2008). Snail *F. ater* is native to Phillipines, Southeast Asia and East Indies (Anon, 2008). Brandt (1974) included *Faunus* in the family Potamididae although some literature included it in the family Thiariidae as it lives in fresh as well as slightly brackish water near the coast in creeks, small rivers and lagoons. They are filter-feeders that use their gills to extract organic matter from the water in which they live. Compared to sediments, molluscs exhibit greater spatial sensitivity and therefore, are the most reliable tool for identifying sources of biologically available heavy metal contamination (Goldberg, 1975; Szefer, 1986; Rainbow, 1997). University of Florida Malacology database (Anon, 2008) noted that, *P. elongata* was collected from Mersing Johore, 1986. The *F. ater*, was obtained from Teluk Dalam, Pulau Perhentian, Malaysia (Valtat, 2006). *F. ater* are herbivores and detritus feeders and typically have a well-defined head with two tiny sensory tentacles and a ventral foot, which gives them their name (Anon, 2008).

Rainbow (1997), concluded that aquatic molluscs are suitable as a biomonitor because it accumulates heavy metals directly from sediments, particles and metals dissolved in water. The molluscs are usually employed as biomonitors of heavy metal pollution due to their ability to accumulate metals in their tissues which are proportional to the degree of environmental contamination. They are suitable 'in situ' biomonitors because they are sedentary or less mobile, abundant, large, easily collected and are consumed by humans (Rainbow, 1997). One of the established biomonitors in this region is the green-lipped mussel, *Perna viridis* (Yap et al., 2003c; 2003b; 2004; 2006a; 2006b; 2006c). However, knowledge on other molluscs such as *P. elongata* and *F. ater* is scarce.

Molluscs accumulate trace metals whether essential or not, all of which have the potential to cause toxic effects at elevated concentrations (Rainbow, 2007) and can be distributed in the different soft tissues of the molluscs (Yap et al., 2006c). Hence, information on the heavy metal concentrations in their tissues is potentially useful in considering toxicological and public health implications of estuarine contamination.

Since there is no reported study on *P. elongata* and *F. ater* from Malaysia in the literature, the objective of this study was to determine the heavy metal concentrations in the different soft tissues and shells of *P. elongata* and *F. ater* collected from Pantai Sri Tujuh, Kelantan.

Materials and Methods

The sampling of clams and snails were conducted at Pantai Sri Tujuh, Kelantan (Figure 1). The main activities in Pantai Sri Tujuh include fish aquaculture, salt fish industry and boat industry. The *P. elongata* and *F. ater* were collected from Pantai Sri Tujuh on 20 August 2006. Identification of both species was based on Anon (2008) for *P. elongata* and Valtat (2006) for *F. ater*.

Thirty individuals were analysed for each species. The ranges of sizes of the clams and the snails were 2.53 cm – 5.82 cm and 3.82 cm – 6.50 cm in length, respectively. Clams were carefully dissected into different parts namely remainder, mantle, muscle, foot, gills and siphon while the snails were dissected into remainder, operculum, muscle, foot, gills and digestive caecum. All of the different soft tissues and shells were placed in aluminum foils. It was then dried in oven for 72 hours at 105°C to constant dry weights (Mo and Neilson, 1994). Dried samples were then stored in clean plastic bags.

About 0.5g of dried soft tissue parts were weighed and placed in acid-washed test digestion tubes. A total of 10ml of concentrated nitric acid (AnalaR grade, BDH 69%) was added to the digestion tube to digest the soft tissues. Three replicates of each soft tissues were prepared for the two species. They were placed in digestion block at 40 °C for 1 hour and were then fully digested at 140°C for 3 hours (Yap et al., 2003a). After cooling the samples diluted to 40ml with double de-ionized water. The digested samples were then filtered through Whatman No. 1 (filter speed: medium) filter papers with funnels into acid-washed pill boxes.

All samples were analyzed for Cd, Cu, Pb and Zn using the air-acetylene Perkin-Elmer™ flame Atomic Absorption Spectrophotometer model AAnalyst 800. Standard solutions were prepared from 1000ppm stock solution (MERCK Titrisol) for Cd, Cu, Pb and Zn and the data were presented in µg/g dry weight.

All equipments and glassware were first acid-washed into 5% of nitric acid solution to avoid contamination. Procedural blanks and quality control samples made from standard solution for each metal were analyzed once in every ten samples to check for sample accuracy (Yap et al., 2003c). The quality of the method used was checked with a Certified Reference Material (CRM) for Dogfish liver-DOLT-3 (National Research Council Canada) and the recoveries were satisfactory (103.07 %, 102.59 % and 95.61 % for Cd, Cu and Zn, respectively). However, the CRM recovery for Pb was not available.

One-way ANOVA was conducted to determine whether the differences among concentrations of each metal in the different parts of two species are significant. Tukey HSD's multiple range tests, was employed to determine which group means did not differ from one another. All the statistical analyses were performed using Statistical Package for Social Science (SPSS) for Windows package.

Results and Discussion

The concentrations of heavy metals (Cd, Cu, Pb and Zn) in different tissues of *P. elongata* and *F. ater* collected from Pantai Sri Tujuh Tumpat are given in Table 1 and 2, respectively.

For the clams *P. elongata*, it was found that hard tissues (shells) accumulated the highest concentrations of Cd and Pb, in contrast to foot which showed the lowest concentrations for these two metals. All the different soft tissue of the clams had higher levels of Zn compared to the shell. Among the different soft tissues, remainder parts exhibited the highest concentrations of Cu and Zn. Gill was also highly accumulative of Cu and Zn. Adams et al. (1981) also found Zn to be preferentially concentrated in the gill tissue of freshwater clams located after a known industrial discharge in Indiana. For Cu, foot was found to be weak accumulators for this metal since the metal levels in this organ were low.

For the snails *F. ater*, the shells of the snails had the highest concentrations of Cd and Pb compared to those of the different soft tissues. Concentrations of Cu and Zn are found to be lower in the shells and operculums of *F. ater* compared to the different soft tissues.

The high levels of metals in the two species may be attributed to sewage and wastes discharged from nearby villages and the boat industry located near the riverside. The bay also receives waste effluent from the domestic and fish aquaculture.

In general, the concentrations of Cd and Pb displayed high metal concentrations in the shells of *P. elongata* and *F. ater*. This could be due to both non-essential metals being highly incorporated into the shell as carbonate or occur as trace amounts within the crystal structure of the shell (Pilkey & Goodell, 1963). Anderson (1977) found similar levels of Pb in both the shells and soft tissue of *Anodonta*. However in this study, Pb displayed a greater Pb affinity to the shell than to the soft tissues of *P. elongata* and *F. ater*.

Similar results were reported by Bourgoïn (1990), Yap et al. (2003d) and Cravo et al. (2004). They proposed that bivalve shells are better than soft tissues for monitoring pollution because of lower degree of trace metal variation. The essential metals in the different soft tissues are incorporated in metabolically important biomolecules (or bonded to their functional groups) with a key role in the organism such as respiratory pigments, proteins, enzymes or metalloenzymes. (Bryan et al., 1985; Rainbow, 1997) whereas in the shell, metal ions may be incorporated into the carbonate crystal lattice by diadochically replacing the calcium ions in the calcite or can also be triggered by metabolic functions (Babukutty and Chacko, 1992).

The concentrations of Cu and Zn were consistently lower in the shells than in the soft tissues. The same results were found even in oysters (Carriker et al., 1980), clams (El-Nady, 1996), mussels *Mytilus edulis* (Giusti et al., 1999; Szefer et al., 2002) and the gastropod *L. sabra* (de Wolf et al. 2001). In contrast for limpets *P. vulgata* (Segar et al. 1971), gastropods *T. lapillus* and *L. littorea* (Ireland and Wootton, 1977) or bivalves *M. edulis*, *Cardium glaucum* and *Astarte borealis* (Szefer and Szefer, 1985; Szefer et al., 1990), the concentrations of Cu and Zn were consistently higher in the shells than in the soft tissues.

The metal distribution in the different soft tissues of *P. elongata* and *F. ater* could be explained by three points. Firstly, the variation distribution is due to differences in the surface of contact of the different soft tissues (Yap et al., 2003c). Secondly, the differences in affinity of metals to the binding sites of metallothioneins found in the different soft tissues. Thirdly, different rates of accumulation and excretion of metals are found in different soft tissues. The differences in the rate of accumulation and depuration indicated that they were the results of internal metal treatment and regulation (Yap et al., 2004).

The essential Zn and Cu which were found in high concentrations in the different soft tissues of molluscs could be due to incorporation in blood pigments (Cu) and proteolytic enzymes (Zn). It is known that the concentrations in essential metals in molluscs can be regulated by homeostatic mechanisms (Prosi, 1979). The regulation of Zn has been reported by several authors (Lobel et al., 1982). The present study shows that the remainder parts always had higher concentrations of Zn and Cu (especially in *P.elongata*) than the other parts. This could be due to their contents included kidney and digestive gland which are known storage site for Cu and Zn (Langston et al., 1998; Blackmore, 2001). It was also reported that detoxification of toxic metals such as Cu and Zn by metallothionein synthesis occurs in molluscs (Roesijadi, 1992).

The present finding indicated (i) the soft tissues represent a major reservoir for Cu and Zn; (ii) the shells could represent a sink for Cd and Pb by removing a relatively higher percentage of this metal from the surrounding medium. This agrees with Langston et al. (1998) that shells are a potential sink for metals due to their capacity to remove bioavailable metals from the environment.

The heavy metal concentrations of the operculums of *F. ater* showed no significant differences with those of shells ($P > 0.05$) for Cu and Zn (Table 2) which could be due to the common structure of the shell and the operculum as both consisted of calcified tissues (Hunt, 1970). As mentioned earlier, incorporation of Cd and Pb occurs in the shells which could be due to the deposition of unwanted or non-essential metal (Yap et al., 2003d). This explains why the operculum, which is not a detoxifying site of the metals, has lower concentrations of Cd and Pb compared to the shell.

Several workers suggested that heavy metals within the tissues of molluscs from a wide range of phyla are first detoxified and then stored in the form of intracellular granules of different shapes and sizes (Simkiss and Mason, 1983; Rainbow and Philips, 1993). In gastropods, the digestive gland was described as the main productive source of granules (Mason and Nott, 1981; Mason et al., 1984).

Conclusion

The variation in the levels of metals in the different parts of *P. elongata* and *F. ater* showed the storage ability of metal concentrations differ among the parts. Cd and Pb were found higher in shells for both species, indicating the shells of gastropods and bivalves can be used as a monitoring tool for these non-essential metals. In contrast to Zn and Cu, the different soft tissues are potential biomonitors for the two essential metals. The findings based on the two species are important future reference since these molluscs can be potentially established as biomonitors of heavy metal pollution in the future.

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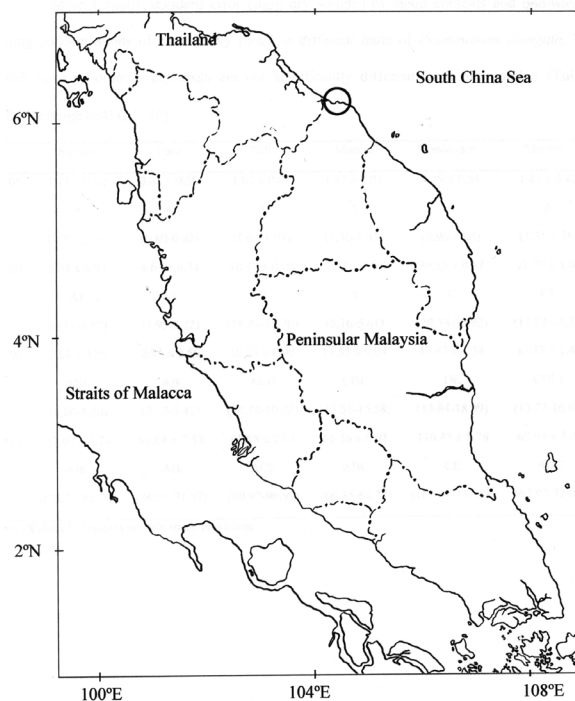


Figure 1 : Sampling map for molluscs of Pantai Sri Tujuh Kelantan (in circle).

Table 1: Mean (range), standard error ($\mu\text{g/g}$ dry weight) of metal contents and one-way ANOVAs among concentrations of each heavy metal in different parts of *Psammotaea elongata*. The tissues which have a common alphabets are not significantly different from one another (Tukey HSD's, multiple range test) ($n = 30$)

Metal	P	Siphon	Foot	Gill	Muscle	Remainder	Mantle	Shell
Cd	< 0.05	1.21 $\pm$ 0.10	0.86 $\pm$ 0.06	1.03 $\pm$ 0.01	1.37 $\pm$ 0.01	1.49 $\pm$ 0.54	1.43 $\pm$ 0.12	7.22 $\pm$ 0.26
		A	A	A	A	A	A	G
		(1.11-1.32)	(0.80-0.92)	(1.02-1.03)	(1.36-1.37)	(0.96-2.03)	(1.31-1.56)	(6.96-7.48)
Cu	< 0.05	5.02 $\pm$ 0.91	4.69 $\pm$ 0.74	18.17 $\pm$ 0.08	1.37 $\pm$ 0.01	30.25 $\pm$ 0.67	12.29 $\pm$ 1.04	20.64 $\pm$ 2.19
		AF	AF	C	A	E	CF	CG
		(4.11-5.92)	(3.95-5.42)	(18.03-18.30)	(5.16-5.61)	(29.58-30.92)	(11.24-13.33)	(18.45-22.83)
Pb	< 0.05	7.65 $\pm$ 0.85	2.91 $\pm$ 0.56	10.33 $\pm$ 0.09	15.55 $\pm$ 0.04	17.42 $\pm$ 1.58	15.17 $\pm$ 1.46	56.39 $\pm$ 2.35
		ABC	AB	ACD	CDE	DE	CDEF	G
		(6.80-8.50)	(2.35-3.47)	(10.24-10.42)	(15.51-15.58)	(15.84-18.99)	(13.72-16.63)	(54.05-58.74)
Zn	< 0.05	77.02 $\pm$ 3.74	64.04 $\pm$ 7.53	90.98 $\pm$ 0.01	66.36 $\pm$ 0.01	110.45 $\pm$ 8.76	69.99 $\pm$ 2.02	12.79 $\pm$ 1.37
		ABC	AB	ACE	ABC	CE	ABC	G
		(73.27-80.76)	(56.51-71.57)	(90.97-90.99)	(66.35-66.37)	(101.69-119.21)	(67.97-72.01)	(11.41-14.16)

Note : Values in brackets are minimum-maximum

Table 2: Mean (range), standard error ($\mu\text{g/g}$ dry weight) of metal contents and one-way ANOVAs among concentrations of each heavy metal in different parts of *Faunus ater*. The tissues which have a common alphabets are not significantly different from one another (Tukey HSD's, multiple range test) ($n = 30$)

Metal	P	Oper	Foot	Gill	Muscle	Remainder	DC	Shell
Cd	< 0.05	2.03 $\pm$ 0.01	0.71 $\pm$ 0.24	1.80 $\pm$ 0.18	1.79 $\pm$ 0.08	3.18 $\pm$ 0.64	2.67 $\pm$ 0.22	8.16 $\pm$ 0.49
		ABEF	AB	ABEF	ABF	AEF	AEF	G
		(2.01-2.05)	(0.47-0.96)	(1.62-1.98)	(1.71-1.86)	(2.55-3.82)	(2.45-2.89)	(7.67-8.66)
Cu	< 0.05	41.62 $\pm$ 0.02	77.92 $\pm$ 11.04	174.27 $\pm$ 22.13	60.31 $\pm$ 2.65	107.89 $\pm$ 1.94	145.02 $\pm$ 38.22	11.90 $\pm$ 1.01
		ABD	ABCDF	BCF	ABDF	ABCDF	BCDF	ABD
		(41.60-41.64)	(66.88-88.96)	(152.15-196.40)	(57.66-62.96)	(105.95-109.825)	(106.80-183.23)	(10.89-12.91)
Pb	< 0.05	25.09 $\pm$ 0.01	4.52 $\pm$ 0.90	14.23 $\pm$ 0.99	19.78 $\pm$ 0.18	27.05 $\pm$ 0.20	20.54 $\pm$ 2.60	66.66 $\pm$ 1.43
		ADEF	B	CDF	ACDF	AEF	ACDEF	G
		(25.08-25.10)	(3.63-5.42)	(13.24-15.23)	(19.61-19.96)	(26.85-27.25)	(17.95-23.14)	(65.24-68.09)
Zn	< 0.05	29.48 $\pm$ 0.02	73.04 $\pm$ 1.26	58.02 $\pm$ 0.87	73.10 $\pm$ 0.70	54.24 $\pm$ 0.70	226.10 $\pm$ 15.42	34.06 $\pm$ 0.71
		AC	BC	ABC	BCD	ABCE	EF	AC
		(29.46-29.50)	(71.78-74.30)	(57.14-58.89)	(72.40-73.79)	(53.54-54.94)	(210.68-241.52)	(33.35-34.76)

Note : Values in brackets are minimum-maximum
: Oper = operculum; DC = Digestive Caccum

Table 3: Decreasing order of metals concentrations in the different parts of *P. elongata*

Cd	Shell	>	Remainder	>	Mantle	>	Muscle	>	Siphon	>	Gill	>	Foot
Cu	Remainder	>	Shell	>	Gill	>	Mantle	>	Muscle	>	Siphon	>	Foot
Pb	Shell	>	Remainder	>	Muscle	>	Mantle	>	Gill	>	Siphon	>	Foot
Zn	Remainder	>	Gill	>	Siphon	>	Mantle	>	Muscle	>	Foot	>	Shell

Table 4: Decreasing order of metals concentrations in the different parts of *Faunus ater*

Cd	Shell	>	Remainder	>	DC	>	Operculum	>	Gill	>	Muscle	>	Foot
Cu	Gill	>	DC	>	Remainder	>	Foot	>	Muscle	>	Operculum	>	Shell
Pb	Shell	>	Remainder	>	Operculum	>	DC	>	Muscle	>	Gill	>	Foot
Zn	DC	>	Muscle	>	Foot	>	Gill	>	Remainder	>	Shell	>	Operculum

Note: DC = Digestive Caecum