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Distribution of Heavy Metal Concentrations in the Different Soft and Hard Tissues of Tropical Mud-Flat Snail *Telescopium telescopium* (Family: Potamididae) Collected From Sepang Besar River

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ABSTRACT

The concentrations of Cd, Cu, Pb, Fe, Ni and Zn were determined in the different parts of the soft tissues (foot, cephalic tentacle, mantle, muscle, gill, digestive caecum and remaining soft tissues) and three parts of hard tissues or shells (anterior shell, middle shell and posterior shell) of the mud-flat snail *Telescopium telescopium* collected from Sepang Besar River. From the cluster analysis, the dendrogram shows that the three parts of the shells are clustered separately from the different parts of the soft tissues, indicating different mechanisms and strategies of metal accumulation and regulation of heavy metals in the shells from the different soft tissues. Among the different soft tissues, the dendrogram also shows that the digestive caecum is clustered differently from other soft tissues, indicating that this organ is distinctly high in metal accumulation and this may probably suggest a different route of metal sequestration from the rest of the soft tissues. The metal distribution found in the different soft tissues of *T. telescopium* is an important knowledge in establishing this mud-flat gastropod as a promising biomonitor of metal contamination and bioavailability for the intertidal area of Peninsular Malaysia.

Keywords: *Telescopium telescopium*, metal distribution, different tissues

INTRODUCTION

It has been widely reported in the literature that gastropods accumulate metals in their tissues in proportion to the degree of environmental contamination and that they can be used as biomonitors of marine metallic pollution (Goldberg *et al*., 1978). The usefulness of molluscs, as sentinel

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organisms in metal biomonitoring studies, is widely recognized (see Rainbow, 1990, 1993; Langston & Spence, 1995; Brown & Depledge, 1998). Snails are good models for examining the effects of pollution on populations because they are in contact with polluted bottom sediments and have short generation time (Lefcort *et al*., 2004). Snails are also known to alter their locations in order to thermoregulate with great accuracy (Lefcort & Bayne, 1991). They are appropriate to be use as biomonitors in situ because they are sedentary, abundant, of relative longevity, large, as well as easily collected and weighed (Hartley & Johnston, 1983).

Most of the biomonitoring studies using gastropods have been directed either to the total soft tissues (e.g. Ismail & Safahieh, 2004) or to the shells, but very few have concurrently addressed trace metal concentrations in the different parts of both the soft and hard tissues. In general, the accumulation and storage of trace metals (e.g. Cd, Cu and Zn) in common biomonitors such as gastropods are strongly associated with the level and metal binding capacity of metallothioneins in their tissues (Roesijadi, 1992; Carpene, 1993; Dallinger *et al*., 1997, 2004a, b).

The objective of this study was to determine the distributions of Cd, Cu, Fe, Ni, Pb and Zn in the different parts of the soft tissues and shells of *T. telescopium* which had been collected from Sepang Besar River.

MATERIALS AND METHODS

Snails were collected from Sepang Besar River (N 02º 36’ 19.41”; E 101º 42’ 11.51”) (*see* Fig.1) on 7th January 2006. These samples were brought back to the laboratory for heavy metal analyses. From the visual observation, this sampling site was close to a restaurant, a jetty and a water irrigation facility. The mean height and width of the shells measured in the snails were 8.35 cm and 4.56 cm, respectively. The shells were cleaned by scrubbing in distilled water with a toothbrush to remove biogenic and inorganic particles (Cravo *et al*., 2004). Meanwhile, total soft tissues of the snail were extracted from the shell and separated into seven different parts (foot, cephalic

TABLE 1
The percentages of weight contributions in the seven soft tissues of *Telescopium telescopium* (N =10).

Soft tissues	%
Digestive caecum	17.33
Foot	15.61
Mantle	8.32
Remaining soft tissues	19.46
Gill	20.07
Cephalic tentacle	5.92
Muscle	13.28

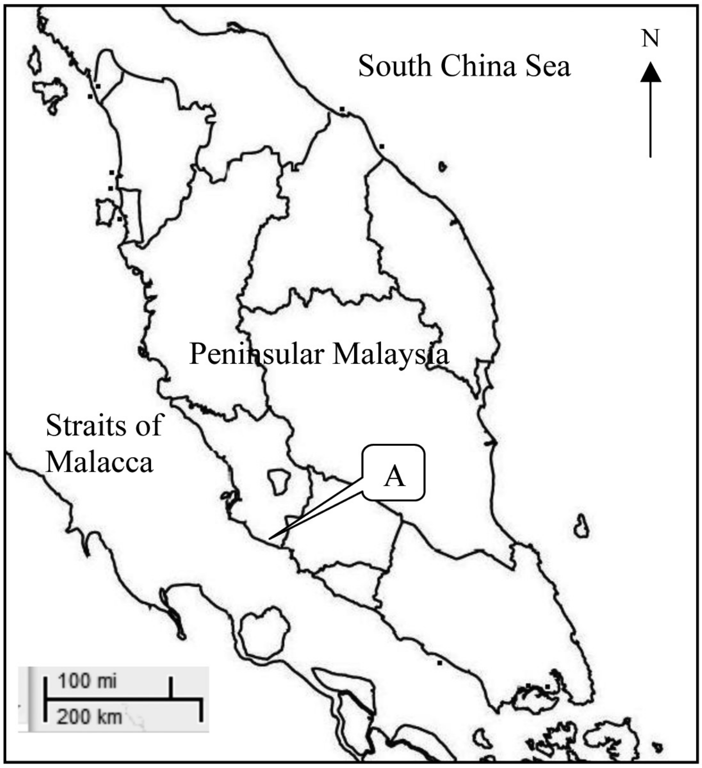

Fig.1: The sampling site of *Telescopium telescopium* at Sepang Besar River (A), Selangor

tentacle, mantle, muscle, gill, digestive caecum and remaining soft tissues) and this were then pooled for each part to form a single batch sample. The percentage of the weight distribution in each separated/dissected soft tissue is given in Table 1. The shells were separated into three parts (body whorl, middle and apex). All the separated samples were dried at 80°C to constant dry weights. Three replicates of each dissected tissue of the snails were then digested in concentrated nitric acid (BDH: 69%) (Yap *et al.*, 2004). The snail samples were put in a hot-block digester at low temperature (40°C) for 1 h and then fully digested at 140°C for 3hrs (Yap *et al.*, 2002, 2004). The digested samples were added up to 40 ml with double distilled water.

After filtration, the prepared samples were determined for Cd, Cu, Fe, Ni, Pb and Zn by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin Elmer Model AAnalyst 800. The data were presented in µg/g dry weight basis. Multi-level calibration standards were analyzed to generate calibration curves against which the sample concentrations were calculated. Standard solutions were prepared from 1000 mg/L stock solutions of each metal (Merck Titrisol).

All the glassware and plastic materials used were acid-washed in 10%

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

For the statistical analysis, the distribution of heavy metals in the different parts was determined using the cluster analysis. The relationships between heavy metals in the different parts were analyzed using the Pearson's correlation coefficient. All the data were $\log_{10} (\times + 1)$ transformed prior to the statistical analysis in order to reduce variance (Zar, 1996). SPSS 12.0 was used to conduct the correlation analysis, while STATISTICA 99 edition was used to conduct the cluster analysis.

RESULTS

The concentrations (µg/g dry weight) of six heavy metals in the different soft tissues and three different parts of shells are presented in Table 3. Based on the correlation analysis in Table 4, Cd, Ni and Pb were shown to be positively and significantly ($P< 0.05$) correlated to each other, while Cu, Fe and Zn were found to be positively and significantly ($P< 0.05$) correlated. Most distinctly, Cd, Ni and Pb were negatively (although mostly were not significant) correlated with Cu and Zn. These results indicated that Cu, Fe and Zn are essential metals which are much needed for the basal metabolism of the snails in contrast to Cd and Pb which are non-essential metals and therefore, their binding sites in the cells are different from the essential metals. Although Ni is now recognized as an essential metal in animals, the results obtained in the current work showed that Ni is more correlated to the non-essential Cd and Pb. Nonetheless, further studies are still required to further investigate this particular finding.

Based on the cluster analysis illustrated in Fig.2, the three parts of the shells were separately grouped from the seven soft tissues to indicate that the accumulation, excretion and sequestration of metals are different between the soft and the hard

TABLE 2
A comparison of the metal concentrations (µg/g dry weight) between Certified Reference Materials (DOLT-3 Dogfish-liver) and their measured values.

Metals	Certified values	Measured values	Percentage % of recovery
Cd	19.4 ± 0.600	20.5 ± 0.439	106 ± 2.26
Cu	31.2 ± 1.00	26.5 ± 2.58	85.0 ± 8.28
Fe	1484 ± 57.0	1070	72.1
Ni	2.72 ± 0.350	2.77 ± 0.741	102 ± 27.2
Zn	86.6 ± 2.40	80.9 ± 1.94	93.4 ± 2.24

Note: The certified reference material for Pb is not available.

TABLE 3
The concentrations (µg/g dry weight) of Cd, Cu, Pb, Fe, Ni and Zn in the different soft tissues of *Telescopium telescopium* collected from Sepang Besar River.

Tissues	Pb	Minimum	Maximum	Mean	Std error
Shells	Body whorl	22.6	24.6	23.5	0.58
	Middle shell	23.2	26.4	24.6	0.94
	Apex	20.7	26.7	23.5	1.75
Soft tissues	Foot	0.00	0.56	0.19	0.19
	Cephalic tentacle	0.00	0.28	0.14	0.08
	Gill	14.4	15.9	15.1	0.44
	Muscle	0.00	0.66	0.22	0.22
	Remainder	5.70	8.25	7.06	0.74
	Digestive caecum	9.77	10.9	10.36	0.35
	Mantle	1.21	1.86	1.55	0.19
	Ni	Minimum	Maximum	Mean	Std error
Shells	Body whorl	21.0	22.6	21.7	0.46
	Middle shell	22.2	24.9	23.1	0.89
	Apex	19.7	23.3	21.4	1.06
Soft tissues	Foot	3.74	3.98	3.85	0.06
	Cephalic tentacle	4.80	6.88	5.59	0.64
	Gill	12.3	13.4	12.7	0.36
	Muscle	4.21	6.05	4.95	0.56
	Remainder	8.60	11.2	9.90	0.77
	Digestive caecum	47.9	51.9	50.3	1.18
	Mantle	4.55	5.04	4.84	0.14
	Cu	Minimum	Maximum	Mean	Std error
Shells	Body whorl	6.91	7.36	7.15	0.13
	Middle shell	8.08	9.41	8.95	0.43
	Apex	7.20	8.84	8.21	0.51
Soft tissues	Foot	98.4	111	106	3.98
	Cephalic tentacle	63.6	85.2	76.8	6.66
	Gill	76.1	97.5	86.9	6.18
	Muscle	43.8	58.7	51.1	4.31
	Remainder	66.5	107	88.1	11.9
	Digestive caecum	128	175	147	14.0
	Mantle	81.7	103	90.8	6.36

Table 3 (*continued*)

	Zn	Minimum	Maximum	Mean	Std error
Shells	Body whorl	6.80	7.19	7.02	0.11
	Middle shell	6.98	11.5	8.62	1.46
	Apex	6.22	8.29	7.56	0.66
Soft tissues	Foot	66.3	72.1	69.6	1.72
	Cephalic tentacle	55.3	67.9	60.9	3.70
	Gill	73.7	74.6	74.1	0.27
	Muscle	78.7	83.7	80.5	1.59
	Remainder	47.5	108	84.1	18.6
	Digestive caecum	215	224	220	2.57
	Mantle	65.3	72.0	68.5	1.93
	Cd	**Minimum**	**Maximum**	**Mean**	**Std error**
Shells	Body whorl	3.18	3.75	3.43	0.16
	Middle shell	3.20	3.47	3.29	0.08
	Apex	2.76	3.22	2.97	0.13
Soft tissues	Foot	0.00	0.02	0.01	0.00
	Cephalic tentacle	0.00	0.06	0.02	0.02
	Gill	0.17	0.65	0.46	0.14
	Muscle	0.04	0.36	0.23	0.09
	Remainder	0.66	0.78	0.72	0.03
	Digestive caecum	2.79	3.11	2.95	0.09
	Mantle	0.40	0.43	0.42	0.01
	Fe	**Minimum**	**Maximum**	**Mean**	**Std error**
Shells	Body whorl	109	195	153	24.9
	Middle shell	63.2	98.9	76.1	11.4
	Apex	67.0	90.5	77.1	6.97
Soft tissues	Foot	161	224	195	18.3
	Cephalic tentacle	161	217	187	16.1
	Gill	1501	1598	1536	31.2
	Muscle	126	264	201	40.4
	Remainder	826	1316	1147	160
	Digestive caecum	1448	1517	1490	21.2
	Mantle	242	318	279	21.8

Note: Remainder= remaining soft tissues.

TABLE 4
The correlation coefficients of heavy metal concentrations ($\log_{10}$ mean +1) based on seven soft tissues and three hard tissues of *Telescopium telescopium* population.

	Pb	Ni	Cu	Zn	Cd	Fe
Pb	1.00	**0.84**	-0.61	-0.56	**0.87**	0.03
Ni		1.00	-0.39	-0.27	**0.91**	0.15
Cu			1.00	**0.97**	**-0.68**	**0.73**
Zn				1.00	-0.58	**0.76**
Cd					1.00	-0.20
Fe						1.00

Note: Values in bold are significant at P> 0.05.

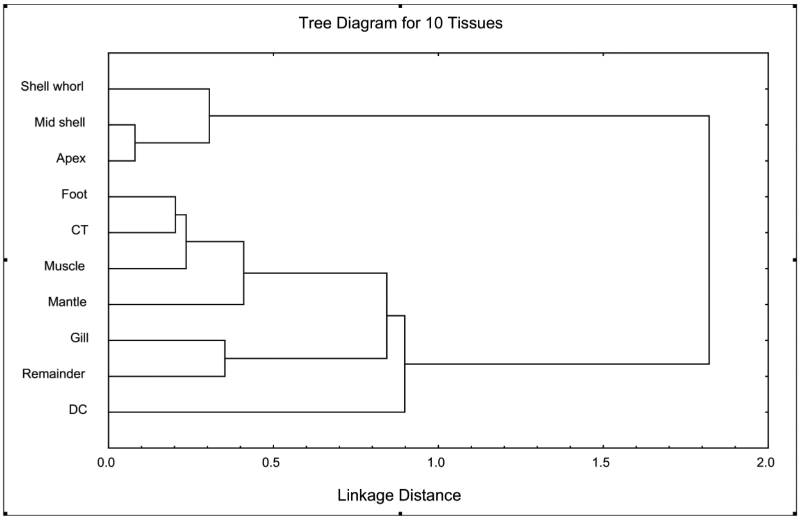

Fig. 2: The clustering pattern of the 10 tissues of *Telescopium telescopium* based on 6 metal concentrations after (log10 [mean +1]) being transformed. Note: CT= Cephalic tentacle; DC = Digestive caecum

tissues. Among the different soft tissues, digestive caecum forms a major cluster differently from the other soft tissues.

DISCUSSION

The discussion of the present data is given based on the following two points.

First, the difference between the clustering pattern in the shells and the soft tissues of T. telescopium.

The metal concentrations found in the shells of the gastropod could be due the different mechanisms of metal accumulations in them. Non-essential metals, like Cd and Pb found in the shell, could be explained by the fact that some trace metals are incorporated into the shells of the gastropod through substitution of calcium ion in the crystalline phase of the shell or are associated with the organic matrix of the shell (Yap *et al*., 2003a). This is totally different from the binding sites for soft tissues (metallothionein). At first, the metals could be distributed in the different soft tissues before they were biodeposited in the shell of the gastropods (Yap *et al*., 2003a).

This study found that the concentrations of Cd, Ni and Pb in the shells were higher than those in the other soft tissues of the snail. Meanwhile, studies on metal accumulation in shells are useful since they can be used as a record of environmental metal levels (Chow *et al.*, 1976). Shells have important practical advantages over the use of soft tissues for monitoring metal contamination in the aquatic environment since they show less variability (Bourgoin, 1990), integrating elemental concentrations over the life of the molluscs and preserving the metals after the death of the organisms. This could give information about the metal concentrations that they were exposed to in the past (Cravo *et al*., 2004) and offer considerable advantages in preservation and storage. Generally, the metal concentrations in the soft tissues show greater variability than those in the shells (Yap *et al*., 2003a), and this is usually due to seasonal weight changes (associated with physiological conditions, reproductive state) and consequently, shells may provide a more realistic indication of the degree of contamination/pollution (Cravo *et al*., 2002).

Second, the different metal concentrations in the different soft tissues of T. telescopium.

The accumulated metal concentrations were regulated in the different parts of the gastropod soft tissues. In this study, it was found that the different parts (e.g. the digestive caecum and mantle) tended to accumulate high concentrations of heavy metals. Besides, Bebianno and Langston (1995) mentioned that, in general, the tissues where absorption takes place have more metal accumulation than other tissues.

Differential affinities of metals to the binding sites may be associated with different metal accumulations found in the different tissues. In more specific, the high level of certain metal found in a particular tissue might be due to the fact that the metal was tightly bound to the metallothionein, as reported by Roesijadi (1992) in the mussels. The formation of a metal-thiolate complex, with the cysteine residues inside the lysosomes, has caused a slower depuration of the metals found in the different tissues (Yap *et al*., 2003b) which could result in the high level of metals found in the above-mentioned tissues. This mechanism would reduce its toxicity by preventing it from disturbing the cell activities (Webb, 1987).

In addition, the important accumulation of the metals in the different tissues mentioned above could also be related to the functions of these organs. The mantles are in contact with the external medium and are responsible for the metal transfer to organism. This further indicates that the differences in the surface of contact of the different soft tissues may affect the accumulations of the metals by the mollusc's tissues (Yap *et al*., 2003b). The digestive caecum, which is a part of the digestive gland, plays an important role in heavy metal metabolism, and this thus contributes to their metal detoxification (Viarengo, 1989; Saha *et al*., 2006). This can explain the high metal concentrations in these organs. The different rates of accumulation and the excretion of the metals in the different tissues also result in the different concentrations found in each of the molluscs' tissues (Yap *et al*., 2003b).

The high concentrations of Cu and Zn found in the digestive caecum may be related to the importance of the two metals in the metabolism of foods in the gastropods since Cu and Zn are essential metals. As for the high concentration of Fe found in the operculum, however, it could be due to its essentiality in forming the corneous plate (Ghesquiere, 2005). The different metal accumulations in the different parts of *T. telescopium* were characterized by the accumulativeness of specific metal as revealed in this study, and thus, might allow the accurate estimation of the metal bioavailability in the coastal area. The bioavailability of the contaminants in the environment is a complicated issue which involves many aspects such as chemical, physical and biological (van Straalen *et al*., 2005). Therefore, the use of the different parts of gastropods that are accumulative of specific metal(s) was strongly recommended in the present study.

Generally, the metal concentrations in the digestive caecum are higher compared to other soft tissues (Table 3). This may be due to the crucial role played by the digestive caecum in the animals' nutritional physiology (Menta & Parisi, 2001). The high Cu concentrations found in the remaining soft tissues, mantle and gill might partly be due to hemocyanin (Dallinger *et al*., 1997). Meanwhile, the metal distribution in the different soft tissues could be due to the environmental metal bioavailability of the habitats and biometric characteristics (Cravo *et al*., 2004). According to Laskowski and Hopkin (1996), the distribution of metals in soft tissues and shells indicated that contamination in the soft tissues could pose a more important threat to higher trophic levels because the protein in the soft tissues is easily soluble and readily available for higher trophic levels during consumption.

CONCLUSION

The results of the present study indicated the ability of the different soft tissues of *T. telescopium* to accumulate Cd, Cu, Fe, Ni, Pb, and Zn. The cluster analysis showed that metal behaviours for Cd, Ni and Pb were different from Cu, Fe and Zn. Meanwhile, the different clusters between the hard and soft tissues indicated that the binding sites and strategies are different. The present study has also shown that the digestive caecum of *T. telescopium* could be potentially used as a better biomonitoring organ for heavy metal bioavailability and contamination in the intertidal area of Malaysia.

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REFERENCES

Bebianno, M. J., & Langston, W. J. (1995). Induction of metallothionein synthesis in the gill and kidney of *Littorina littorea* exposed to cadmium. *J. Mar. Biol. Assoc. UK.*, *75*, 173-186.

Bourgoin, B. P. (1990). *Mytilus edulis* shell as a bioindicator of lead pollution: considerations on bioavailability and variability. *Mar. Ecol. Prog.,* *61*(3), 253-262.

Brown M. T., & Depledge M. H. (1998) Determinants of trace metal concentrations in marine organisms. In W. Langston, & M. J. Bebianno (Eds.), *Metal metabolism in aquatic environments*, (pp. 185–217). London: Chapman & Hall.

Carpene, E. (1993). Metallothionein in marine molluscs. In R. Dallinger, & P.S. Rainbow (Eds.), *Ecotoxicology of Metals in Invertebrates* (pp. 55–72). London: Lewis Publishers.

Chow, T. J., Snyder, H. G., & Snyder, C. B. (1976). Mussels (*Mytilus* sp.) as an indicator of lead pollution. *Sci. Total Environ., 6*, 55-63.

Cravo, A., Bebianno, M. J., & Foster, P. (2002). Minor and trace elements in the shell of *Patella asprera* (Roding 1798). *Environ. Int.*, 28, 295-302.

Cravo, A., Bebianno, M. J., & Foster, P. (2004). Partitioning of trace metals between soft tissues and shells of *Pastella aspera*. *Environ. Int., 30*, 87-98.

Dallinger, R., Berger, B., Hunziker, P., & Kagi, J. H. (1997). Metallothionein in snail Cd and Cu metabolism. *Nature, 388*(6639), 237-238..

Dallinger, R., Chabicovsky, M., & Lagg, B. (2004a). Isoform-specific quantification of metallothionein in the terrestrial gastropod *Helix pomatia*. I. Molecular, biochemical and methodical background. *Environ. Toxicol. Chem., 23*, 890–901.

Dallinger, R., Chabicovsky, M., Lagg, B., & Schipelinger, R. (2004b). Isoform-specific quantification of metallothionein in the terrestrial gastropod *Helix pomatia*. II. A differential biomarker approach under laboratory and field conditions. *Environ. Toxicol. Chem., 23*, 902–910.

Goldberg, E. D., Bowen, V. T., Farrington J. W., Harvey, G., Martin, J. H., Parker, P. L., Risebrough, R. W., Robertson, W., Schneider, W., & Gamble, E. (1978). *The Mussel Watch. Environ. Conser., 5*, 101-125.

Hartley, D. M., & Johnston, J. B. (1983). Use of the fresh water clam *Corbicula manilensis* as a monitor for organochlorine pesticides. *Bull. Environ. Contam. Toxicol., 31*, 33-40.

Ismail, A., & Safahieh, A. (2004). Copper and Zinc in intertidal surface sediment and *Telescopium telescopium* from Lukut River, Malaysia. *Coast. Mar. Sci., 29*(2), 111-115.

Langston, W. J., & Spence, K. (1995). Biological factors involved in metal concentrations observed in aquatic organisms. In A. Tessier, & D. R. Turner (Eds.), *Metal speciation and bioavailability in aquatic systems* (pp. 407-78). New York: Wiley.

Laskowski, R., & Hopkin, S. P. (1996). Accumulation of Zn, Cu, Pb and Cd in the garden snail (*Helix aspersa*): Implications for predators. *Environ. Pollut., 91*(3), 89-297.

Lefcort, H. D., Abbott, P., Cleary, D. A., Howell, E., Keller, N. C., & Smith, M. M. (2004). Aquatic snails from mining sites have evolved to detect and avoid heavy metals. *Arch. Environ. Contam. Toxicol.*, *46*, 478–484.

Lefcort, H., & Bayne, C. J. (1991). Thermal preferences of resistant and susceptible strains of Biomphalaria glabrata (Gastropoda) exposed to Schistosoma mansoni (Trematoda). Parasitology, 103, 357–362.

Menta, C., & Parisi, V. (2001). Metal concentrations in *Helix pomatia*, *Helix aspersa* and *Arion rufus*: A comparative study. *Environ. Pollut., 115*, 205-208.

Rainbow, P. S. (1990). Heavy metals in marine invertebrates. In R. W. Furness, & P. S. Rainbow (Eds.), *Heavy metals in the marine environment* (pp. 67 – 79). Boca Raton, FL: CRC Press.

Rainbow, P. S. (1993). The significance of trace metal concentrations in marine invertebrates. In R. Dallinger, & P. S. Rainbow (Eds.), *Ecotoxicology of metals in invertebrates* (pp. 3-23). Boca Raton: Lewis Publication.

Roesijadi, G. (1992). Metallothioneins in metal regulation and toxicity in aquatic animals. *Aquat. Toxicol., 22*, 81–114.

Saha, M., Sarkar, S. K., & Bhattacharya, B. (2006). Interspecific variation in heavy metal body concentrations in biota of sunderban mangrove wetland, northeast India. *Environ. Int., 32*, 203-207.

Stijn Ghesquiere, A.I. (2005). *Applesnails*. Retrieved on February 2007 from www.applesnail.net.

van Straalen, N. M., Donker, M. H., Vijver, M. G., & van Gestel, C. A. M. (2005). Bioavailability of contaminants estimated from uptake rates in soil invertebrates. Environ. Pollut., 136, 409-417.

Viarengo, A. (1989). Heavy metals in marine invertebrates: mechanisms of regulation and toxicity at the cellular level. *CRC Critical Rev. Aquat. Sci., 1*, 295-317.

Webb, M. (1987). Toxicological significance of metallothionein. Experientia Supplement, 52, 109–134.

Yap, C. K., Ismail, A. Tan, S. G., & Omar, H. (2002). 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., & Abdul Rahim, I. (2003a). Can the shell of the green-lipped mussel *Perna viridis* 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. (2003b). Different soft tissues of the green-lipped mussel *Perna viridis* (L.) as biomonitoring agent of copper: Field and laboratory studies. *Malays. Appl. Biol., 32*(2), 9-18.

Yap, C. K., Ismail, A., Tan, S. G., & Rahim Ismail, A. (2004). The impact of anthropogenic activities on heavy metal (Cd, Cu, Pb and Zn) pollution: Comparison of the metal levels in green-lipped mussel *Perna viridis* (Linnaeus) and in the sediment from a high activity site at Kg. Pasir Puteh and a relatively low activity site at Pasir Panjang. *Pertanika J. Trop. Agric. Sci., 27*(1), 73-78.

Zar, J. H. (1996). *Biostatistical Analysis (3rd Ed.)*. New Jersey: Prentice Hall.