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# HEAVY-METAL CONCENTRATIONS IN THE MANGROVE SNAIL, *Nerita lineata* AND SURFACE SEDIMENTS COLLECTED FROM KLANG RIVER ESTUARY, SELANGOR, MALAYSIA.

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**Abstract:** The mangrove snail, *Nerita lineata*, and the surface sediments were collected from the Klang River Estuary, potentially receiving anthropogenic inputs such as domestic, sewage and industrial waste. The snails were divided into three parts, namely soft tissues, operculum and shells and analysed for heavy-metal (Cd, Cu, Fe, Ni, Pb and Zn) concentrations. The total concentrations (µg/g dry weight) of heavy-metals in the surface sediments collected from the four sampling sites ranged from 0.21-1.45, 5.29-53.9, 22121-24175, 14.2-19.5, 30.4-62.3 and 46.4-207 for Cd, Cu, Fe, Ni, Pb and Zn, respectively. The ranges of heavy-metal concentrations (µg/g dry weight) in the shells were 3.45-7.48, 5.54-7.27, 39.8-49.6, 23.8-28.6, 66.3-71.8 and 4.10-7.33 for Cd, Cu, Fe, Ni, Pb and Zn, respectively. The ranges of heavy-metal concentrations (µg/g dry weight) in the operculum were 3.28-5.81, 6.67-11.2, 45.4-68.8, 23.5-24.7, 62.5-66.2 and 14.5-24.9, for Cd, Cu, Fe, Ni, Pb and Zn, respectively. As for the soft tissues, the heavy-metal concentrations (µg/g dry weight) ranges were 1.54-4.47, 17.3-24.4, 627-716, 4.41-5.32, 10.8-43.6 and 87.5-109 for Cd, Cu, Fe, Ni, Pb and Zn, respectively. It was found that the concentrations of Cu, Fe and Zn were higher in the soft tissues while the concentrations of Cd, Ni and Pb were higher in the operculum and shells of the snails.

KEYWORDS: Heavy metals, *Nerita lineata*, Klang River Estuary, sediment

## Introduction

Selangor is a well-developed state where many anthropogenic activities, such as shipping, industry, agriculture and urbanisation are concentrated (Yap *et al.,* 2008a). One of the areas subjected to a variety of pollutants, such as heavy metals, is the Klang River which empties into the Straits of Malacca (Abdullah *et al.,* 1999). Over the years, increasing industrial and shipping activities have been reported along the Klang River Estuary and these have further aggravated the pollution problem in this area.

The snail, *Nerita lineata*, belongs to the order Archaeogastropoda and family Neritidae (Oliver, 1980). It is one of the common herbivorous snails in mangrove areas and grazes on algae at night during low tide. They are usually inactive during the day, and often stay above water during high tide. *Nerita* is an aquatic gastropod that has a distinctive and hard calcareous operculum (Oliver, 1980) with a body size that ranges from 2 to 3 cm. With sturdy and rounded shell, it can be distinguished by the grey shell with neat, black grooved lines. Recently, Yap *et al.* (2009a) found that *N. lineata* can be used as a potential biomonitor of heavy-metal pollution in the intertidal area of Peninsular Malaysia since they can be found in abundance near jetties, below rocks and on the sediments and roots of mangroves.

Several studies have rated molluscs as being the most suitable animal for use as biomonitors of contaminants in aquatic habitats (Kondo *et al.,* 1990; AbdAllah and Mustafa, 2002; Ng *et al.,* 2007). Since there has been no report on heavy metals in *N. lineata* in the Klang River Estuary, the objective of this study is to provide such information.

**Materials and Methods**

The sampling of snails and surface sediments was done at four sampling sites in the Klang River Estuary (Selangor, Peninsular Malaysia) on the 2nd December 2007. Surface sediments (0-10 cm) near snail habitats were collected from each sampling site. Sampling site descriptions are given in Table 1 while the three allometric parameters measured in the snails analysed are presented in Table 2.

Triplicates of physico-chemical parameters, including temperature, conductivity, salinity and total dissolved solid were measured *in situ* using a physico-chemical meter model YSI 556 MPS. The samples collected from the four sites (Figure 1) were stored in clean plastic bags and brought back to the laboratory in an ice compartment. In the laboratory, all the samples were kept at -10ºC until analysis. Before dissection, the samples were thawed at room temperature.

The surface-sediment samples were dried at 60ºC in an oven to constant dry weights, ground with pestle and mortar, sieved through a stainless-steel sieve with a mesh-size of 63 µm and shaken vigorously for homogenisation. The snails were dissected and separated into three different parts, namely soft tissue, hard tissue and operculum. The three different dissected parts of the snails were pooled and dried in an oven for 72 hours at 60 ºC to constant dry weights (Mo and Neilson, 1994). Triplicates of about 1 g of the sediment sample from each site were digested in a mixture of concentrated nitric acid (AnalaR grade, BDH 69%) and perchloric acid (AnalaR grade, BDH 60%) in the ratio of 4:1 while triplicates for each pooled part from each snail population were digested in concentrated nitric acid (AnalaR grade, BDH 69%). Both sediment and snail samples were digested in a hot-block digestor at low temperature (40º C) for one hour and then fully digested at high temperature (140º C) for at least three hours (Yap *et al.,* 2002a, b; Yap *et al.,* 2005). All the digested samples (sediments and snails) were diluted to a certain volume (40 ml) with double-distilled water and then filtered through Whatman No. 1 (medium) filter papers into pill boxes.

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 geochemical fractions were 1) Easily, freely, leacheable or exchangeable (EFLE), 2) 'Acid-reducible', 3) 'Oxidisable-organic' and 4) Resistant. 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.

Subsequently, the samples were analysed for Cd, Cu, Fe, Ni, Pb and Zn by using a flame Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. All data were presented in µg/g dry weight. Procedural blanks and quality-control samples made from the standard solutions for all metals were analysed once in every five samples in order to check for accuracy. The percentages of recoveries for the analysis were 103% for Cd, 103% for Cu, 72% for Fe, 80% for Ni, and 116% for Zn. The quality of the method used was checked with the Certified Reference Material (CRM) for dogfish liver-DOLT-3 (National Research Council Canada) and the recoveries of the CRM were 103 %, 103 %, 72 %, 141 % and 96 % for Cd, Cu, Fe, Ni and Zn, respectively. However, the CRM for Pb was not available.

**Results and Discussion**

The physico-chemical parameters (temperature, conductivity, salinity, total dissolved solid) are shown in Table 3 while the concentrations of four geochemical fractions of Cd, Cu, Fe, Ni, Pb and Zn in the surface sediments are given in Table 4. The values of water-quality parameters recorded were 27.2–29.1 °C, 9285–14653 µs/cm, 2.28–8.02 ppt, 2.73–9.06 mg/L for temperature, conductivity, water salinity and total dissolved solid, respectively. In addition, the physico-chemical data showed higher conductivity, water salinity and total dissolved solid in the upstream (site 1 and site 2) sites when compared to the downstream (site 3 and site 4). This phenomenon could be due to freshwater discharge at site 3 since site 3 was situated about 1 km away from a water tank, and therefore, possibly receiving freshwater influence. When sites 3 and 4 are compared, site 4 (located nearest to the estuary) had the higher salinity and conductivity values, indicating higher seawater influence, which was expected. Generally, the higher salinity and conductivity values recorded at sites 1 and 2 were attributable to seawater inflow to the upstream during high tide.

The ranges of the total concentrations (µg/g dry weight) of heavy metals in the surface sediment collected from the four sampling sites for Cd, Cu, Fe, Ni, Pb and Zn were 0.21–1.45, 5.29-53.90, 22121.00-24175.00, 14.20-19.50, 30.40-62.30 and 46.40-207.00, respectively. In order to estimate possible environmental consequences of the analysed metals at the sampling sites, the concentrations of Cd, Cu, Ni, Pb and Zn were compared to the Sediment Quality Guidelines of Effect Range Low (ERL) and Effect Range Median (ERM) proposed by Long et al. (1995). The Cd concentrations in three sampling sites were still below the values for ERL (1.20 µg/g) and ERM (9.60 µg/g). Only site 2 slightly exceeded the ERL but was still well below ERM Cd value. For Cu and Zn, sites 1, 2 and 3 exceeded the ERL values (34.0 µg/g for Cu; 150.0 µg/g for Zn) and all the four sites were still below the ERM value (270.0 µg/g for Cu; 410.0 µg/g for Zn). For Ni, all the four sites had Ni concentrations below the ERL values (20.9 µg/g). Concentrations of Pb in sediments at sites 2 and 3 exceeded the ERL value (46.7 µg/g). This comparison with the guidelines proposed by Long *et al*. (1995) indicates that follow-up monitoring should be continued at Klang River estuary. The heavy-metal concentrations in the sediments of the four sites along the Klang River Estuary showed low metal concentrations except for site 3 which displayed higher concentrations of Cu (53.9), Ni (19.5), Pb (62.3) and Zn (207.0). It is suspected that the high concentrations of heavy metals were due to the industrial activities found further up the river but only further monitoring study can answer this phenomenon.

From Table 4 again, it is clearly shown that the nonresistant fraction of Zn covered more than 50% in all the four sites, indicating major Zn contribution was possible from anthropogenic input. On the other hand, the nonresistant fractions for Cu and Fe in all the four sites were below 50%, indicating less anthropogenic input. For Cd and Pb, all the four sites, except for site 4, had nonresistant fractions below 50%. Interestingly, the total concentrations of Cd and Pb at site 4 were dominated by nonresistant fractions (72% for Cd and 87% for Pb) although their total concentrations for both metals were generally low or considered as unpolluted. For Ni, sites 2 and 4 had slightly higher than 50% contribution due to nonresistant fractions.

The heavy-metal concentrations in the soft tissues, operculums and shells of *N. lineata* collected from the four sites are given in Tables 5-10. Heavy-metal concentrations (µg/g dry weight) in the shells ranged from 3.45-7.48, 5.54-7.27, 39.8-49.6, 23.80-28.60, 66.30-71.80 and 4.10-7.33 for Cd, Cu, Fe, Ni, Pb and Zn, respectively. The heavy metal concentrations (µg/g dry weight) in the operculum ranged from 3.28-5.81, 6.67-11.2, 45.4-68.8, 23.6-24.7, 62.5-66.2 and 14.50-24.90, for Cd, Cu, Fe, Ni, Pb and Zn, respectively. As for the soft tissues, the heavy metal concentrations (µg/g dry weight) ranged from 1.54-4.47, 17.40-24.40, 626.00-716.00, 4.41-5.32, 10.80-43.60 and

87.50-109.00 for Cd, Cu, Fe, Ni, Pb and Zn, respectively. Results of the study show that the shells and operculums have higher concentrations of Cd, Ni and Pb compared to the same metals in the soft tissues of *N. lineata*. The shells of the snails recorded higher concentrations of Pb, Ni and Cd compared to the operculum but the contrary is true for Cu, Zn and Fe. The general patterns found in decreasing orders of Cu, Fe and Zn in *N. lineata* were: Soft tissue > Operculum > Hard tissue while for Cd, Ni and Pb, their orders were: Hard tissue > Operculum > Soft tissue. The above findings were in agreement with those reported by Yap *et al.* (2009a) for 15 populations of *N. lineata* collected from along the west coast of Peninsular Malaysia.

Generally, it has been reported in other molluscs species that essential metals such as Cu, Fe and Zn were higher in the soft tissues when compared to the shells and operculums of mollusks (Cravo *et al.,* 2002; Amin *et al.,* 2006a; Yap *et al.,* 2009b). This could possibly be due to the requirements for essential metal by the snail to carry out important metabolic pathways and cell or body functions (Rainbow, 1997; Astorga *et al.,* 2007; Yap *et al.,* 2009b). James (1991) reported that the maximum concentrations of Zn in molluscs were usually associated with the gland, kidney, gill and gonad rather than with the foot and muscle tissues. Therefore, higher concentrations of essential metals could be found in the soft tissues. As for the non-essential metals, the shells of *N. lineata* showed higher concentrations probably due to the incorporation of the metals during shell growth (Koide *et al.,* 1982; Yap and Cheng, 2008b). The hard tissues and the operculum shared the same accumulation of heavy metals (Cd, Ni and Pb) which could be due to some similarities in structures of the shell and the operculum as both consisted of calcified tissues (Amin *et al.,* 2006b). The shells also serve as a biodeposition site of unwanted chemical species such as elevated levels of heavy metals (Bertine and Goldberg, 1972; Yap *et al.,* 2003). For this reason, we can expect higher concentrations of Cd, Ni and Pb in the shells when compared to the soft tissues as well as the operculums.

Generally, the heavy-metal (Cd, Cu, Fe, Ni, Pb and Zn) concentrations of the geochemical fraction in sediments do not show a clear pattern in relation to the heavy-metal concentrations in the snails. However, the concentrations of Cu, Fe and Pb were higher in all the three parts of *N. lineata* collected from site 2 when compared to the other sites. This indicates that higher bioavailabilities of Cu, Fe and Pb to *N. lineata* were found in site 2 of the Klang River Estuary than in the other sampling sites. This could possibly be due to the dumping of domestic waste that was observed at this site (Table 1). However, the EFLE fraction which is also known to be the most bioavailable fraction (Yap *et al.,* 2002a) in sampling site 2 was found not to have the highest concentrations of Cu, Fe and Pb when compared to the other sites. Therefore, further studies should be carried out to investigate this finding.

Gastropods bioaccumulate metals in their tissues in proportion to the degree of environmental contamination (Bu-Olayan and Subrahmanyam, 1997) from sea water, suspended particles, and sediments and through the food chain (Louma, 1983; Blackmore, 2001). Therefore, heavy-metal levels in other gastropods had been widely reported from all over the world (Conti and Cecchetti, 2003; Cravo *et al.,* 2004; Hamed and Emara 2006). The heavy-metal levels found in this study are generally comparable to those reported from Singapore, Indonesia and Peninsular Malaysia (Table 11). The heavy-metal concentrations recorded at the Klang River Estuary are still within the ranges of those found in 15 other geographical populations of *N. lineata* from along the west coast of Peninsular Malaysia as reported by Yap *et al.* (2009a).

**Conclusion**

From the present study, the heavy-metal concentrations found in the soft tissues, operculums and shells of the *N. lineata* could serve as important data for future reference. As part of a biomonitoring study in Malaysia, studies using *N. lineata* in other estuaries of Malaysia should be implemented in the near future. The use of *N. lineata* is also part of our effort to provide baseline data for the sustainable environmental management of the estuarine resources of Malaysia.

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**References**

AbdAllah, A.T., M.A. Moustafa. (2002). Accumulation of lead and cadmium in the marine prosobranch *Nerita saxtilis*, chemical analysis, light and electron microscopy. *Environ. Int*. 116: 185–191.

Abdullah, A.R., N.M. Tahir, S.L. Tong, T.M. Hoque, A.H. Sulaiman. (1999). The GEF/UNDP/IMO Malacca Straits Demonstration Project: Sources of Pollution. *Mar. Pollut. Bull*. 39: 229-233.

Amin, B., A. Ismail, A. Arshad, C.K. Yap, M.S. Kamarudin. (2006a). Heavy metals concentrations in *Telescopium telescopium* from Dumai coastal waters, Indonesia. *Pertanika J. Trop. Agri. Sci*. 28(1): 33-39.

Amin, B., A. Ismail, Arshad, A., C.K. Yap, M.S. Kamarudin. (2006b). A comparative study of heavy metal concentrations *Nerita lineata* from the intertidal zone between Dumai Indonesia and Johor Malaysia. *J. Coast. Develop.*10: 19-32.

Astorga, E.M.S., R.E.M. Rodríguez, R.C. Díaz. (2007). Comparison of mineral and trace element concentrations in two molluscs from the Strait of Magellan (Chile). *J. Food Composit. Analys*. 20: 273-279.

Bertine, K.K., E.D. Goldberg. (1972). Trace elements in clams, mussel and shrimp. *Limnol. Oceanogr*. 17: 877-884.

Blackmore, G. (2001). Interspecific variation in heavy metal body concentrations in Hong Kong marine invertebrates. *Environ. Pollut*. 114: 303-311.

Bu-Olayan, A.H., M.N.V. Subrahmanyam. (1997). Accumulation of copper, nickel, lead and zinc by snail, *Lunellu coronatus* and pearl oyster, *Pinctada radiata* from the Kuwait coast before and after the gulf war oil spill. *Sci. Tot. Environ*. 197: 161-165.

Conti, M.E., G. Cecchetti. (2003). A biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. *Environ. Res.* 93: 99-112.

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

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

Cuong, D.T., S. Bayen, O. Wurl, K. Subramanian, K.K.S. Wong, N. Sivasothi, J.P. Obbard. (2005). Heavy metal contamination in mangrove habitats of Singapore. *Mar. Pollut. Bull*. 50: 1713-1744.

Hamed, M.A., A.M. Emara. (2006). Marine molluscs as biomonitors for heavy metal levels in the Gulf of Suez, Red Sea.. *J. Mar. Syst*. 60(3-4): 220-234.

James, W.M. (1991). Inorganic contaminants of surface water- Research and monitoring activities. New York, USA. Springer-Verlag.

Koide, M., D.S. Lee, E.D. Goldberg. (1982). Metal and transuranic records in mussel shells, byssal threads and tissues. *Estuar. Coast. Shelf Sci*. 15: 679-695.

Kondo, M., M. Imagawa, K. Maruyama, Y. Okada. (1990). Biochemical and immunochemical characterization of *Caenohabditis elegans* metallothionine induced by cadmium. *Biomed. Environ. Sci*. 3: 315-325.

Long, E.R., D.D. MacDonald, S.L. Smith, F.D. Calder. (1995). Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environ. Manage*. 19: 81-97.

Louma, S.N. (1983). Bioavailability of trace metals to aquatic organisms– a review. *Sci. Tot. Environ*. 28: 1-22.

Mo, C., B. Neilson. (1994). Standardization of oyster soft dry weight measurements. *Wat. Res*. 28: 243-246.

Ng, T.Y., P.S. Rainbow, C. Amiard-Triquet, J.C. Amiard, W.X. Wang. (2007). Metallothionein turnover, cytosolic distribution and the uptake of Cd by the green mussel *Perna viridis. Aquat. Toxicol*. 84: 153-161.

Oliver, A.P.H. (1980). *The Hamlyn Guide to the Shell of the World.* The Hamlyn Publishing Group Ltd. 42-43 pp.

Rainbow, P.S. (1997). Trace metal accumulation in marine invertebrates: marine biology or marine chemistry? *J. Mar. Biol. Assoc. UK*. 77: 195-210.

Yap, C.K., A. Ismail, H. Omar. (2002a). Correlation between speciation of Cd, Cu, Pb and Zn in sediment and their concentrations in total soft tissue of green-lipped mussel (Linnaeus) from the west coast of Peninsular Malaysia. *Environ. Int*. 28: 117-126.

Yap, C.K., A. Ismail, S.G. Tan, H. Omar. (2002b). Concentrations of Cu and Pb in the offshore and intertidal sediments of the west coast of Peninsular Malaysia. *Environ. Int*. 28: 467-479.

Yap, C.K., A. Ismail, S.G. Tan, R.I. Abdul. (2003). 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., A. Ismail, S.G. Tan. (2005). Cadmium, copper, lead and zinc levels in the green-lipped mussel *Perna viridis* (L.) from the west coast of Peninsular Malaysia: Safe as food? *Pertanika J. Trop. Agri. Sci*. 28(1): 41-47.

Yap, C.K., M.S. Fairuz, W.H. Cheng, S.G. Tan. (2008a). Distribution of Ni and Zn in the surface sediments collected from drainages and intertidal areas in Selangor. *Pertanika J. Trop. Agri. Sci*. 31(1): 79-90.

Yap, C.K., W.H. Cheng. (2008b). Heavy metal concentrations in *Nerita lineata*: the potential as a biomonitor for heavy metal bioavailability and contamination in the tropical intertidal area. *JMBA Biodiversity Records*: Ref no. 6292.

Yap, C.K., W.H. Cheng, A. Ismail, A.R. Ismail, S.G. Tan.( 2009a). Biomonitoring of heavy metal (Cd, Cu, Pb, and Zn) concentrations in the west intertidal area of Peninsular Malaysia by using *Nerita lineata*. *Toxicol. Environ. Chem*. 91(1): 29-41.

Yap, C.K., A. Noorhaidah, A. Azlan, A.A. Nor Azwady, A. Ismail, A.R. Ismail, S.S. Siraj, S.G. Tan. (2009b). *Telescopium telescopium* as potential biomonitors of Cu, Zn, and Pb for the tropical intertidal area. *Ecotoxicol. Environ. Safety* 72: 496-506.

Table 1: Descriptions of sampling sites for the snail, *Nerita lineata*, in the Klang River Estuary collected on 2nd December 2007.

| Sampling sites no. | Longitude | Latitude | Description of sampling site |
|---|---|---|---|
| 1. | N 03º 01.343’ | E 101º 22.511’ | Mangrove area, near bridge |
| 2. | N 03º 01.546’ | E 10º 22.680’ | Mangrove area, domestic waste observed. |
| 3. | N 03º 01.615’ | E 10º 21.150’ | Mangrove area, about 1 km away water tank. |
| 4. | N 02º 59.570’ | E 10º 23.098’ | Estuary area, near industry area, shipping activities |

Table 2: Allometric data for *Nerita lineata* analysed in the present study.

| Sampling site no. | N | Total wet weight (g) | Shell length (cm) | Shell width (cm) |
|---|---|---|---|---|
| 1. | 16 | 4.86 ± 0.45<br>(2.40-7.91) | 2.54 ± 0.15<br>(1.74-3.52) | 1.72 ± 0.07<br>(1.32-2.11) |
| 2. | 20 | 3.62 ± 0.26<br>(2.25-6.18) | 2.54 ± 0.07<br>(2.27-3.29) | 1.48 ± 0.02<br>(1.32-1.78) |
| 3. | 8 | 6.39 ± 0.48<br>(4.42-7.91) | 3.12 ± 0.13<br>(2.59-3.51) | 2.13 ± 0.10<br>(1.67-2.37) |
| 4. | 19 | 3.79 ± 0.26<br>(2.01-5.62) | 2.24 ± 0.05<br>(1.88-2.61) | 1.41 ± 0.06<br>(1.05-2.02) |

Note: N = number of individuals analysed.
Length are measured from anterior hard shell to posterior hard shell
Width are measured from the opening of operculum to the top hard shell

Table 3: The physico-chemical parameters (temperature, conductivity, salinity and total dissolved solid) of the water in the Klang River Estuary.

| No. | Parameters | Site 1 | Site 2 | Site 3 | Site 4 |
|---|---|---|---|---|---|
| 1. | Temperature (ºC) | 27.2 | 27.7 | 27.1 | 29.1 |
| 2. | Conductivity (µs/cm) | 14402 | 14652 | 4408 | 9285 |
| 3. | Water salinity (ppt) | 7.96 | 8.02 | 2.28 | 5.17 |
| 4. | Total dissolved solid (mg/L) | 8.98 | 9.06 | 2.73 | 5.60 |

Table 4: The mean concentrations (µg/g dry weight ± SE) of Cd, Cu, Fe, Ni, Pb and Zn in the geochemical fractions and total aqua regia of the surface sediments collected from Klang River Estuary.

| No. | Sampling site 1 | Cd | Cu | Fe | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|
| 1. | F1 | 0.27 ± 0.01 | 0.78 ± 0.01 | 199 ± 31.8 | 0.99 ± 0.08 | 0.89 ± 0.02 | 19.5 ± 0.03 |
| | F2 | 0.19 ± 0.00 | 0.01 ± 0.01 | 974 ± 5.46 | 1.20 ± 0.01 | 0.58 ± 0.04 | 46.4 ± 0.41 |
| | F3 | 0.11 ± 0.01 | 7.65 ± 0.39 | 2659 ± 3.55 | 6.76 ± 0.45 | 17.6 ± 0.72 | 54.1 ± 1.93 |
| | F4 | 0.92 ± 0.24 | 32.03 ± 0.88 | 24542 ± 708 | 10.1 ± 0.36 | 32.7 ± 0.62 | 72.7 ± 0.40 |
| | Sum of SET | 1.50 ± 0.27 | 40.49 ± 0.48 | 28374 ± 674 | 19.1 ± 0.72 | 51.8 ± 0.17 | 192.7 ± 1.10 |
| | Total Aqua-Regia | 0.73 ± 0.05 | 37.55 ± 0.25 | 24175 ± 869 | 15.7 ± 0.10 | 45.9 ± 0.90 | 159 ± 0.02 |
| | Nonresistant (%) | 38.3 | 20.9 | 13.5 | 46.9 | 36.9 | 62.3 |
| No. | Sampling site 2 | Cd | Cu | Fe | Ni | Pb | Zn |
| 2. | F1 | 0.28 ± 0.00 | 1.01 ± 0.01 | 260 ± 0.70 | 0.85 ± 0.01 | 1.04 ± 0.20 | 27.2 ± 0.13 |
| | F2 | 0.28 ± 0.05 | 0.03 ± 0.01 | 1783 ± 984 | 1.23 ± 0.06 | 0.79 ± 0.18 | 49.3 ± 0.32 |
| | F3 | 0.16 ± 0.01 | 10.07 ± 0.12 | 2408 ± 34.0 | 6.75 ± 0.17 | 19.2 ± 0.57 | 52.1 ± 1.23 |
| | F4 | 1.20 ± 0.24 | 31.43 ± 0.62 | 21222 ± 189 | 8.39 ± 0.12 | 21.3 ± 5.19 | 66.5 ± 1.84 |
| | Sum of SET | 1.92 ± 0.21 | 42.54 ± 0.75 | 25673 ± 760 | 17.2 ± 0.25 | 42.4 ± 4.64 | 195 ± 0.42 |
| | Total Aqua-Regia | 1.45 ± 0.04 | 37.92 ± 0.14 | 22122 ± 97.7 | 15.9 ± 0.94 | 48.6 ± 0.68 | 168 ± 0.13 |
| | Nonresistant (%) | 37.5 | 26.1 | 17.3 | 51.3 | 49.6 | 65.9 |
| No. | Sampling site 3 | Cd | Cu | Fe | Ni | Pb | Zn |
| 3. | F1 | 0.32 ± 0.01 | 1.68 ± 0.01 | 375 ± 6.65 | 0.92 ± 0.14 | 1.10 ± 0.07 | 41.1 ± 0.06 |
| | F2 | 0.18 ± 0.03 | 0.04 ± 0.03 | 922 ± 19.2 | 2.05 ± 0.05 | 1.19 ± 0.11 | 54.7 ± 0.11 |
| | F3 | 0.10 ± 0.02 | 15.41 ± 4.43 | 1378 ± 521 | 6.48 ± 1.69 | 20.6 ± 4.71 | 47.9 ± 9.08 |
| | F4 | 0.68 ± 0.04 | 35.90 ± 2.87 | 23549 ± 54.4 | 10.1 ± 0.26 | 36.5 ± 0.81 | 70.7 ± 1.89 |
| | Sum of SET | 1.28 ± 0.11 | 53.04 ± 7.27 | 26225 ± 588 | 19.6 ± 1.86 | 59.4 ± 5.48 | 214 ± 11.0 |
| | Total Aqua-Regia | 0.72 ± 0.14 | 53.99 ± 0.41 | 23095 ± 381 | 19.5 ± 0.88 | 62.3 ± 0.55 | 207 ± 0.66 |
| | Nonresistant (%) | 46.9 | 32.3 | 10.2 | 48.4 | 38.6 | 67.0 |
| No. | Sampling site 4 | Cd | Cu | Fe | Ni | Pb | Zn |
| 4. | F1 | 0.16 ± 0.04 | 0.24 ± 0.00 | 272 ± 70.7 | 0.32 ± 0.09 | 1.03 ± 0.13 | 6.86 ± 1.31 |
| | F2 | 0.15 ± 0.00 | 0.01 ± 0.00 | 2173 ± 37.2 | 0.91 ± 0.01 | 0.98 ± 0.32 | 10.9 ± 0.73 |
| | F3 | 0.10 ± 0.01 | 0.01 ± 0.00 | 9844 ± 749 | 6.41 ± 0.16 | 16.8 ± 1.62 | 18.8 ± 0.90 |
| | F4 | 0.16 ± 0.08 | 7.17 ± 0.90 | 12822 ± 999 | 6.19 ± 0.92 | 2.70 ± 1.06 | 22.8 ± 2.84 |
| | Sum of SET | 0.58 ± 0.13 | 7.43 ± 0.90 | 25112 ± 1711 | 13.8 ± 1.16 | 21.4 ± 2.25 | 59.4 ± 3.16 |
| | Total Aqua-Regia | 0.21 ± 0.01 | 5.29 ± 0.11 | 22767 ± 1123 | 14.2 ± 0.28 | 30.3 ± 0.50 | 46.4 ± 0.38 |
| | Nonresistant (%) | 71.9 | 3.50 | 48.9 | 55.2 | 87.4 | 61.6 |

Note: F1= Easily, freely, leacheable or exchangeable (EFLE), F2= acid-reducible, F3= oxidisable-organic, F4= resistant. SUM of SET= summation of F1, F2, F3 and F4 fractions using Sequential Extraction Technique (SET). Total Aqua-Regia= total metal concentrations obtained by using direct digestion of aqua-regia method. Nonresistant (%)= percentage of nonresistant fraction ($\frac{F1+F2+F3}{F1+F2+F3+F4}\times 100\%$).

Table 5: The mean concentrations (µg/g dry weight) of Cd in hard tissue (shell), soft tissue and operculum of *Nerita lineata*.

| Sampling site no. | Parts | Mean | SE | Min | Max |
|---|---|---|---|---|---|
| 1. | Hard tissue | 7.48 | 0.16 | 7.19 | 7.78 |
| | Soft tissue | 4.46 | 0.73 | 3.06 | 5.55 |
| | Operculum | 3.65 | 0.73 | 2.41 | 4.97 |
| 2. | Hard tissue | 3.45 | 0.03 | 3.39 | 3.50 |
| | Soft tissue | 2.16 | 0.08 | 2.03 | 2.31 |
| | Operculum | 3.28 | 0.05 | 3.23 | 3.34 |
| 3. | Hard tissue | 6.11 | 0.77 | 4.83 | 7.51 |
| | Soft tissue | 4.47 | 0.09 | 4.37 | 4.67 |
| | Operculum | 5.81 | 0.41 | 5.07 | 6.52 |
| 4. | Hard tissue | 4.80 | 0.43 | 4.18 | 5.63 |
| | Soft tissue | 1.54 | 0.42 | 0.84 | 2.32 |
| | Operculum | 4.11 | 0.30 | 3.51 | 4.46 |

Table 6: The mean concentrations (µg/g dry weight) of Cu in hard tissue (shell), soft tissue and operculum of *Nerita lineata*.

| Sampling site no. | Parts | Mean | SE | Min | Max |
|---|---|---|---|---|---|
| 1. | Hard tissue | 5.54 | 0.21 | 5.15 | 5.88 |
| | Soft tissue | 19.0 | 0.31 | 18.40 | 19.40 |
| | Operculum | 10.9 | 1.13 | 9.23 | 13.10 |
| 2. | Hard tissue | 7.27 | 0.04 | 7.19 | 7.32 |
| | Soft tissue | 24.40 | 0.56 | 23.30 | 25.20 |
| | Operculum | 11.20 | 0.62 | 10.50 | 11.80 |
| 3. | Hard tissue | 6.13 | 0.89 | 5.03 | 7.91 |
| | Soft tissue | 18.60 | 1.75 | 15.60 | 21.60 |
| | Operculum | 10.40 | 0.43 | 9.58 | 11.10 |
| 4. | Hard tissue | 5.56 | 0.03 | 5.49 | 5.60 |
| | Soft tissue | 17.36 | 1.17 | 16.20 | 19.60 |
| | Operculum | 6.67 | 0.04 | 6.62 | 6.77 |

Table 7: The mean concentrations (µg/g dry weight) of Fe in hard tissue (shell), soft tissue and operculum of *Nerita lineata*.

| Sampling site no. | Parts | Mean | SE | Min | Max |
|---|---|---|---|---|---|
| 1. | Hard tissue | 44.3 | 4.49 | 35.3 | 49.3 |
| | Soft tissue | 664 | 20.8 | 622 | 686 |
| | Operculum | 45.5 | 2.18 | 41.8 | 49.4 |
| 2. | Hard tissue | 49.6 | 6.50 | 36.9 | 58.4 |
| | Soft tissue | 716 | 16.4 | 684 | 738 |
| | Operculum | 68.8 | 1.38 | 67.5 | 70.2 |
| 3. | Hard tissue | 47.7 | 5.12 | 38.4 | 56.1 |
| | Soft tissue | 713 | 12.8 | 689 | 734 |
| | Operculum | 58.9 | 8.52 | 42.6 | 71.3 |
| 4. | Hard tissue | 39.8 | 3.21 | 35.1 | 45.9 |
| | Soft tissue | 627 | 13.2 | 612 | 653 |
| | Operculum | 53.2 | 1.33 | 51.4 | 55.8 |

Table 8: The mean concentrations (µg/g dry weight) of Ni in hard tissue (shell), soft tissue and operculum of *Nerita lineata*.

| Sampling site no. | Parts | Mean | SE | Min | Max |
|---|---|---|---|---|---|
| 1. | Hard tissue | 26.3 | 0.37 | 25.7 | 27.0 |
| | Soft tissue | 5.32 | 0.65 | 4.37 | 6.58 |
| | Operculum | 24.7 | 0.14 | 24.5 | 24.9 |
| 2. | Hard tissue | 23.8 | 0.44 | 23.0 | 24.6 |
| | Soft tissue | 5.15 | 0.75 | 3.65 | 6.06 |
| | Operculum | 23.6 | 0.06 | 23.5 | 23.6 |
| 3. | Hard tissue | 28.6 | 3.30 | 24.8 | 35.2 |
| | Soft tissue | 4.91 | 0.59 | 3.75 | 5.72 |
| | Operculum | 24.7 | 0.74 | 23.2 | 25.6 |
| 4. | Hard tissue | 24.8 | 0.92 | 22.9 | 25.9 |
| | Soft tissue | 4.41 | 0.58 | 3.40 | 5.43 |
| | Operculum | 24.0 | 0.26 | 23.6 | 24.5 |

Table 9: The mean concentrations (µg/g dry weight) of Pb in hard tissue (shell), soft tissue and operculum of *Nerita lineata*.

| Sampling site no. | Parts | Mean | SE | Min | Max |
|---|---|---|---|---|---|
| 1. | Hard tissue | 68.2 | 0.51 | 67.2 | 68.9 |
| | Soft tissue | 19.8 | 1.19 | 17.4 | 21.1 |
| | Operculum | 62.6 | 0.71 | 61.3 | 63.8 |
| 2. | Hard tissue | 71.8 | 0.71 | 70.6 | 73.0 |
| | Soft tissue | 43.6 | 4.20 | 37.9 | 51.8 |
| | Operculum | 66.2 | 0.37 | 65.8 | 66.6 |
| 3. | Hard tissue | 71.3 | 3.81 | 64.9 | 78.1 |
| | Soft tissue | 12.4 | 0.52 | 11.6 | 13.4 |
| | Operculum | 64.1 | 1.28 | 62.5 | 66.7 |
| 4. | Hard tissue | 66.3 | 2.49 | 63.2 | 71.3 |
| | Soft tissue | 10.8 | 1.16 | 8.51 | 12.1 |
| | Operculum | 62.5 | 1.22 | 60.1 | 64.2 |

Table 10: The mean concentrations (µg/g dry weight) of Zn in hard tissue (shell), soft tissue and operculum of *Nerita lineata*.

| Sampling site no. | Parts | Mean | SE | Min | Max |
|---|---|---|---|---|---|
| 1. | Hard tissue | 4.58 | 0.76 | 3.31 | 5.94 |
| | Soft tissue | 98.4 | 0.94 | 97.1 | 100 |
| | Operculum | 24.9 | 3.22 | 18.6 | 29.3 |
| 2. | Hard tissue | 4.10 | 0.44 | 3.22 | 4.57 |
| | Soft tissue | 97.5 | 0.68 | 96.8 | 98.9 |
| | Operculum | 14.5 | 0.15 | 14.3 | 14.7 |
| 3. | Hard tissue | 6.06 | 0.86 | 5.03 | 7.79 |
| | Soft tissue | 109 | 1.76 | 106 | 112 |
| | Operculum | 16.7 | 2.46 | 13.7 | 21.6 |
| 4. | Hard tissue | 7.33 | 0.41 | 6.77 | 8.13 |
| | Soft tissue | 87.5 | 2.57 | 84.3 | 92.6 |
| | Operculum | 15.4 | 1.19 | 13.5 | 17.6 |

Table 11: Comparisons of heavy metal concentrations (µg/g) in *Nerita linaeta* from different areas reported in the literature.

| No. | Location | Part analysed | WB | Cd | Cu | Fe | Ni | Pb | Zn | References |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Sungai Buloh, Singapore | Soft tissues | ww | 0.03 | 7.50 | NA | 2.70 | 0.49 | 31.0 | Cuong et al. (2005) |
| 2. | Sungai Khatib Bongsu | Soft tissues | ww | 0.02 | 8.80 | NA | 10.0 | 1.10 | 680 | Cuong et al. (2005) |
| 3. | Dumai, Indonesia | Soft tissues | dw | 0.71 | 15.6 | 398 | 5.08 | 9.34 | 94.7 | Amin et al. (2006b) |
| | | Operculums | dw | 4.16 | 7.31 | 30.6 | 23.5 | 51.8 | 17.6 | |
| | | Shells | dw | 4.14 | 5.90 | 24.9 | 20.7 | 44.4 | 3.74 | |
| 4. | Johore, Malaysia | Soft tissues | dw | 1.24 | 18.0 | 474 | 5.57 | 19.8 | 95.1 | Amin et al. (2006b) |
| | | Operculums | dw | 4.73 | 6.51 | 34.9 | 20.7 | 60.6 | 19.5 | |
| | | Shells | dw | 4.18 | 5.06 | 31.6 | 19.3 | 59.8 | 4.81 | |
| 5. | Peninsular Malaysia (15 sites) | Soft tissues | dw | BDL-2.80 | 11.5-25.2 | NA | NA | 1.40-1251 | 60.6-113 | Yap et al. (2009a) |
| | | Operculums | dw | 1.60-6.20 | 1.00-10.1 | NA | NA | 20.6–80.8 | 9.30-27.9 | |
| | | Shells | dw | 1.60-6.40 | 1.30-11.1 | NA | NA | 22.3-84.4 | 6.10-9.90 | |
| 6. | Klang River, Malaysia (4 sites) | Soft tissues | dw | 1.54-4.47 | 17.4-24.4 | 627-716 | 4.41-5.32 | 10.8-43.6 | 87.5-109 | This study |
| | | Operculums | dw | 3.28-5.81 | 6.67-11.2 | 45.5-68.8 | 23.6-24.7 | 62.5-66.2 | 14.5-24.9 | |
| | | Shells | dw | 3.45-7.48 | 5.54-7.27 | 39.8-49.6 | 23.8-28.7 | 66.3-71.8 | 4.10-7.33 | |

Note: dw= dry weight basis; ww= wet weight basis.

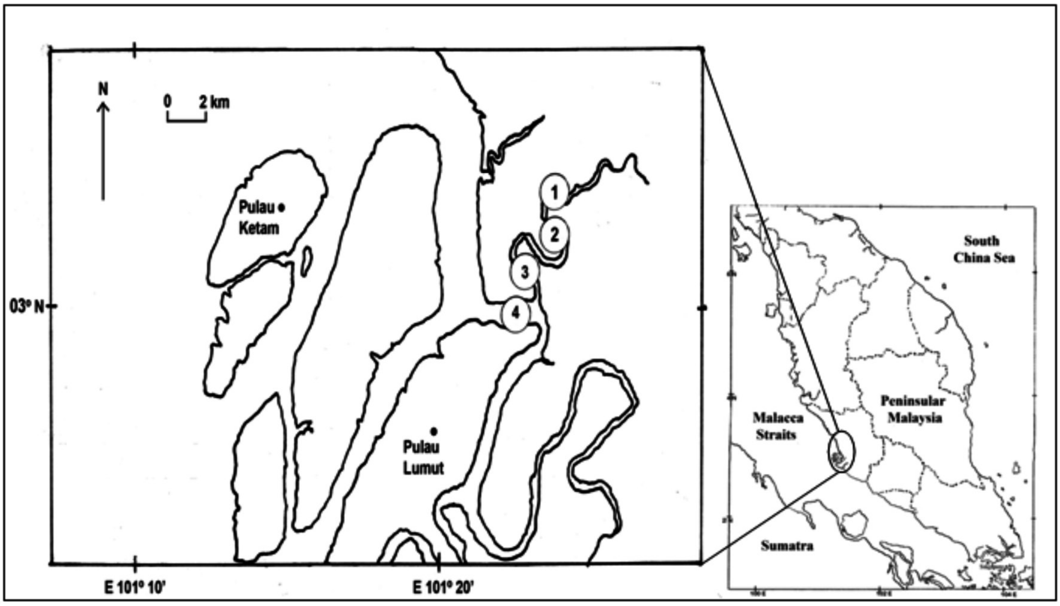


Figure 1: Map showing the four sampling sites of *Nerita lineata* in the Klang River Estuary, Selangor, Peninsular Malaysia.