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Distributions of Heavy Metal Concentrations in Different Tissues of the Mangrove Snail *Nerita lineata*

(Taburan Kepekatan Logam Berat dalam Tisu yang Berlainan bagi Siput Bakau *Nerita lineata*)

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ABSTRACT

This study focused on the distributions of Cd, Cu, Fe, Ni, Pb and Zn in the various tissues (shell, operculum, muscle, digestive cecum, foot, cephalic tentacles, mantle, radular and remainder) of the mangrove snail Nerita lineata *collected from Sungai Janggut, Selangor. Copper and Zn levels in all soft tissues were found to exceed those in the sediment, indicating bioaccumulation of these metals. Fe levels in all soft tissues of the snails were found to be lower than those in the sediment even though Fe is the most abundant metal among the six metals investigated. Cd and Pb levels in soft tissues were also found to be lower than those in the sediment but opposite trends were observed for Cd and Pb levels in the shells. Ni, Cd and Pb levels in the shells were significantly (*$p< 0.05$*) higher than those in the soft tissues and sediment. However, digestive cecum and remainder showed higher Ni level as compared with sediment. The biota sediments accumulation factor (BSAF) has identified that the shell and operculum were macroconcentrators for Cd, Ni and Pb while all the soft tissues of* N. lineata *were macroconcentrators for Cu (except for muscle) and Zn. The findings indicated that the differences in metal distribution could be attributed to the differences in tissue physiology and metal handling, storage and detoxification strategies.*

Keywords: Different tissues; metal distribution; Nerita lineata

ABSTRAK

Kajian ini memfokus kepada taburan Cd, Cu, Fe, Ni, Pb dan Zn di dalam tisu berlainan (cangkerang, operkulum, otot, sekum pencernaan, kaki, tentakel, mantel, radula dan tinggalan) bagi siput bakau Nerita lineata *yang disampel dari Sungai Janggut, Selangor. Aras Cu dan Zn dalam semua tisu lembut didapati melebihi aras di sedimen, menunjukkan berlakunya biopenimbunan logam tersebut. Aras Fe dalam semua tisu lembut didapati lebih rendah daripada aras di sedimen walaupun Fe adalah logam paling banyak antara enam logam yang dikaji. Aras Cd dan Pb dalam tisu lembut pula didapati rendah daripada aras pada sedimen, namun trend yang berlawanan diperhatikan bagi Cd dan Pb dalam cengkerang. Tahap Ni, Cd dan Pb di dalam cangkerang adalah lebih tinggi secara signifikan (*$p< 0.05$*) daripada tahap di tisu lembut yang berlainan dan sedimen. Namun, sekum pencernaan dan tinggalan menunjukkan kepekatan Ni yang lebih tinggi berbanding sedimen. Faktor akumulasi biota sedimen (BSAF) telah menunjukkan bahawa cengkerang dan operkulum ialah makrokonsentrator bagi Cd, Ni dan Pb manakala tisu lembut ialah makrokonsentrators bagi Cu (kecuali otot) dan Zn. Penemuan di atas menunjukkan bahawa perbezaan taburan logam mungkin disebabkan oleh perbezaan fisiologi tisu, strategi pengendalian logam serta penyimpanan dan mekanisme detoksifikasi yang berlainan antara tisu-tisu tersebut.*

Kata kunci: Nerita lineata*; pelbagai tisu; taburan logam*

INTRODUCTION

Gastropods are among the most promising candidates used in biomonitoring studies focusing on heavy metals apart from the well established green-lipped mussel, *Perna viridis*, in the mussel watch approach (Yap et al. 2003a, 2006). Among the intertidal molluscs, *Nerita lineata* (Figure 1) has been evidenced to be one of the most potential biomonitoring species in Sumatera (Indonesia) (Amin et al. 2006, 2008, 2009) and west coast of Peninsular Malaysia (Yap & Cheng 2008; Yap et al. 2009a, 2010). This is due to the fact that *N. lineata* can be widely found in the tropical mangrove areas and can readily accumulate heavy metals (Yap et al. 2009a). Gastropods are one of the most important taxonomic groups which are potential biomonitors of heavy metal pollution and there are several important features or characteristics of the gastropods which supports *N. lineata* used as a biomonitor of heavy metal pollution (AbdAllah & Moustafa 2002; Bu-Olayan & Subrahmanyam 1997; Yap & Cheng 2008). However, all the above studies focused on total soft tissues, operculum and total shells in the mangrove snail *N. lineata* and detailed study on the heavy metal concentrations in the different soft tissues of the snails has not been reported yet in the literature. Therefore, the objective of this paper was to provide such information in the *N. lineata* as baseline data as well as to verify the potential of the snail as a good biomonitor.

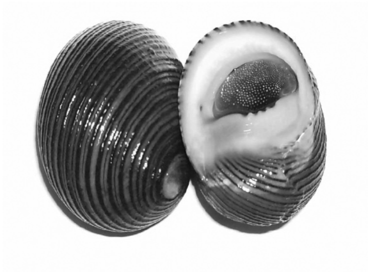


FIGURE 1. *Nerita lineata* collected from Sungai Janggut, Selangor

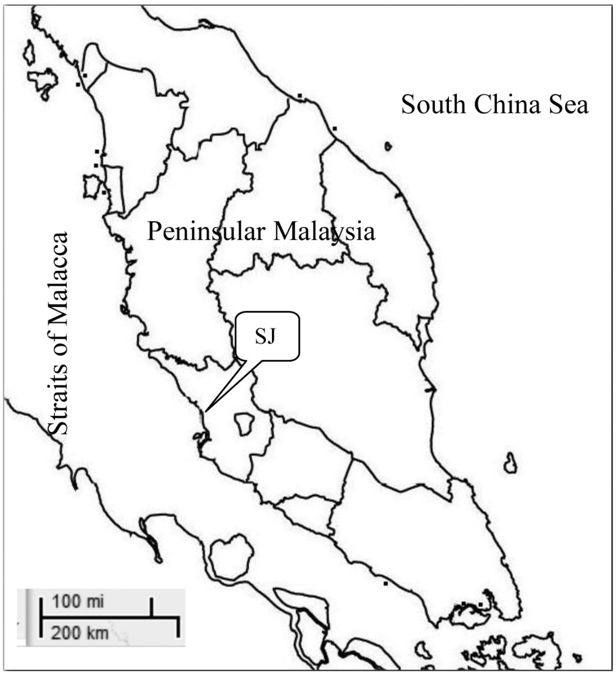


FIGURE 2. Sampling map for *Nerita lineata* at Sungai Janggut (SJ) in Selangor, Peninsular Malaysia

MATERIALS AND METHODS

The snails, *N. lineata* and surface sediments were collected, on 20 March 2006, from the mangrove area at Sungai Janggut, Kuala Selangor (N04º 8´10˝; E101º 22´31˝), west coast of Peninsular Malaysia (Figure 2). The sampling site was characterized by a typical fishing village with no direct observable human activities in the vicinity. A total of 83 individuals were dissected and pooled into shell, operculum, remainder, muscle, digestive cecum, foot, cephalic tentacles, mantle and radular (Figure 3). All the pooled samples were then dried at 60ºC to constant dry weights (Yap et al. 2006). The snail samples were obtained in three replicates (shell, operculum, remainder, muscle, digestive cecum and foot), two replicates (cephalic tentacles) and one replicate (mantle and radular) of the dried pooled tissue parts. The lengths, widths and heights of the shells were measured by using a vernier caliper to an accuracy of 0.1 mm. The lengths, widths and heights of the shells were 14.00-32.22, 11.30-22.0 and 7.90-17.80 mm, respectively. All dried category of gastropod tissues were digested in concentrated HNO_3 (Analar grade, BDH 69%).

For the sediment samples, about 1 g of each oven-dried sample was weighed and placed in acid-washed test digestion tubes. A combination of concentrated HNO_3 (AnalaR grade, BDH 69%) and concentrated $HClO_4$

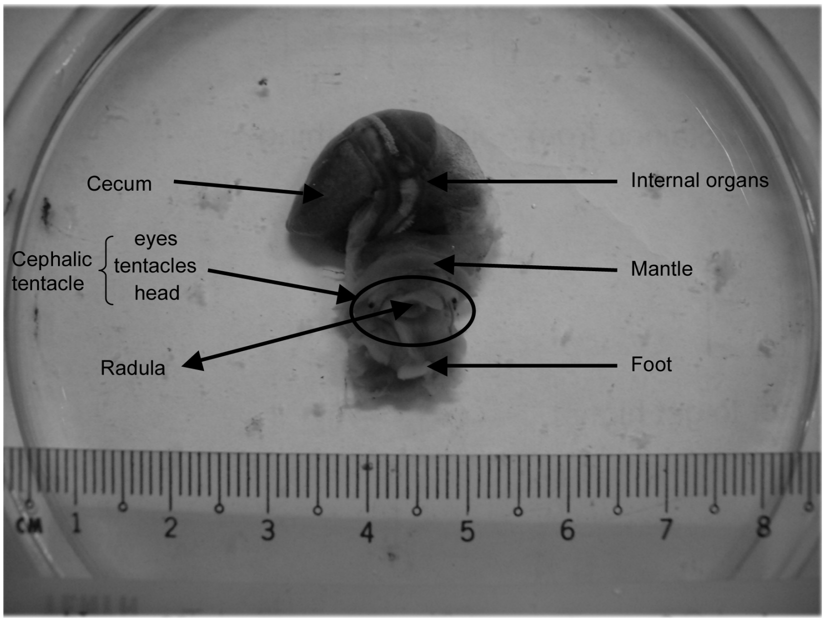


FIGURE 3. Different parts of the soft tissues of the *Nerita lineata*

(AnalaR grade, BDH 60%) in the ratio of 4:1 was added for the digestion of the sediment samples.

All the test tubes with samples were placed in digesting blocks at 40ºC for 1 h and were then fully digested at 140ºC for 3 h. It was then diluted to 40 mL with double de-ionized water. Later it was filtered through Whatman No. 1 (filter speed: medium) filter paper into acid-washed pill box. The concentrations of Cd, Cu, Fe, Ni, Pb and Zn were determined by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin Elmer model analyst 800. The analytical procedures for the gastropods were checked with the certified reference material (CRM) for dogfish liver (DOLT-3, National Research Council Canada) and the recoveries of all the metals were being satisfactory (70-110%). The analytical procedures for the sediment was also checked with a certified reference material for soil (International Atomic Energy Agency, Soil-5, Vienna, Austria) and the recoveries of all the metals were being satisfactory (88-144%). The detection limits of the AAS for Cd, Cu, Fe, Ni, Pb and Zn were 0.009, 0.010, 0.010, 0.010, 0.009 and 0.007 mgL^{-1}, respectively.

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

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

where Cx and Cs are the mean metal concentrations in the different tissues of snails and in associated sediment, respectively.

Statistical analyses were done by using statistical software, SPSS software version 15.0 for Windows. The Student-Newman-Keuls (SNK) test was applied to the data from the dissected parts of the tissues (9 parts) of the snails to compare the mean values of heavy metal concentrations between the different parts of the snails used. All comparisons were made at least at the 95% ($p< 0.05$) level of significance.

Results and Discussion

The concentrations (µg/g dry weight) of heavy metals (Cd, Cu, Fe, Ni, Pb and Zn) in the different parts of the soft tissues of *N. lineata* collected from Sg. Janggut are shown in Figure 4. The concentrations (µg/g dry weight) of Cd, Cu and Fe in the different tissues of the snails ranged at 1.10-6.83, 4.56-19.12 and 27.29-1353.54, respectively. As for Ni, Pb and Zn, the ranges (µg/g dry weight) were 3.37-28.67, 5.69-67.29 and 10.26-133.76, respectively.

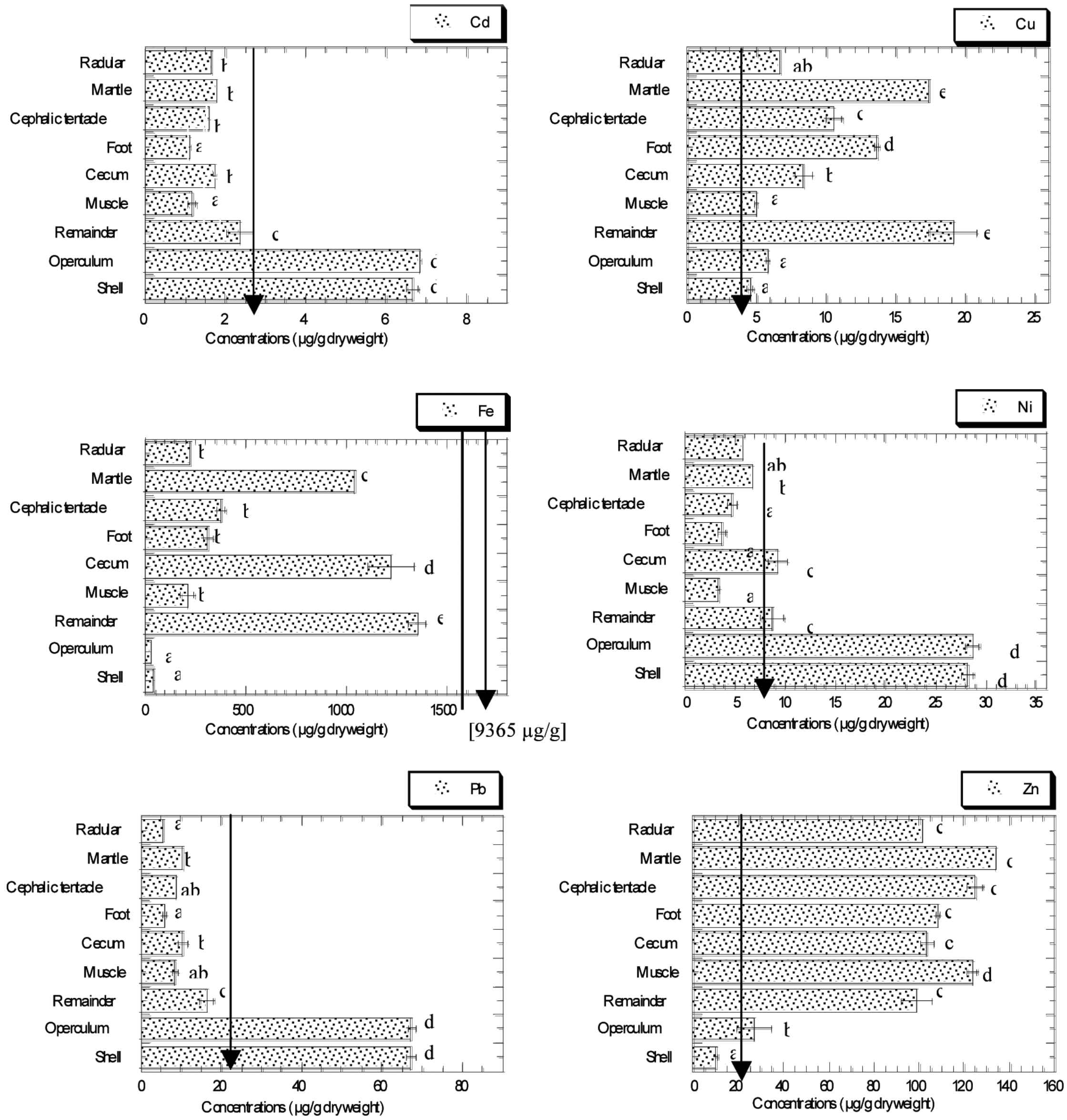
The foot and muscle were recorded to have low concentrations (µg/g dry weight) (Figure 4) of Cd (foot = 1.10, muscle = 1.18), Ni (foot = 3.37, muscle = 3.75) and Pb (foot = 1.10, muscle = 1.18). The SNK comparison also showed that there were no significant differences ($p> 0.05$) between the foot and muscle for the levels of Cd, Ni and Pb. One possible explanation for the low concentrations of metals in foot and muscle could be due to the low affinities of metals to binding sites of metallothioneins in foot and muscles of the snail (Roesijadi 1992).

The remainder showed the highest concentrations (µg/g dry weight) of Cu (19.12) and Fe (1353.54) (Figure 4). Biochemically, Cu resembles or even overlaps with the role of Fe in the metabolism of molecular oxygen in molluscs because the oxygen carrying pigment of molluscs' blood is not hemoglobin but Cu-containing cuproprotein, hemocyanin (Cheung & Wong 1992). Therefore the Cu level were high in the remainders as it is consisted of the left over of the soft tissues which are rich in blood such as gills, internal organs and digestive system.

The mantle accumulated the highest level of Zn and among the highest for Cu. The mantle forms the shell of gastropods by secretion of mineral ions and organic components of the shell matrix (Marin & Luquet 2005) including heavy metals. This explains why the mantle has high concentration of Zn and Cu. The shells on the other hand, act as a biodeposition site of unwanted chemical species (Bertine & Goldberg 1972; Yap et al. 2003b). This is supported by the relatively high Zn and Cu accumulations of shells of *N. lineata* collected from Sg. Janggut population. According to Foster and Cravo (2003), the chemical can enter the shell from surrounding environment and they have reported higher concentration of Zn in the shells of *Nerita* species when compared with other gastropod species.

In addition to that, the heavy metal concentrations in the shells and operculums (Figure 4) show similar pattern of accumulation when compared with soft tissues of *N. lineata*. The levels of Cd, Ni and Pb in the operculums and shells of *N. lineata* were significantly higher ($p< 0.05$) than the rest of the soft tissues. As for Cu, Fe and Zn, the levels of these metals in the operculums and shells were significantly lower ($p< 0.05$) than the rest of the soft tissues. This is in agreement with the higher Cd and Pb levels reported in the *P. viridis* shell by Yap et al. (2003b). It is generally recognized that the molluscs soft tissues accumulate higher concentrations of Cu, Zn and Fe than those in the shells (de Wolf et al. 2001; Fishelson et al. 1999; Giusti et al. 1999; Puente et al. 1996; Szefer & Szefer 1985, 1990; Szefer et al. 2002; Yap et al. 2009b).

In comparison to the metal concentrations in the surface sediments at Sungai Janggut (Figure 4 and Table 1), the Cd and Pb concentrations in the different soft tissues of *N. lineata* are lower than those in the surface sediment. This indicates that these nonessential metals did not exceed the background levels in the habitat. For Cu and Zn, their concentrations in all tissues (including operculum and shell) are higher than those in the surface sediment. The concentration of Fe in all the tissues (including operculum and shell) are significantly lower than that in the surface sediment. It should be noted that Fe is the most abundant metal in the tissues of *N. lineata* when compared with Cd, Cu, Ni, Pb and Zn and this corresponds to the similar abundance pattern



Note : Objects indicated with different alphabet : $p<0.05$, Objects indicated with same alphabet : $p>0.05$

FIGURE 4. Concentrations (µg/g dry weight) of heavy metals (Cu, Zn, Ni, Cd, Fe and Pb) and comparison (SNK) of means (µg/g dry weight) in the different tissues in the *Nerita lineata* collected from Sg. Janggut, Selangor. Vertical lines are the metal concentrations of the surface sediments collected from the snail habitat at Sungai Janggut (Table 1). Double vertical line for Fe indicates Fe concentration in the sediment is significantly higher than those in the snail tissues and not within the X-scale

for surface sediment Fe>Pb>Zn>Ni>Cu>Cd (Table 1). However, a better representation of *N. lineata* tissue's accumulation capacity can be illustrated based on their BSAF (Table 2), with increasing values. The snail tissues can be classified into macroconcentrator (BSAF > 2), microconcentrators (1<BSAF<2) or deconcentrators (BSAF< 1), as proposed by Dallinger (1993). In this study, the shell and operculum can be classified as macroconcentrators for Cd, Ni and Pb while all the soft tissues of *N. lineata* were macroconcentrators for Cu (except for muscle) and Zn. On the other hand, all the soft tissues of the snails were deconcentrators for Cd (except for remainder as microconcentrator), Fe, Ni (except for remainder and cecum as microconcentrators) and Pb. The results indicated that the selected soft tissues were macroconcentrators of Cu and Zn and thus provide a further claim that the selective tissues of the *N. lineata* as good biomonitors.

The presence of high levels of Fe, Cu and Zn than Pb, Ni and Cd in the soft tissues of *N. lineata* could be due to their roles as components of metabolically important biomolecules including enzymes, metalloenzymes and respiratory pigments (Catsiki et al. 1994; Depledge et al. 1994; Langston et al. 1998; Rainbow 1997). In particular, the soft tissues of the snail could be the target organs for Cu and Zn since their concentrations exceeded

TABLE 1. Concentrations (μg/g dry weight) of heavy metals in the surface sediment collected from Sg. Janggut, Selangor. Values in brackets are minimum to maximum concentrations

Metals	Mean
Cd	2.13 ± 0.1 (1.95-2.31)
Cu	3.25 ± 0.18 (2.94-3.55)
Fe	9365 ± 130 (9140-9590)
Ni	7.96 ± 0.27 (7.49-8.44)
Pb	23.5 ± 0.45 (22.7-24.3)
Zn	21.8 ± 0.41 (21.1-22.6)

TABLE 2. Biota sediment accumulation factors in the different tissues of *Nerita lineata*

Cd	Foot 0.52	Muscle 0.55	CT 0.74	Radular 0.78	Cecum 0.81	Mantle 0.84	Remainder 1.11	Shell 3.13	Operculum 3.21
Cu	Shell 1.40	Muscle 1.53	Operculum 1.78	Radular 2.06	Cecum 2.57	CT 3.31	Foot 4.20	Mantle 5.35	Remainder 5.88
Fe	Operculum 0.00	Shell 0.00	Muscle 0.02	Radular 0.02	Foot 0.03	CT 0.04	Mantle 0.11	Cecum 0.13	Remainder 0.14
Ni	Muscle 0.42	Foot 0.47	CT 0.58	Radular 0.73	Mantle 0.86	Remainder 1.10	Cecum 1.17	Shell 3.54	Operculum 3.60
Pb	Radular 0.24	Foot 0.25	Muscle 0.36	CT 0.37	Mantle 0.44	Cecum 0.44	Remainder 0.70	Shell 2.86	Operculum 2.86
Zn	Shell 0.47	Operculum 1.24	Remainder 4.53	Radular 4.65	Cecum 4.74	Foot 4.96	Muscle 5.67	CT 5.77	Mantle 6.14

environmental concentration which indicated the occurrence of bioaccumulation process (Gundacker 2000).

Marine gastropods normally accumulate and store Cu and use it in the synthesis of hemocyanin, a blood pigment. The similar Cu concentrations in the different soft tissues of *N. lineata* may in part be attributed to Cu in hemocyanin (Dallinger & Wieser 1984). According to Pyatt et al. (2003), the foot of *Lymnaea pregra* had the highest concentrations of Cu, hence exhibiting well-defined enhanced Cu bioaccumulation as a response to increasing concentrations of Cu. The same explanation could be applied to the muscular foot of *N. lineata,* the higher Cu level in the foot was found more than in the muscle as a response to higher Cu bioavailability in the Sg. Janggut sampling site.

Although the total concentration of metals in the soft tissues of molluscs can be a measure of metal bioavailability originating from both natural and anthropogenic sources (Rainbow 1995), their distributions in the different soft tissues of *N. lineata* is more informative in determining the target organ that is specific, selective and sensitive to metal accumulation (Szefer & Szefer 1990; Yap et al. 2009b).

CONCLUSION

The present findings indicated that the differences in metal distribution could be attributed to the differences in tissue physiology and metal handling, storage and detoxification strategies. In particular, the concentrations of Cu and Zn in the soft tissues and Cd, Ni and Pb in the operculum and shells were to be significantly higher than those in the sediments. These tissues are classified as macroconcentrators and could be used as potential biomonitors metal accumulation. Therefore, these findings are informative for further ecotoxicological studies aimed at establishing *N. lineata* a good biomonitor and bioindicator species for heavy metal contamination.

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