Sains Malaysiana 44(7)(2015): 965–971

Beryllium Levels in the Mangrove Snail, *Nerita lineata* and Surface Sediments from Peninsular Malaysian Mangrove Area

(Tahap Berilium dalam Siput Bakau, *Nerita lineata* dan Sedimen Permukaan daripada Kawasan Bakau Semenanjung Malaysia)

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ABSTRACT

The beryllium (Be) concentrations were determined in the shells, opercula and soft tissues of the Nerita lineata *and in the surface sediments collected from nine geographical sites of Peninsular Malaysian mangrove area in April 2011. The ranges of Be concentrations (µg/kg dry weight) were: <1.00- 4.00 for shells, <1.00-2.00 for opercula and 3.00-41.00 for soft tissues of* N. lineata. *For sediments, Be ranges were found between 1200 and 3810 µg/kg dry weight. The distribution of Be was found to be: sediment> soft tissues > shells ≥ opercula. The insignificant* (p>*0.05) relationships of Be between snails and sediments and low bioaccumulation factor (ratio of soft tissues to sediment) (<1.00), could be attributed to the fact that Be contamination being not serious in the Peninsular Malaysian mangrove area. The snail* N. lineata *as a good biomonitor of Be needs further investigations. Lastly, Peninsular Malaysia mangrove was not contaminated by Be based on the present findings.*

Keywords: Be; biomonitoring; mangrove snails; sediment

ABSTRAK

Kepekatan berilium (Be) telah ditentukan di dalam cangkerang, operkulum dan tisu lembut bagi Nerita lineata *dan di dalam sedimen permukaan disampel dari sembilan kawasan geografi di kawasan paya bakau di Semenanjung Malaysia pada April 2011. Julat kepekatan Be (µg/kg berat kering) adalah: <1.00-4.00 bagi cangkerang, <1.00-2.00 bagi operkulum dan 3.00-41.0 bagi tisu lembut dalam* N. lineata. *Bagi sedimen, julat Be adalah didapati antara 1200 dan 3810 µg/kg berat kering. Taburan bagi Be didapati sebagai: sedimen> tisu lembut> cangkerang ≥ operkulum. Korelasi yang tidak bererti (*p>*0.05) antara siput dan sedimen dan faktor biopenimbunan yang rendah (nisbah bagi tisu lembut kepada sedimen) (<1.00), mungkin disebabkan oleh pencemaran Be tidak serius di dalam kawasan paya bakau Semenanjung Malaysia. Siput* N. lineata *sebagai penunjuk pemantauan bagi Be memerlukan kajian lanjutan. Akhir sekali, paya bakau Semenanjung Malaysia tidak dicemari Be berdasarkan hasil kajian ini.*

Kata kunci: Be; pemantauan; sedimen; siput bakau

INTRODUCTION

Beryllium (Be) can be found in the Earth's crust at an average concentration of approximately 2.8-5.0 mg/kg (ATSDR 1993). It occurs in rocks and minerals at concentrations ranging from 0.038 to 11.4 mg/kg (Drury et al. 1978). Beryllium and Be compounds are not metabolized (WHO 2001) and therefore, it is considered a non-essential metal to living organisms.

The metal Be is used primarily in the aerospace, weapons and nuclear industries. Be alloy, mostly beryllium-copper, is used in the aerospace, electronics and mechanical industries due to its unique properties, such as high specific heat and excellent dimensional stability (WHO 2001). Natural sources of Be release to the atmosphere, such as windblown dust and volcanic particles, are estimated to account for 5.2 tonnes per year, or 2.6% of total emissions (WHO 2001). Be is carried to rivers, lakes and oceans by the process of land erosion and acid deposition has been shown to accelerate chemical weathering of soil and bedrock into drainage outflow, increasing the mobility of Be.

Anthropogenic sources of Be include landfill disposal of coal ash (Griffitts et al. 1977) and municipal waste combustor ash, land burial of industrial wastes (ATSDR 1993) and land application of Be-enriched sewage sludge. Deposition of atmospheric Be is also a source of Be in soil (WHO 2001). In particular, Be particles produced from anthropogenic processes such as ore crushing and coal combustion/ fossil fuels are generally emitted as Be oxide (ATSDR 1993). In fact, over 99% of beryllium emitted into the atmosphere is the result of oil or coal combustion for electric power generation (IPCS 1990), thus becoming the primary source in the atmosphere. Be is released to water in some industrial wastewater effluents, most notably treated wastewaters from iron and steel manufacturing and non-ferrous manufacturing industries (ATSDR 1993). Coal combustion is regarded as the main anthropogenic source

for Be in the environment (Clarke & Sloss 1992). Long-term exposure of various animal species has shown that Be exhibits toxic and carcinogenic effects, depending on the kind of exposure (Nordberg 1982). Human can be exposed to trace amounts of Be by inhalation of air and ingestion of drinking water and food. 'Berylliosis' or 'chronic berylliosis', is an inflammatory lung disease that resulted from inhalation exposure to both soluble and insoluble forms of Be (WHO 2001).

In this study, sediments were collected because it is known to be the ultimate sink and agent of transport for trace metals and can be used to represent the degree of metal pollution of an environment (Sultan & Shazili 2010; Yap & Pang 2011). Whereas, most gastropod species have been employed as biomonitor of trace metal pollution in the coastal environment because they have fulfilled most of the criteria for a good biomonitor including the ability to accumulate various trace metals in their tissues, limited mobility thus representative of the study site, easy sampling, wide distribution and enough tissue for metal analysis (Yap et al. 2009a, 2009b). Since there has been no report on Be in snails and sediments from Malaysia, the objective of this study was to provide the baseline levels of Be in the mangrove snail *Nerita lineata* and surface sediments from Peninsular Malaysia.

MATERIALS AND METHODS

The snails, *N. lineata* and sediments were randomly collected from nine sampling sites in the mangrove area of Peninsular Malaysia in April 2011 (Figure 1). About 20 individuals of the snails of similar size were selected from each sampling site, dissected and pooled into soft tissues, opercula and shells.

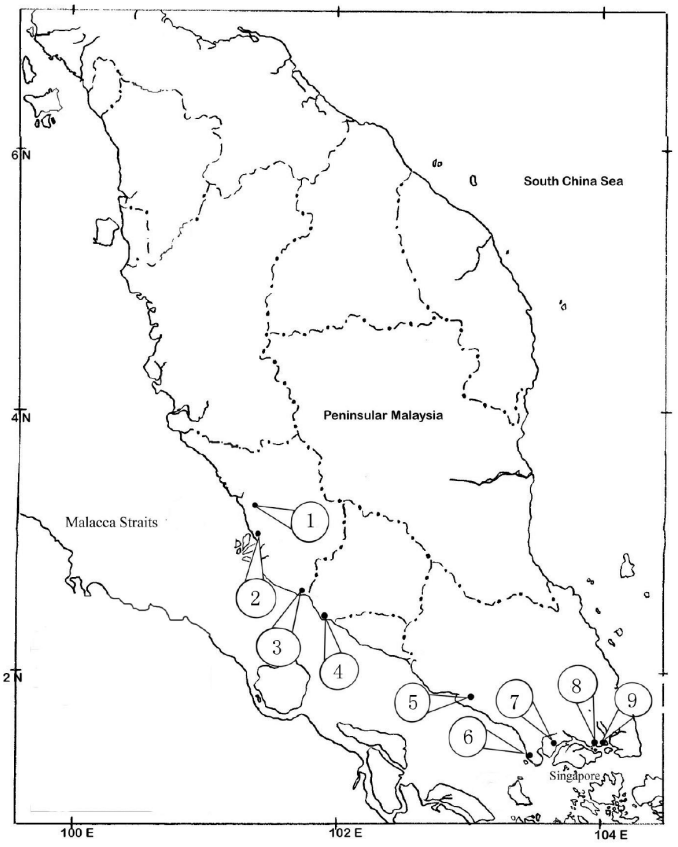

FIGURE 1. Sampling map for snails and surface sediments in this study

No.	Sampling sites
1	SJanggut
2	JKetam
3	Sepang
4	Lukut
5	SAyam
6	Kukup
7	KSMelayu
8	KPPuteh
9	TLangsat

The dissected tissue parts were then dried at 60°C until constant dry weights. Three aliquots of each tissue parts were measured, with an approximate amount of 0.5 g each and placed in the TFM vessels. A mixture of acids (7 mL of HNO_3 65% + 1 mL H_2O_2 30%) were added to the dried samples before inserting them into the microwave cavity. For the sediment samples, they were dried at 60°C until constant dry weights and sifted with a stainless steel sift of 63 µm in mesh. Triplicates of 0.5 g each were obtained from the sampling sites and placed in TFM vessels. A mixture of acids (9 mL of HCl + 3 mL of HNO_3 65%) were added to the dried sediment samples.

For digestion of snail and sediment samples, the microwave digester used was the Milestone ETHOS labstation with easyWAVE or easyCONTROL software HPR1000/10S high pressure segmented rotor. To digest the snail samples, the microwave digester were set to increase the temperature to 200°C for the first 10 min and maintained at 200°C for the following 20 min, with the application of 1000 W of microwave power. Similar procedures from the snail preparation were applied to the sediments but with a temperature raised to 200°C for the first 10 min and maintained at 200°C for the following 15 min. The samples were left in the microwave digester to cool down to room temperature for 10 min after the digestion is completed. Digested samples were then diluted to 100 mL with double distilled water (DDW) and filtered with Whatman No. 1 filter paper before they were stored for metal analysis. All the prepared samples were determined for Be and Fe by using the Perkin Elmer SCIEX ELAN DRC-e ICP-MS. For quality control, the precision and quality of the method of Be analysis was checked with Standard Reference Material for Estuarine sediments (NIST SRM 1646) (recovery %= 92.72%).

The index of geoaccumulation (Igeo) which is a quantitative measure of the degree of metal pollution in aquatic sediments (Müller 1969), was calculated as:

$$Igeo = \log_2\left(\frac{Sample}{1.5 \times Background}\right).$$

In this equation, sample is the concentration measured in the soil and Background is the background concentration in the upper continental crust (UCC) materials, and 1.5 is a correction factor due to lithogenic effluents. For the background values, the values from UCC materials were used, which gives 3100 µg/g for Be, and 3.09% for Fe (Wedepohl 1995). The following classification is given for the Igeo by: < 0 practically unpolluted, 0-1 unpolluted to moderately polluted, 1-2 moderately polluted, 2-3 moderately to strongly polluted, 3-4 strongly polluted, 4-5 strongly to very strongly polluted and >5 very strongly polluted.

A bioconcentration factor (BCF) was calculated for Be to estimate the degree by each metal accumulated in the snails and associated sediments (Szefer & Szefer 1990). This was calculated based on BCF= Cx/Cs, where Cx and Cs are concentrations in biota and sediments, respectively. If the BCF is higher than one, bioaccumulation of contaminant is considered to happen.

The calculation of enrichment factor (EF) of the sediments in this study followed that defined by Ergin et al. (1991):

$$EF = \frac{\left(\frac{Me}{Fe}\right)Sample}{\left(\frac{Me}{Fe}\right)Background},$$

where (Me/Fe)Sample is the metal to Fe ratio in the soil samples while the background concentrations of metal and Fe in the UCC were taken from Wedepohl (1995). The above equation used in the present study can estimate the EF of Be in the sediments of the sampling sites using Fe as a normalizer to correct for differences in soil grain size and mineralogy (Schi & Weisberg 1999). Five contamination categories have been established (Sutherland 2000) namely EF<2, depletion of mineral enrichment; 2 ≤ EF< 5, moderate enrichment; 5 ≤ EF< 20, significant enrichment; 20 ≤ EF< 40, very high enrichment; and EF> 40, extremely high enrichment.

For statistical analysis, Pearson's correlation coefficient (IBM SPSS Statistic version 19) and Single Linkage Euclidean distances (STATISTICA version 8.0) were used to determine the relationship of Be between the snails and sediments.

Results and Discussion

The Be concentrations of the snails and sediments are shown in Table 1. The ranges of Be concentrations (µg/kg dry weight) were: <1.00-4.00 for shells, <1.00-2.00 for opercula and 3.00-41.00 for total soft tissues of *N. lineata*. Higher levels of Be (38-41 µg/kg dry weight) in the soft tissues of the snails are found at Lukut and KSAyam. However, almost all of the shells and opercula from all sampling sites are below 1.00 µg/kg dry weight. For sediments (Table 2), Be ranges were found between 1200 and 3810 µg/kg dry weight with KSAyam being recorded the highest and Tg. Langsat recorded the lowest. In general, Be concentrations follows sediment> ST> shell = opercula. Since other biomonitoring studies on Be concentrations in marine gastropods are not available at present, it is difficult to classify the Be concentrations determined in *Nerita* snails as either natural or elevated. However, the present range of Be levels (3-41 µg/kg dry weight) in *Nerita* soft tissues are comparable to those reported for marine and freshwater mussels. For the marine mussels, Richir and Gobert (2014) reported Be levels as 5- 37 µg/kg dry weight of rope-grown *M. galloprovincialis* collected from a salt pond with good chemical water quality in Diane pond, east Corsica. Be levels were reported as 8-20 µg/kg dry weight in the freshwater mussel *Pletholophus swinhoei* in Vietnam (Wagner & Boman 2004). Wagner and Boman (2004) found a significantly higher mean concentration of 20 µg/

TABLE 1. Beryllium concentrations (mean±SE, µg/kg dry weight) in the shells, opercula and total soft tissue (ST) of *Nerita lineata* and surface sediments (SED) collected from nine sampling sites in Peninsular Malaysia. Bioconcentration factor (BCF) was calculated based on ratio of total soft tissues to sediment

Sites	Shell	Opercula	ST	Fe(%)SED	BCF
Sg. Janggut	<1.00	<1.00	13.0±1.00	1.35	5.72×10^{-3}
Jetty to Pulau Ketam	<1.00	<1.00	12.0±2.00	1.75	6.11×10^{-3}
Sepang	<1.00	<1.00	11.0±1.00	1.30	7.71×10^{-3}
Lukut	<1.00	<1.00	41.0±1.00	2.69	23.92×10^{-3}
Sg. Ayam	<1.00	<1.00	38.0±7.00	2.34	9.98×10^{-3}
Kukup	<1.00	<1.00	8.00±0.0	3.09	3.38×10^{-3}
Kpg. Sg. Melayu	<1.00	2.00±0.50	26.0±1.00	2.69	13.16×10^{-3}
Kpg. Pasir Puteh	2.00±1.00	<1.00	3.00±0.30	3.36	2.13×10^{-3}
Tg. Langsat	4.00±1.00	2.00±0.10	7.00±3.00	4.89	5.81×10^{-3}

TABLE 2. Enrichment factor (EF), index of geoaccumulation (Igeo) and contamination factor (CF) of all sampling sites based on upper continental crust materials (UCCM) value by Wedepohl (1995) as background value

Site	Be (µg/kg dw)	Fe (%)	EF	Igeo	CF
Sg. Janggut	2270	1.35	1.68	-1.03	0.73
Jetty to Pulau Ketam	1960	1.75	1.12	-1.24	0.63
Sepang	1430	1.30	1.09	-1.70	0.46
Lukut	1710	2.69	0.64	-1.44	0.55
Sg. Ayam	3810	2.34	1.62	-0.29	1.23
Kukup	2370	3.09	0.76	-0.97	0.76
Kpg. Sg. Melayu	1980	2.69	0.73	-1.24	0.64
Kpg. Pasir Puteh	1410	3.36	0.42	-1.73	0.45
Tg. Langsat	1200	4.89	0.25	-1.95	0.39
UCCM (Wedepohl 1995)	3100	3.09	-	-	-

kg dry weight in the freshwater mussels collected near a coal power plant and thus explained the most probable anthropogenic source for the higher Be concentrations of the power plant as a pollution source on the sampling site at An Thin. Luy et al. (2012) reported the Be levsls in the endemic seagrass shoots (*Posidonia oceanica*) of the Mediterranean from 18 sampling sites as 3.7-11.3 µg/kg dw. Beryllium has been measured in rice at 80 µg/kg, head lettuce at 330 µg/kg and potatoes at 0.30 µg/kg (Reeves 1986).

Based on EF in Table 2, the values ranged from 0.25-1.68, in which all are EF ≤ 2. They are all categorized as 'deficiency to minimal enrichment' according to Buat-Menerd and Chesselt (1979). Based on Igeo, the values are all ≤ 0.1, indicating 'practically uncontaminated' according to Muller (1969). Lastly, based on CF, the values ranged from 0.39-1.23, in which all sites are ≤ 1 (Low contamination) except for KSAyam (1 < CF ≤ 3; Moderate contamination), according to Hakanson (1980).

The relationship of Be between the soft tissue of snails and sediments is presented in Figure 2. It is found that there is positive correlation for Be levels between soft tissue snails and surface sediment (R=0.533) although the relationship is not significant (p> 0.05). The positive relationship does indicate the potential of *N. lineata* which could reflect the environmental sediment. The insignificant correlation could be due to the fact that low level of Be being found in the environmental mangrove of Peninsular Malaysia. Further studies incorporating controlled experimental laboratory study are needed to investigate the linear positive relationship between the snail and their environment.

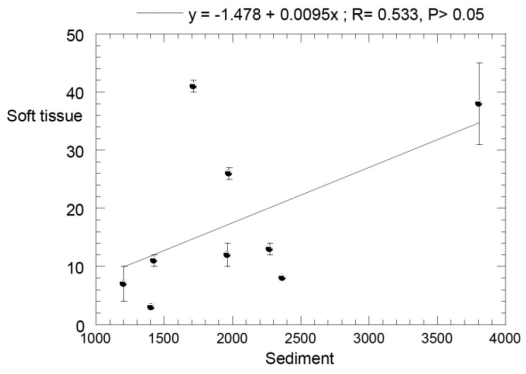

FIGURE 2. Relationships of Be concentrations (µg/kg dry weight) between total soft tissues of *Nerita lineata* and surface sediments

In order to estimate the Be status, the present ranges of Be in the sediment are compared to the established Upper Continental Crusts Materials (UCCM) values, as given in Figure 3. Since there is no established sediment quality values/guidelines for Be are found in the literature, the

present range (1200-3810 µg/kg dw) is compared to the Be background values, the UCCM values for Be which are reported as 3100 µg/kg dw (Wedepohl 1995), 1300 µg/kg dw (Shaw et al. 1976, 1967), 1950 µg/kg dw (Gao et al. 1998a), 3000 µg/kg dw (Taylor & McLennan 1995, 1985) and 2100 µg/kg dw (Rudnick & Gao 2003). It is found that sampling sites at KPPuteh, Tg.Langsat and Sepang exceeded all UCCM values except for Shaw et al. (1976, 1967) while KSAyam appeared to exceed all UCCM values. Based on Wedepohl (1995), only KSAyam exceeded the UCCM value. Thus, sampling area at KSAyam should be given a further environmental concern in future.

The comparison of Be concentrations (µg/kg dw) in the soils and sediments of different areas and average natural soils, with the Peninsular Malaysia mangrove sediments is shown in Figure 4. The present Be ranges in sediments (1200-3810 µg/kg dw) are comparable to Be data in the literature. The present Be ranges are lower than Terengganu River basin, Sri Lanka gem fields, Florida acidic soils (Chen et al. 1999), surface soils of Thailand, former Yugoslavia and Czech Rep. (Asami & Kubota 1995), and Nagoya Harbor estuaries. According to Itoh (1986), the highest Be (16100 µg/kg dw) was reported at mouth of most dominantly polluted river in Nagoya

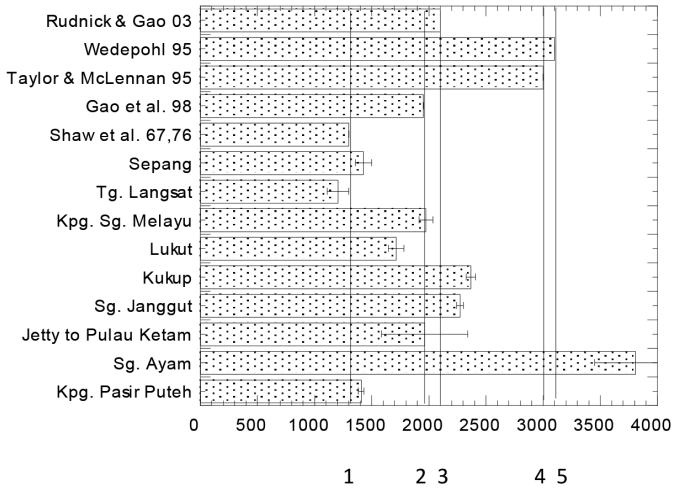

FIGURE 3. Comparison of Be concentrations (µg/kg dw) in the sediments of sampling sites with established upper continental crusts materials values. 1) 1300 µg/kg dw (Shaw et al. 1976, 1967), 2) 1950 µg/kg dw (Gao et al. 1998a), 3) 2100 µg/kg dw (Rudnick & Gao 2003); 4) 3000 µg/kg dw (Taylor & McLennan 1995, 1985) and 5) 3100 µg/kg dw (Wedepohl 1995)

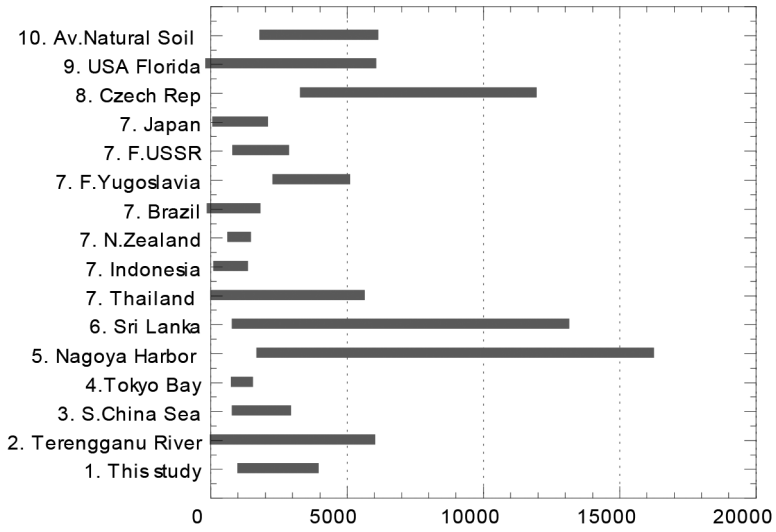

FIGURE 4. Comparison of Beryllium concentrations (µg/kg dw) in the soils and sediments of different area and average natural soils, with the Peninsular Malaysian mangrove sediments. 1. This study/Peninsular Malaysia mangrove, 2. Sultan & Shazili (2010); 3. Rezaee et al. (2011)/ Offshore; 4. Asami & Fukazawa (1985); 5. Itoh (1986)/ Estuaries; 6. Rupasinghe et al. (1984)/ Gem fields; 7. Asami & Kubota (1995)/ surface soils; 8. Borovec (1993)/ Labe (Elbe) River in Central Bohemi; 9. Chen et al. (1999)/ acidic soils and 10. Kabata-Pendias & Mukherjee (2007)

city and is located 4 km downstream of a Be smelting industry which stopped its operation in 1975. However, the present Be ranges are higher than Tokyo Bay and Sagami Bay sediments, Japan (Asami & Fukazawa 1985), South China Sea offshore (Rezaee et al. 2011) and surface soils of Indonesia, New Zealand, Brazil and former USSR (Asami & Kubota 1995), but within the average natural soils concentrations (2000-6000 µg/kg dw; Kabata-Pendias & Mukherjee 2007) and far below the Pyroclastic Rocks (8100-79000 µg/kg dw; Armiento et al. 2013) which is related to Pleistocene activity of the Vico volcano and Be levels in four Piedmont soil profiles in the USA (6300-30500 µg/kg dw; Anderson et al. 1990).

The bioconcentration factor (BCF), a value that represents the concentration ratio of Be (concentration in the soft tissues/total concentration in sediment) in naturally habitats of the snails were determined and are also presented in Table 1. Since all the BCF values were below 1.00 (2.13×10^{-3} - 13.16×10^{-3}), all the values of BCF are categorized as deconcentrators (Dallinger 1993). The shells and opercula were not calculated for the BCF values because most of the concentrations were below detection limit. Moreover, there is significant difference of Be levels between snail soft tissues (mean= 17.7 µg/kg dw) and surface sediment (mean= 2020 µg/kg dw) in which those in the sediment are significantly ($p<0.001$, T-test) higher. Therefore, the snails are generally not a good bioaccumulator of Be and the present BCF values showed that Be was not significantly bioaccumulated in the *Nerita* from their environmental habitats.

This was well supported by some studies previously reported in some aquatic species (Kenaga 1980). It was also reported to be apparently not bioaccumulated from sediment by bottom feeders such as clams and oysters from Lake Pontchartrain, USA (Byrne & DeLeon 1986). However, the *Nerita* snails which are deposit feeders was recorded to possess even lower (about 3-4 orders of magnitude lower), than the Be levels in the surface sediments. Newer literature on Be levels in gastropods and sediments is lacking and thus comparison is difficult to make.

Conclusion

This study provided the baseline data of Be in the shells, opercula and total soft tissues of *N. lineata* and surface sediments from Peninsular Malaysia. Peninsular Malaysia mangrove is not enriched and not contaminated by Be. Thus, the Malaysia mangrove area is not an environmental Be concern at the moment. The present insignificant relationships of Be between snails and sediments and low BCF, could be attributed to the fact that Be contamination is not serious in the Peninsular Malaysia mangrove area. Thus, high bioaccumulation of Be was not recorded and the positive and significant relationship between snails and environmental sediments could hardly been established. Lastly, the mangrove snail as a good biomonitor of Be needs further studies.

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Received: 27 June 2014
Accepted: 4 March 2015