UNIVERSITI PUTRA MALAYSIA

DISTRIBUTION AND CONCENTRATION OF SEVERAL HEAVY METALS IN SNAILS (*NERITA LINEATA*) FROM THE INTERTIDAL AREAS OF PENINSULAR MALAYSIA

CHENG WAN HEE

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By

CHENG WAN HEE

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfillment of the Requirement for the Degree of Master of Science

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The aim of this study is to determine the background concentrations of heavy metals in the soft and hard tissues of *Nerita lineata* collected from the west intertidal area of Peninsular Malaysia and to study its potential use as a biomonitor of Cd, Cu, Fe, Ni, Pb and Zn. From this study, the snails *N. lineata*, and sediments were collected from 15 sites along the west coast of Peninsular Malaysia from December 2005 until April 2006. Snails collected from each sampling site were dissected and pooled into three parts (total soft tissues, operculums and shells) and were analyzed for Cd, Cu, Fe, Ni, Pb and Zn in addition to sediment samples.

The mean metal concentrations (μg/g dry weight) in the soft tissues were 1.18 ± 0.17, 15.43 ± 0.86, 546.21 ± 61.98, 6.69 ± 0.45, 94.42 ± 46.81 and 87.07 ± 4.08 for Cd, Cu, Fe, Ni, Pb and Zn, respectively. As for operculums, the mean metals (μg/g dry weight) were 2.99 ± 0.22, 6.38 ± 0.33, 35.05 ± 2.49, 23.34 ± 0.92, 48.22 ± 1.99 and 16.59 ± 1.03 for Cd, Cu, Fe, Ni, Pb and Zn, respectively. In the shells, it was found that the mean metal concentrations (μg/g dry weight) were 3.00 ± 0.23, 6.25 ± 0.54, 70.20 ± 14.04, 23.33 ± 1.14, 47.17 ± 2.30 and 13.54 ± 3.21 for Cd, Cu, Fe, Ni, Pb and Zn, respectively. The means of heavy metal concentrations (μg/g dry weight) in sediments were 2.86 ± 0.36, 18.95 ± 3.49, 25499.63 ± 1895.43, 12.35 ± 0.95, 47.24 ±5.87 and 64.61 ± 4.77 for Cd, Cu, Fe, Ni, Pb and Zn, respectively.

The snails were good biomonitors for heavy metals as significant correlations were found between sediments and the soft tissues for Cu ($P<0.05$) and Pb ($P<0.01$), while operculums and shells were significantly ($P<0.01$) correlated with sediments for Cd and Pb.

Different patterns of heavy metal distribution were found in the different tissues (shell, operculum and soft tissues) of *N. lineata* as well as spatial differences and distributions. This shows that the distribution of metals in shells and total soft tissues of *N. lineata* were not similar and this could be due to different rates of metal accumulation, excretion and sequestration. Since *N. lineata* can be abundantly found in the rocky shores, below jetties and mangrove trees along the intertidal area of the west coast of Peninsular Malaysia and are accumulative of Cd, Cu, Fe, Ni, Pb and Zn, the snail species is therefore a potential biomonitor of heavy metal pollution in the west coast of Peninsular Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master of Science

DISTRIBUTION AND CONCENTRATION OF SEVERAL HEAVY METALS IN SNAILS (*NERITA LINEATA*) FROM THE INTERTIDAL AREAS OF PENINSULAR MALAYSIA

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Tujuan utama kajian ini adalah untuk menentukan kandungan kepekatan logam berat dalam tisu keras dan lembut *N. lineata* yang dikumpulkan dari pesisiran pantai barat Semenanjung Malaysia dan mengkaji potensi spesis ini sebagai agen penunjuk biologi bagi Cd, Cu, Fe, Ni, Pb and Zn. Melalui kajian ini, *N. lineata*, dan sedimen telah disampel dari 15 kawasan sepanjang pantai barat Semenanjung Malaysia dalam bulan Disember 2005 hingga April 2006. Siput yang disampel dari setiap kawasan dibedah dan diasingkan kepada 3 bahagian (keseluruhan tisu lembut, operculum and cengkerang) dan analisis dijalankan ke atas tisu dan juga sedimen untuk kandungan Cd, Cu, Fe, Ni, Pb dan Zn.

Min kandungan logam berat (μg/g berat kering) dalam tisu lembut adalah 1.18 ± 0.17, 15.43 ± 0.86, 546.21 ± 61.98, 6.69 ± 0.45, 94.42 ± 46.81 dan 87.07 ± 4.08 untuk Cd, Cu, Fe, Ni, Pb dan Zn. Bagi operculum, min kandungan logam berat (μg/g berat kering) adalah 2.99 ± 0.22, 6.38 ± 0.33, 35.05 ± 2.49, 23.34 ± 0.92, 48.22 ± 1.99 dan 16.59 ±

1.03 untuk Cd, Cu, Fe, Ni, Pb dan Zn. Dalam cengkerang, didapati bahawa min kandungan logam berat (μg/g berat kering) adalah 3.00 ± 0.23, 6.25 ± 0.54, 70.20 ± 14.04, 23.33 ± 1.14, 47.17 ± 2.30 dan 13.54 ± 3.21 untuk Cd, Cu, Fe, Ni, Pb dan Zn. Kandungan logam berat (μg/g berat kering) dalam sedimen adalah dalam lingkungan 0.96 - 9.27, 2.94 - 58.69, 254.38 - 31976.95, 6.02 - 24.2, 21.89 - 152.20 dan 21.13 - 113.43 untuk Cd, Cu, Fe, Ni, Pb dan Zn.

Siput ini merupakan penunjuk biologi yang baik kerana ia menunjukkan korelasi yang signifikan antara sedimen dan tisu lembut untuk Cu (P<0.05) dan Pb (P<0.01), dan antara operculum, cengkerang dan sedimen (P<0.01) untuk Cd dan Pb.

Terdapat perbezaan antara kandungan logam berat dalam tisu (tisu lembut, operculum dan cengkerang) yang mungkin disebabkan oleh perbezaan kadar pengumpulan, penyingkiran dan pengasingan logam-logam tersebut. Memandangkan *N. lineata* mudah didapati dengan banyak di kawasan pantai berbatu, di bawah jeti dan di kawasan paya bakau sepanjang pantai barat Semenanjung Malaysia dan berkemampuan untuk mengumpul logam berat, maka spesies ini adalah berpotensi sebagai penunjuk biologi bagi pencemaran logam berat di Semenanjung Malaysia.

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LIST OF ABBREVIATIONS

µm	micrometre
µg/g	microgram per gram
µg/L	microgram per litre
CF	conversion factor
CI	condition index
CV	coefficient of variation
DDW	double distilled water
g/cm^3	gram per centimetres cubic
H_2SO_4	sulphuric acid
HCl	hydrochloric acid
HNO_3	nitric acid
ppm	part per million
rpm	rotation per minute
se	standard error
SNK	Student-Newman-Kuels
ST	soft tissues

CHAPTER I

INTRODUCTION

Marine pollution has always been a concern in most developed countries. In the marine system, the intertidal zone is continuously influenced by tidal changes and inputs from riverine systems which are closely associated to the inputs from anthropogenic sources (Yap *et al.*, 2004a). Although the sea covers more than 70% of the surface of our planet, the continental shelf is the most important portion as far as human activities are concerned since it is the main source of living marine resources used as food for humans and it is most significantly impacted by anthropogenic factors (Zoller, 2006). The west coast of Peninsular Malaysia is a principle repository for agricultural, industrial and domestic wastes originating from land-based and sea-based activities (Abdullah *et al.*, 1999). The land-based activities such as agriculture, industry and urbanisation and the role of the west coast of Peninsular Malaysia as the major international shipping lane (sea-based activities), has caused the input of a great variety of pollutants (Abdullah *et al.*, 1999) to the coastal area here. These are the reasons why pollution levels were monitored mostly in the west coast of Peninsular Malaysia (Yap *et al.*, 2002a).

These anthropogenic activities have brought to the excessive input of toxicants such as heavy metals into the marine environment. Heavy metals are inorganic chemicals that are non-biodegradable, cannot be metabolized and will not break down into harmless forms (Depledge *et al.*, 1994). Metals differ from other toxic substances in that they are neither created nor destroyed by human (Hamed and Emara, 2006).

Exposure of heavy metals to marine ecosystem will not only cause hazardous effects to the organisms in the ecosystem but also to humans as it flows through the food web (Agusa *et al.*, 2005). Nevertheless, utilization by human influences the potential for health effects in at least two major ways: first, by environmental transport that is, by human or anthropogenic contributions to air, water, soil and food and second, by altering the speciation or the biochemical form of the element (Beijer and Jernelov, 1979; Hamed and Emara, 2006). Therefore biomonitoring of heavy metals in the aquatic environment is necessary.

Biomonitoring programs, for instance the 'Mussel Watch' program, were first initiated by Goldberg *et al.* (1978) in which bivalves was used as sentinels of coastal chemical pollution. According to Hung *et al.* (2001), molluscs can accumulate numerous pollutants from natural in quantities that are orders of magnitude higher than the background levels. In addition to that the levels of heavy metals in molluscs and other invertebrates are often considerably higher than in other constituents of the marine environment (Hamed and Emara, 2006). Compared to sediments, molluscs exhibit greater spatial sensitivity and therefore, are the most reliable tool for identifying sources of biologically available heavy metal contamination (Goldberg *et al.*, 1978; Koide *et al.*, 1982; Thomson *et al.*, 1984; Hamed and Emara, 2006). Generally, molluscs accumulate metals in their tissues in proportion to the degree of environmental contamination which signifies their importance as biomonitors of heavy metal pollution (Goldberg *et al.*, 1983; Elder and Mattraw, 1984; Bu-Olayan and Subrahmanyam, 1997).

In Peninsular Malaysia, biomonitoring work and studies of the background concentrations of heavy metals were mainly focused on marine mussels (Yap *et al.*, 2002b; 2003a; 2003b; 2003c; 2004a; 2006a; 2006b). Apart from mussels, other intertidal molluscs on the west coast of Peninsular Malaysia such the gastropod *N. lineata* can be widely found. However, a detailed biomonitoring study of heavy metals on this snail species is not available in the literature. A few studies done by researchers from all over the world has reported on the background heavy metal concentrations of nerites. 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 (Hartley and Johnston, 1983; Bu-Olayan and Subrahmanyam, 1997; AbdAllah and Moustafa, 2002) which supports *N. lineata* being used as a biomonitor of heavy metals pollution. The objectives of this study are to:-

1. determine the background concentrations of Cd, Cu, Fe, Ni, Pb and Zn in the soft tissues, operculums and shells of *N. lineata* collected from different locations in Peninsular Malaysia,

2. study its potential use as a biomonitor of Cd, Cu, Fe, Ni, Pb and Zn based on correlation analysis of heavy metals concentration between snails and sediments, and

3. evaluate the potential use of *Nerita*'s shells as a biomonitoring material for heavy metals.

CHAPTER II

LITERATURE REVIEW

Tropical Intertidal Pollution in Malaysia

Human anthropogenic activities have always been the main source of pollution to all developing countries in this region. These developments have made heavy metals pollution in tropical coastal waters a major cause of concern from the ecotoxicological point of view.

Pollution can be defined as any 'change in the physical, chemical and biological properties of any habitat or ecosystem, due to any discharge of liquid, gaseous or solid substances, which is liable to create harmful effects on living organisms or that may affect the domestic agricultural, industrial and recreational activities of human being (Ho *et al.*, 1980). Introduction of toxic material aquatic ecosystems produces a variety of complex responses governed by several basic factors: (1) nature of the toxicant; (2) concentration; (3) exposure time; (4) environmental characteristics of the receiving system; (5) age, condition, etc., of exposure organisms; and (6) the present of other toxicants (Hart and Fuller, 1974).

Increased coastal population, rapid urbanization, oil and gas production, tourism development, heavy rainfall throughout the year and various economic activities have created numerous environmental and ecological problems in Malaysia's coastal areas

(Cicin-Sain and Knecht, 1998; Edward et al., 2008). Malaysia's economic growth is rapidly increasing and thus, resulted in the increment of production and usage of toxic chemicals such as trace elements (Agusa *et al.*, 2005) to the marine system of Malaysia. In the west coast of Peninsular Malaysia or better known as the Straits of Malacca, the sea-based sources of pollution arise mainly from operation and accidental discharges of oily pollutants from shipping vessels plying the Straits (Abdullah *et al.*, 1999). While land-based inputs such as deforestation, disposal of agricultural and industrial wastes, sewage disposal, solid waste disposal, mangrove swamp conversion and land reclamation and sea-based inputs (shipping, dumping, mining, oil exploration, mariculture and fishing) were the human activities that contributed to the adverse environmental changes in the west coast of Peninsular Malaysia and the Straits of Malacca (IMPAK, 1998; Yap *et al.*, 2002a). Most heavy metals discharged into coastal waters rapidly become associated with particulate matter and are incorporated into the bottom sediments (Yap *et al.*, 2005). Therefore heavy metal pollution in the intertidal areas of Peninsular Malaysia it has always been a concern. Reports of waste of Cu oxide and Cu slag were reported in the late 1990s while in 2000, there were 34% in Penang and 53% in Langkawi Island where these percentages were exceeding the Interim Standard for Marine Water Quality for Cu (0.10 mg/L) (DOE, 2001; Yap *et al.*, 2003f).

Various studies regarding biomonitoring have been carried out in order to monitor contaminations status. Contaminations of organochlorines (Monirith *et al.*, 2003), butyltin (Ismail *et al.*, 2004), and heavy metals (Ismail, 1993; Ismail and Rosniza, 1997; Yap *et al.*, 2002b; 2003a; 2003b; 2003c; Amin *et al.*, 2006a) have been reported in coastal area of Malaysia.

Heavy metals

The term 'heavy metal' is synonymously used with 'trace metal' which includes both essential and non-essential trace metal and can be defined by chemical criteria (Rainbow, 1995). Whether essential or not, all trace metals are potentially toxic at a threshold bioavailability (Rainbow, 1990). Heavy metals or trace metals are a group name for metals or metalloids which are related to the toxicity and pollution and it is defined as metals atomic density (> six g cm^{-3}) (Alloway, 1990). Heavy metals are often introduced into the aquatic ecosystems as byproducts of industrial processes and acid mine drainage residues (Hart and Fuller, 1974). In contrast with organic pollutants, heavy metals cannot be biologically or chemically degraded, and thus may either accumulate locally or be transported over long distances (Marchand *et al.*, 2006).

Heavy metals can be divided into essential and non-essential metals. Essential metals are elements required by most living organisms in small but critical concentrations for normal health but excess concentrations cause toxicity (Alloway and Ayres, 1997). Hence, a deficient supply of essential metals will result in shortage of the enzyme, which leads to metabolic dysfunction causing disease (Alloway and Ayres, 1997). The essential metals include Na, K, Ca, Mg, Fe, Cu and Zn (Astorga et al., 2007). As for non-essential metals, they are elements with no known essential biochemical function and do not cause deficiency disorder to organisms at low concentrations (Alloway and Ayres, 1997). Non-essential metals, includes As, Cd, Pb, Ni, Mn (Astorga et al., 2007), are sometimes also referred to (incorrectly) as 'toxic' elements.

The cycling of heavy metals, because of their toxicity, bio-accumulation capacity and persistence, is a serious question recently addressed by many studies on mangrove environments (Marchand *et al.*, 2006). Consequently high concentrations of heavy metals can accumulate in sediments, and especially in fine-grained oozes, which present high mineral specific surfaces (Marchand *et al.*, 2006). They may act as a sink or a source of heavy metals in coastal environments because of their variable physical and chemical properties (Marchand *et al.*, 2006).

Gastropoda (*Nerita lineata*)

Taxonomy

As well as being the largest class of molluscs, the gastropoda are easily the most varied (Morton, 1968). Gastropods range from snails with robust shells and slugs without external shells, to vermiform endoparasites and they are classified into three main groups: the prosobranchs, opistobranchs and pulmonates (Hughes, 1986).

In Malaysia, the *N. lineata* is locally known with different names. People from the northern part of Malaysia called it 'siput bulan' or 'siput mata kerbau'. The snails were commonly known by the locals from the southern part of Peninsular Malaysia as 'siput timba' (bucket snail) adopted from the shape of the snail. The following is the taxonomy of the snail *N. lineata* (Oliver, 1980).

Phylum : Mollusca

Class : Gastropoda

Subclass : Prosobranchia

Order : Archaeogastropoda

Superfamily : Neritacea

Family : Neritidae

Subfamily : Neritinae

Genus : *Nerita*

Species : *Nerita lineata*

Figure 1 : *Nerita lineata*

Morphology

Generally, the body of gastropods is symmetrical with a mantle and calcareous shell (Khanna and Yadav, 2004). The shells are thick, porcellanous, semi-globose with a small spire (Macpherson and Gabriel, 1962). The shell is brownish grey in colour and is about 2 cm in width. The species *N. lineata* has dark brown operculum with fine granules (Oliver, 1980).

Habitat

Snails of the family Neritidae live in tropical and subtropical areas (Oliver, 1980). Nerites are principally inhabitants of warmer shores, living on the upper surface of rocks between tide marks (Macpherson and Gabriel, 1962) and in deep or brackish water in mangrove swamps, rivers and seas (Oliver, 1980). In rocky beaches, the snails hide behind crevices of rock pools during day time to avoid heat stress. In mangrove areas, they were observed, naturally attached to the roots of the mangrove trees or on the ground. The snails were exposed in the open air during low tides. They were found on tree trunks or submerged into the sea water during high tide.