



UNIVERSITI PUTRA MALAYSIA

**AN ASSESSMENT OF METAL DISTRIBUTION AND METAL SOLUBLE
FRACTIONS IN THE EDIBLE MOLLUSCS FROM MALAYSIA**

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By

FRANKLIN BERANDAH ANAK EDWARD THOMAS

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
fulfillment of the Requirements for the degree of Master of Science**

July 2009



Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

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July 2009

Chairman: Associate professor Yap Chee Kong, PhD.

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The present study focused on the heavy metal concentrations in the different parts of 12 species of Malaysian molluscs, six species of bivalves and gastropods, respectively. The aim of the present study was to provide information on the concentrations of the essential metals: Cu, Fe and Zn and the non-essential metals: Cd, Ni and Pb in the edible tissues of molluscs with particular reference to the food safety and ecotoxicological points of views. For bivalves, Cu, Fe and Zn concentrations in the edible parts ranged at 1.80-79.8 $\mu\text{g/g dw}$, 42.9-4895 $\mu\text{g/g dw}$ and 28.3-379 $\mu\text{g/g dw}$, respectively. While for Cd, Pb and Ni, they ranged at 0.253-22.4 $\mu\text{g/g dw}$, 0.558-46.5 $\mu\text{g/g dw}$ and 0.656-23.6 $\mu\text{g/g dw}$, respectively. As for gastropods, Cu, Fe and Zn concentrations in the edible parts ranged at 1.97-686 $\mu\text{g/g dw}$, 51.2-2921 $\mu\text{g/g dw}$, 22.8-337 $\mu\text{g/g dw}$, respectively. While for Cd, Pb and Ni, they ranged at 0.159-32.9 $\mu\text{g/g dw}$, 1.20-43.0 $\mu\text{g/g dw}$ and 0.222-27.9 $\mu\text{g/g dw}$, respectively.

The study on the soluble and insoluble heavy metal fractions revealed that an abundance of soluble metals like Cd, Pb and Ni were consistently found in some tissues of the



molluscs such as in the foot, mantle and muscle and they could potentially be transferred through the food web (predators).

The total metal concentrations in the different parts were compared with the food permissible limits set by six organizations around the world. However, to overcome the overestimation of food safety based on the total metal concentrations in the different edible tissues, determination of the metal soluble fractions in the tissues were further investigated in this study. The soluble concentrations were compared with the permissible limits set by the Environmental health Criteria (1998, 2001) and the FAO/WHO (1984). From the present findings, it was found that consumption of large amounts of *Per. viridis*, *G. expansa* and most of the gastropods could pose metal toxic to their consumer. The elevated of Cu and Zn concentrations in most of the edible parts of the gastropods suggested that the consumption of large amounts of most gastropods were not advisable. As for Cd levels, it was found that the byssus of *Per. viridis* and *D. faba*; and the digestive gland and mantle of *Chi. capucinus* were not safe for continuous consumption (for example: more than a week) since the levels would exceed the permissible limit. Besides, the continuous consumption of the byssus of *Per. viridis* and *D. faba* would also potentially cause Pb toxicity.

The information on the metal distributions in the different parts obtained by using the cluster analysis is important to facilitate the biomonitoring of the marine environment, which based on the use of different tissues in the species of molluscs of Malaysia.

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

**PENILAIAN TERHADAP TABURAN LOGAM BERAT DAN PECAHAN
LOGAM BERAT TERLARUT DALAM MOLLUSK BOLEH-MAKAN
DARIPADA MALAYSIA**

Oleh

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Kajian ini menfokuskan terhadap taburan logam berat di dalam pelbagai bahagian 12 spesies mollusk di Malaysia, masing-masing enam spesis bivalve dan gastropod. Tujuan utama kajian ini ialah menyediakan maklumat tentang kepekatan Cd, Cu, Fe, Ni Pb dan Zn di dalam tisu-tisu boleh makan dengan perbincangannya tertumpu khas kepada keselamatan pemakanannya selain dari segi ekotoksikologi. Untuk bivalve, julat kepekatan Cu, Fe dan Zn di dalam tisu-tisu boleh makan masing-masing adalah 1.80-79.8 $\mu\text{g/g dw}$, 42.9-4895 $\mu\text{g/g dw}$ dan 28.3-379 $\mu\text{g/g dw}$. Manakala untuk Cd, Pb dan Ni, kepekatan logam berat tersebut masing-masing berjulat antara 0.253-22.4 $\mu\text{g/g dw}$, 0.558-46.5 $\mu\text{g/g dw}$ dan 0.656-23.6 $\mu\text{g/g dw}$. Untuk gastropod pula, julat kepekatan Cu, Fe dan Zn di dalam tisu-tisu boleh makan masing-masing adalah 1.97-686 $\mu\text{g/g dw}$, 51.2-2921 $\mu\text{g/g dw}$ dan 22.8-337 $\mu\text{g/g dw}$. Manakala untuk Cd, Pb dan Ni, kepekatan logam berat tersebut masing-masing berjulat antara 0.159-32.9 $\mu\text{g/g dw}$, 1.20-43.0 $\mu\text{g/g dw}$ dan 0.222-27.9 $\mu\text{g/g dw}$.



Kajian terhadap logam berat terlarut dan tak terlarut mendapati kehadiran logam terlarut yang tinggi Cd, Pb dan Ni secara konsisten di dalam tisu-tisu mollusk seperti kaki, mantel dan otot dan berpotensi dipindahkan melalui jaringan makanan (oleh pemangsa).

Kepekatan keseluruhan logam berat di dalam pelbagai tisu dibandingkan dengan tahap yang dibenarkan oleh enam organisasi dari seluruh dunia. Walaubagaimanapun, untuk mengelakkan anggaran yang kurang tepat yang berdasarkan jumlah keseluruhan logam berat di dalam pelbagai tisu boleh makan tersebut, kajian tentang pecahan terlarut logam di dalam tisu-tisu itu telah dicadangkan. Kepekatan pecahan terlarut tersebut dibandingkan dengan sukatan yang dibenarkan oleh Environmental health Criteria (1998, 2001) dan FAO/WHO (1984). Melalui perbandingan tersebut, didapati pengambilan *Per. viridis*, *G. expansa* dan kebanyakan gastropod mungkin boleh menyebabkan ketoksikan kepada si pemakannya. Kehadiran Cu dan Zn yang banyak di dalam kebanyakan gastropod menunjukkan pengambilannya yang banyak juga tidak digalakkan. Untuk tahap Cd pula, didapati bisus *Per. viridis* dan *D. faba*; dan kelenjar pencernaan dan mantel *Chi. capucinus* adalah tidak selamat diambil secara berterusan (Contohnya: satu atau lebih minggu) kerana tahapnya akan melebihi tahap yang dicadangkan. Selain itu, pengambilan berterusan bisus *Per. viridis* dan *D. faba* mungkin juga berpotensi menyebabkan ketoksikan Pb.

Maklumat tentang taburan logam di dalam pelbagai bahagian tisu yang diperolehi melalui analisis kluster adalah penting untuk panduan dalam mengawal kawasan marin, yang mengaplikasikan pelbagai tisu spesis mollusk di Malaysia.

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I certify that an Examination Committee has met on **13 July 2009** to conduct the final examination of **Franklin Berandah Anak Edward Thomas** of his degree thesis entitled “**AN ASSESSMENT ON THE METAL DISTRIBUTION AND METAL SOLUBLE FRACTIONS IN THE EDIBLE MOLLUSCS FROM MALAYSIA**” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science (Ecotoxicology)

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

FRANKLIN BERANDAH ANAK EDWARD THOMAS

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LIST OF ABBREVIATIONS

μm	micrometre
$\mu\text{g/g}$	microgram per gram
$\mu\text{g/L}$	microgram per litre
CI	condition index
DDW	double distilled water
g/cm^3	gram per centimetres cubic
H_2NO_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 1

INTRODUCTION

1.1 Background of Study

Marine molluscs are a major internationally traded seafood commodity. They inhabit their natural habitat, the intertidal area, which are usually close to estuaries. Hence, the chance of their exposure to various type of contaminants and pollutants from anthropogenic (land-based activities) through the riverine system and the sea-based sources is very high. Moreover, the tissues of molluscs are well known for their accumulation of a wide range of contaminants such as heavy metals (Goldberg *et al.*, 1978). Hence, the information on the safety of molluscs as food is important and crucial.

In the literature, studies on the food safety of molluscs focused on the total soft tissues such as the studies conducted by Storelli (2008) in the Adratic Sea (Cephalopod molluscs), Amiard *et al.* (2008) in France, UK and Hong Kong (*Buccinum undatum*, *Crassostrea gigas*, *Ostrea edulis*, *Saccostrea cucullata*, *Perna viridis*, *Marcia hiantina* and *Chlamys nobilis*), Espana (2007) in the Straits of Magellan (*Mytilus chilensis* and *Perunytitus purpuratus*), Fung *et al.* (2004) in the east coast of China (*Perna viridis* and *Mytilus edulis*), Chiu *et al.* (2000) in Hong Kong (*Per. viridis*). In Malaysia, a detailed study on the food safety of *Per. viridis* from Peninsular Malaysia was conducted by Yap *et al.* (2004).

However, studies on the food safety of molluscs which are based on the different edible tissues are scarce in the literature. The only such study found was conducted

by Amiard *et al.* (2008) who evaluate the remaining soft tissue and digestive gland of *S. cucullata*, *Per. viridis* and *M. hiantina*; and the adductor muscle of *C. nobilis*. The present study aims to provide information on food safety of Malaysian tropical molluscs based on different edible tissues to overcome the overestimation of the toxicological risk in the evaluation of the total soft tissues. The possible metals bioavailable to the consumer which could be toxic (or trophic bioavailability) could also be estimated based on the soluble fractions of the different edible tissues (Reinfelder *et al.*, 1998; Bragigand *et al.*, 2004).

The term trophic bioavailability (or soluble fraction) is used to describe the proportion of a chemical ingested with food which enters the systemic circulation (Amiard *et al.*, 2008). The release of a chemical from an ingested food is a prerequisite for its uptake and assimilation. Thus, as was described by Amiard *et al.* (2008), the determination of the bioavailability of a food-bound contaminant, as measured by its extractability from the food, can be used as an indicator of its maximum trophic bioavailability. In this study, the bioavailabilities of heavy metals in Malaysian molluscs were estimated by measuring their extractabilities based on the modified method described by Bragigand *et al.* (2004).

From the ecotoxicological point of view, numerous factors may affect the concentrations of heavy metal in molluscs. The concentrations of heavy metal accumulated by marine organisms are functions of not only water quality, also of seasonal factors, temperature, salinity, diet, spawning and individual variations (Hamed and Emara, 2006). The bioaccumulation of heavy metals and their subsequent bioavailabilities are highly dependent on geochemical and biological

factors. Among the biological factors, there are distinct differences in the accumulation of heavy metal between molluscs species; and within a single species, accumulation can be a function of age, size, sex, genotype, phenotype, feeding activity and reproductive state (Boening, 1999). The geochemical factors that influence bioaccumulation are organic carbon, water hardness, temperature, pH, dissolved oxygen, sediment grain size and hydrologic features of the system (Elder and Collins, 1991; Martoja *et al.*, 1988; Boening, 1999).

Due to the numerous factors discussed in the previous paragraph, studies of heavy metals in the different parts of molluscs were suggested when a potential biomonitor which could indicate metal contamination in the environment is proposed. The use of different tissues, could overcome the inaccuracies incurred when determining heavy metal levels in the total soft tissues since this may not accurately reflect the contaminant concentrations in the individual target tissues of the organism. This was based on the fact that different tissues accumulate different metals at different rates and that the biological half-lives of metals at each type of soft tissues also differ from one another (Lakshmanan and Nambisan, 1989; Yap *et al.*, 2007).

1.2 Objectives of Study

1. To determine the heavy metal concentrations (Cu, Cd, Zn, Pb, Ni and Fe) in the different soft tissues of molluscs (bivalves and gastropods) collected from the coastal areas of Malaysia.
2. To Determine the soluble and insoluble heavy metal concentrations in the different soft tissues of molluscs.

3. To evaluate the safety of the consumption of molluscs as food based on the metal distributions and the soluble fractions of the edible tissues of molluscs from Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy metals Studies in Malaysian Molluscs

The eastern coast of Peninsular Malaysia and the western coast of East Malaysia are characterized by well-developed sandy beaches with clay-composed soils and mudflats (Cicin-Sain and Knecht 1998). Mangrove trees are found along the west coast of Peninsular Malaysia, generally associated with mudflats and clay swamps, which provide good environments for living organisms including molluscs. According to the Department of Fisheries, Malaysia (2005), in 2005-06, the Malaysia Fisheries Directory documented 27 species of bivalves and 18 species of gastropods in Malaysian coastal areas. The high diversity of molluscs is an advantage for use in biomonitoring studies in Malaysia.

In Malaysia, few researchers had conducted studies of heavy metals in local molluscs to biomonitor the coastal water areas. Below are some of the studies of heavy metals in Malaysian molluscs:

Table 2.10: Some previous studies of heavy metal concentrations ($\mu\text{g/g}$) in Malaysian marine molluscs

Species/location	Tissue	Cu	Cd	Zn	Pb	Ni	Fe
<i>Cerithidea obtusa</i> / Sepang River (Ismail and Ramli, 1997)	Total soft tissues	10.0-32.0	0.02-0.67	20.0-40.0	10.0-19.0	NA	NA
<i>Nerita lineata</i> / Sepang River (Ismail and Ramli, 1997)	Total soft tissues	8.00-55.0	0.04-0.38	21.0-38.0	10.0-14.0	NA	NA
<i>Perna viridis</i> / Peninsular Malaysia (Yap <i>et al.</i> , 2002a)	Total soft tissues	6.31-20.1	0.51-1.22	69.4-129	2.00-8.76	NA	NA
<i>Anadara granosa</i> / Peninsular Malaysia (Yusof <i>et al.</i> , 2004)	Total soft tissues	1.67-9.10	0.18-4.43	41.8-158	0.13-3.04	NA	NA
<i>Perna viridis</i> / Peninsular Malaysia (Yusof <i>et al.</i> , 2004)	Total soft tissues	2.09-8.55	0.10-2.88	52.1-90.2	0.20-1.69	NA	NA
<i>Isognoman alatus</i> / Sepang Besar River (Saed <i>et al.</i> , 2004)	Total soft tissues	17-45	1.5-5.6	148-564	1.4-6.4	NA	NA
<i>Isognoman alatus</i> / Sepang Kecil River (Saed <i>et al.</i> , 2004)	Total soft tissues	11	0.3-0.6	23-24	0.3-0.5	NA	NA
<i>Nerita lineata</i> / Johor (Amin <i>et al.</i> , 2006)	Total soft tissues	18.0	1.24	95.09	19.8	5.57	474
<i>Perna viridis</i> / The Johore Straits (Yap <i>et al.</i> , 2006a)	Different soft tissues	6.98-11.5	1.43-1.69	59.9-91.9	10.9-15.0	16.1-23.8	89.8-453
<i>Perna viridis</i> / Pasir Panjang (Yap <i>et al.</i> , 2007)	Different soft tissue	1.01-6.36	NA	3.92-24.0	0.59-2.70	NA	NA
<i>Crassastrea iredalei</i> / Setiu Wetland (Najiah <i>et al.</i> , 2008)	Total soft tissues	38.9 \pm 13.2	1.60 \pm 0.28	785 \pm 286	0.17 \pm 0.15	NA	NA
<i>Telescopium telescopium</i> / South-western Peninsular Malaysia (Yap <i>et al.</i> , 2009)	Total soft tissues	25.4-79.3	NA	12.6-120	0.78-10.1	NA	NA

Note: NA= Not available

2.2 Heavy Metals in Molluscs: Differences by Species, Habitat and Location

Most biomonitoring programs do not study the metal concentrations in each of the harvested species and instead use one or a few species that are assumed to be bioindicator species (Saavedra *et al.*, 2004). Following this approach, metal concentrations in a mollusc are used to assess water quality and it is assumed that all molluscs in good-quality water are safe for human consumption. This method does not hold when the metal accumulation characteristics of the target species are not similar to those of the bioindicator species, making the estimation of the toxicity risk incorrect (Saavedra *et al.*, 2004).

Several studies have demonstrated different molluscs species to differ substantially in their capabilities to accumulate various metals (Reinfelder *et al.*, 1997; Wang and Fisher, 1999; Chong and Wang, 2000; Wang, 2001). According to Blackmore (2001), different organisms respond to different sources, correlating with different feeding types and prey taken and it is difficult to ascertain the relative importance of each of the major routes of uptake. Body concentrations may vary between metals and, for the same metal, between species, including many that are closely related (Rainbow, 1990), and even individuals (Depledge and Bjerregaard, 1989). Such variability may be accounted for by differences in permeability, metabolic rate and the quantities and types of metal-binding ligands present either at the membrane surface or intracellularly (Brown and Depledge, 1998; Langston *et al.*, 1998). Based on a study conducted by Blackmore (2001), the researcher found that Cd concentrations were high in neogastropods and filter feeders. Filter feeders have a high proportion of permeable surfaces and large volumes of water pass over them

through the gills allowing the organisms to take up elevated concentrations of metal. Meanwhile, the predatory neogastropods had greater Cd concentrations than the grazers did which could be due to the animal prey of the neogastropods (Blackmore, 2001).

Besides, Blackmore (2001) also explained that metal concentrations in molluscs were slightly higher in individuals collected from a sheltered shore which indicated that metal bioavailabilities were elevated in such a place when compared with the exposed shore. This also suggested that habitat played an important role in affecting metal concentrations in a mollusc which is dependent on the food availability in its living habitat.

Variations of metal concentrations in molluscs could also be observed in different locations, such as polluted and non-polluted sites. In Malaysia, a study on the different soft tissues of *Per. viridis* from the coastal waters of Peninsular Malaysia, namely the crystalline style and byssus, showed a significance different of Ni concentrations between the contaminated and uncontaminated samples (Yap *et al.*, 2006a). A few more studies were conducted in the Johore Straits (Yap *et al.*, 2006b) and Kuala Juru (Ibrahim and Mat, 1995) which also resulted in similar findings.

2.3 Heavy Metals in Molluscs: Food Safety Concern

Most marine molluscs provide a cheap source of protein for consumers. From the nutritional point of view, the marine molluscs is an important food source for supplying essential trace metals such as Ca and Fe and certain vitamins such as