UNIVERSITI PUTRA MALAYSIA

HEAVY METALS (ZN, CU PB AND CD) CONCENTRATION IN MERETRIX MERETRIX OF BINTULU WATERS

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HEAVY METALS (ZN, CU PB AND CD) CONCENTRATION IN MERETRIX MERETRIX OF BINTULU WATERS

By

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A Project Report Submitted in Partial Fulfilment of the Requirement for the Degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences Universiti Putra Malaysia Bintulu Sarawak Campus

2008

DEDICATION

I dedicate this final year research project to my mother and my father, Mrs. Rosanani bt. Mohamed and Mr. Azizan bin Ab. Aziz. All your hard work will not be forgotten. May all of you be blessed by Allah.

ABSTRACT

Mussels (*Meretrix meretrix*) and sediment samples collected in Tanjung Kidurong beach of Bintulu from September to November 2007 were analyzed for Zn, Cu, Pb and Cd using Atomic Absorption Spectrophotometer (AAS). The highest heavy metal concentration in *Meretrix meretrix* was Zn with mean value of 52.29 µg/g, followed by Cu, Pb and Cd with mean concentration values of 5.54 µg/g, 5.43 µg/g and 1.59 µg/g respectively. Zinc showed the highest concentration with a mean value with 51.30 µg/g in smaller *Meretrix meretrix* (20-40 mm) followed by (Pb) 6.40 µg/g, (Cu) 4.83 µg/g and (Cd) 1.66 µg/g. *Meretrix meretrix* sizes ranged between 41 – 80 mm showed heavy metals (Zn, Cu, Pb and Cd) concentration with mean value of 53.29 µg/g, 6.25 µg/g, 4.47 µg/g and 1.51 µg/g respectively. The highest concentration of heavy metal in the sediment recorded was Zn with mean value of 33.04 µg/g followed by (Pb) 20.12 µg/g, (Cu) 3.87 µg/g and (Cd) 3.47 µg/g. Since all the concentrations of the heavy metals (Zn, Cu, Pb dan Cd) in the flesh of *Meretrix meretrix* collected from the Tanjung Kidurong were still within the permissible limits set by most countries, this indicated that the consumption of mussels from Tanjung Kidurong is unlikely to cause health problems in the short-term or if they are consumed in small amount.

ABSTRAK

Sampel *Meretrix meretrix* dan sediment yang dikumpul dari Pantai Tanjung Kidurung, Bintulu pada bulan September hingga November 2007 dianalisis untuk mengesan kehadiran Zn, Cu, Pb dan Cd menggunakan 'Atomic Absorption Spectrophotometer' (AAS). Zink merekodkan kepekatan logam berat tertinggi dengan jumlah min 52.29 µg/g. Ini diikuti oleh Cu, Pb dan Cd dengan kepekatan min masing – masing 5.54 µg/g, 5.43 µg/g dan 1.59 µg/g. *Meretrix meretrix* bersaiz dalam lingkungan 21 – 40 mm menunjukkan kepekatan logam berat (Zn, Pb Cu, dan Cd) dengan nilai min masing – masing 51.30 µg/g, 6.40 µg/g, 4.83 µg/g dan 1.65 µg/g. *Meretrix meretrix* bersaiz dalam lingkungan 41–80 mm menunjukkan kepekatan logam berat (Zn, Cu, Pb dan Cd) dengan nilai min masing – masing 53.29 µg/g, 6.52 µg/g, 4.47 µg/g dan 1.51 µg/g. Bagi sampel sedimen, zink merekodkan kepekatan tertinggi dengan nilai min 33.04 µg/g diikuti oleh Pb, Cu dan Cd dengan nilai min masing – masing 20.12 µg/g, 3.87µg/g dan 3.47µg/g. Kepekatan logam berat (Zn, Cu, Pb dan Cd) yang terdapat dalam daging *Meretrix meretrix* masih di bawah takat dibenarkan yang disediakan oleh kebanyakan negara dan ini menunjukkan dengan jumlah pengambilan yang sedikit atau untuk jangkamasa yang pendek ia tidak memberi kesan kepada kesihatan.

ACKNOWLEDGEMENTS

Alhamdulillah, thanks to Allah S.W.T for giving me the opportunity to complete this final year research project. First of all, I would like to express my gratitude to my thesis supervisor, Mr. Mohd Saufi Mohd Ramli and for his guidance, understanding and patience throughout the completion of this thesis. I also would like to thank Mr. Mohd Ikram for his advises and inspiration. Without them this thesis could not be done completely.

Gratitude also goes to my family especially to mak, abah, and angah, all my friends especially to Fatimah, Larry, Iqbal, Deli, Opie, Aajmaly, and also to seniors and staff in the Faculty of Agriculture and Food Sciences for all their kind in helping me completing this thesis, such as Mrs. Raesah, Ms. Ana, Mr. Awang Nizam, Mr. Hamzah, Mr. Meruni, Ms. Elizabeth, Ms. Susie and Mr. Harris.

Grateful to my love and source of inspiration; Norfahiah binti Musa for her sacrifice, patience, understanding and love.

Finally, I also like to thank anyone who helps me along this project being done.

APPROVAL SHEET

I certify that this research project report entitled "Heavy Metals (Zn, Cu, Pb and Cd) concentration of *Meretrix meretrix* in Bintulu" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

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Date: 24-09-2008

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
Cd	cadmium
cm	centimeter
Cr	chromium
Cu	cooper
dw	dry weight
g	gram
g/mol	gram per mol
HCl	hydrochloric acid
Hg	mercury
HNO_3	nitric acid
kg	kilogram
L	liter
mg	miligram
ml	mililiter
NA	Not Available
ppm	part per million
Pb	plumbum
SD	standard deviation
SPSS	Statistical Package for Social Sciences
Zn	zinc
^{o}C	degree Celsius
µg/g	microgram per gram
>	more than
<	less than
%	percentage

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), copper (Cu), and lead (Pb).

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain.

Ingestion is the most common route of exposure in children. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues (Roberts, 1999).

Excessive consumption of metal-contaminated seafood may cause toxicity to humans. Since heavy metals are inorganic chemicals that are non-biodegradable, it cannot be metabolized and will not break down into harmless forms (Kromhout *et al*., 1985).

Heavy metals (e.g. copper, cobalt, lead, mercury, and zinc) are a concern because of their toxicity to estuarine organisms above a threshold availability as they have been implicated in the development of the following biotic disorders; feeding, digestive, and respiratory dysfunctions; aberrant physiological, neurological and reproductive activities; tissue inflammation and degeneration; neoplasm formation; and genetic derangement (Kennish, 1997).

Meretrix meretrix is a filter feeder organism that filters the surrounding water which practically can accumulate heavy metals such as Cd, Cu, Pb and Zn and have the potential as a bioindicator for those metals (Böhlmark, 2003; Wang *et al*., 2005).

In Bintulu, *Meretrix meretrix* is known as *kunau*. It is considered as one of the sources of food and provides small incomes to local inhabitants. Locals eat the edible muscle which also provides an alternative source of protein other than fish and is commonly sold in local markets. Earlier studies of heavy metals concentration in *Mereterix meretrix* tissues and its surrounding sediments in Bintulu showed that the concentration of heavy metals was presumably high (Nurulrashidah, 2006). Therefore it plays an important and interesting health perspective view in terms of heavy metals bioaccumulation along the food chain.

Figure 1.1: Locals search for *Meretrix meretrix* in Tanjung Kidurong beach

Further study is appropriate to access a baseline data regarding the present status of heavy metals in Tanjung Kidurong since there are a lot of industrial base activities that has been running within this vicinity for many years, for example the ASEAN Fertilizer Sdn. Bhd., Austral Edible Oil, and Bintulu PORT Sdn. Bhd. These activities could possibly lead to devastating results due to the release of domestic effluence and waste which in time will result to contamination problems. This information is hope to serve as a guideline and database on the heavy metals concentrations in *Meretrix meretrix* and whether the concentration is still within the guidelines of the Permissible Limits of food regulations. Thus, the objectives of study are:

1.2 Objective

- To determine Zn, Cu, Pb and Cd concentration in the flesh of *Meretrix meretrix* from Tanjung Kidurong beach.
- To determine Zn, Cu, Pb and Cd concentration level in the flesh of *Meretrix meretrix* according to two different sizes.
- To compare of present data with reported studies in the literature and to determine if the mussels are safe for human consumption by comparing with safety guideline.
- To determine Zn, Cu, Pb, and Cd concentration in sediments surrounding *Meretrix meretix*.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy metals

Heavy metals are the group name for metals or metalloid which is related to the toxicity and pollution. According to Nieboer and Richardson (1980), heavy metals are the elements that have density of more than 4 or 5 gcm^{-3} excluding Lactanide and Actanide.

Most metals are generally concentrated several times over within an organism's soft tissue, rather than the shell. Vast majority of studies also concentrate on the soft tissue. However, a few studies of the shell material have also been conducted, and many authors propose that shells can provide a more accurate indication of environmental alteration and pollution; they exhibit less variability than the living organism's tissue, and they provide a historical record of metal content throughout the organism's lifetime, with this record still preserved after death (Thorn *et al*., 1995; Huanxin *et al*., 2000; Yasoshima and Takano, 2001).

Some metals, such as Cd and Pb, have long been known to accumulate within the aquatic food chain. Since Cd, Cu, Pb and Zn are widely distributed in the coastal environment, both from natural geological processes and anthropogenic activities; this is of much interest to public health, since the metals are readily accumulated in the soft tissues of *Meretrix meretrix* (Nurulrashidah, 2006). Goldberg (1975) mention that mussels are well known to accumulate a wide range of contaminants in their soft tissues.

Pb, Zn, Cd and Cu are the parameters that will be measured in this study. According to Nriagu (1979) and Adriano (1986), these 4 elements are the most toxic and frequently being observed by earlier studies.

2.1.1 Zinc

Zinc is an essential trace element for all forms of life. The significance of zinc in human nutrition and public health was recognized relatively recently. Zinc insufficiency has been recognized by a number of experts as an important public health issue, especially in developing countries (Prasad, 1998).

Zinc plays an important role in the structure of proteins and cell membranes. For example, copper provides the catalytic activity for the antioxidant enzyme copper-zinc superoxide dismutase (CuZnSOD), while zinc plays a critical structural role (King *et al.*, 1999). The structure and function of cell membranes are also affected by zinc. O'Dell (2000) show that loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function.

Zinc also plays a role in cell signaling and has been found to influence hormone release and nerve impulse transmission. Recently zinc has been found to play a role in apoptosis (gene-directed cell death), a critical cellular regulatory process with implications for growth and development, as well as a number of chronic diseases (Truong *et al.*, 2000)

King *et al*. (1999) found that the zinc in whole grain products and plant proteins is less bio-available due to their relatively high content of phytic acid, a compound that inhibits zinc absorption. The enzymatic action of yeast reduces the level of phytic acid in foods. Therefore, leavened whole grain breads have more bio-available zinc than unleavened whole grain breads.

Zinc toxicity can cause a lot of frazzle to our body. Isolated outbreaks of acute zinc toxicity have occurred as a result of the consumption of food or beverages contaminated with zinc released from galvanized containers. Signs of acute zinc toxicity are abdominal pain, diarrhea, nausea, and vomiting. Profuse sweating, weakness, and rapid breathing may develop within 8 hours of zinc oxide inhalation and persist 12-24 hours after exposure is terminated (King *et al*., 1999).

2.1.2 Copper

Copper is a chemical element in the periodic table that has atomic number 29. It is a ductile metal with excellent electrical conductivity, and finds extensive use as an electrical conductor, heat conductor, as a building material, and as a component of various alloys.

Copper is an essential nutrient to all high plants and animals. In animals, including humans, it is found primarily in the bloodstream, as a co-factor in various enzymes, and in copper-based pigments. In sufficient amounts, copper can be poisonous and even fatal to organisms.

Perwak *et al.* (1980) mentioned that much of the copper that enters the environmental waters will be associated with particulate matter. Copper is a natural constituent of soil and will be transported into streams and waterways in runoff either due to natural weathering or anthropogenic soil disturbances. Sixty-eight percent of releases of copper to water is estimated to derive from these processes. Copper sulfate use represents 13% of releases to water and urban runoff contributes 2%.

Copper binds primarily to organic matter in estuarine sediment, unless the sediment is low in organic matter content. A study evaluated the importance of the absorption properties of different nonlithogenic components of aerobic estuarine sediment to copper binding by determining copper's adsorptivity to model components (phases) in artificial seawater (Davies-Colley *et al.*, 1984).

Giusti *et al.* (1993) provided estimates of global anthropogenic and natural copper inputs into oceans that are derived from two sources, atmospheric deposition and riverine input.

Cu can affect human health as been stated by Eisler (1997) that in a low level of Cu can result to anemia, gastrointestinal disturbances, bone development abnormalities, and death. However, according to Sellers *et al.* (1975) at higher levels in fish, copper can cause sublethal effects on respiration, osmoregulation (Reid and McDonald, 1988), behavior, growth, and metabolism (Eisler, 1997), and death (Handy, 1993).

2.1.3 Plumbum

Lead is a naturally occurring bluish-gray metal found in small amounts in the earth's crust, with strong metallic lust when newly cut but which tarnishes on exposure to moist air. Lead is very soft, may be drawn to wire or rolled to sheet, has little tenacity. Its melts at low heat.

Underwood (1986) found that lead has no beneficial or desirable nutritional effects in humans, and all known effects of lead on biological systems are deleterious. Lead is widely used, resulting in discharges to the environment.

According to Liston and Maher (1986), lead enters the marine environment via industrial discharges, non-ferrous metal smelters, and highway run-off and sewage effluent. Urban runoff constitutes an important non-point source of trace metals to aquatic ecosystems. Other pathways include wet deposition and dry deposition of atmospheric lead. Nriagu, (1978) state that volcanic emanations from active oceanic ridges also enrich the sediments with lead.

Lead has a short half-life in seawater. It undergoes adsorption, desorption, precipitation, dissolution and biological incorporation, which reduce its concentration in the seawater.

Lead is also strongly associated with ferromanganese oxides in estuaries and oceans (Nriagu, 1978). Hydrous ferric and manganese oxide becomes soluble and bound lead may be released under anaerobic condition. A study by Windom *et al.* (1988) on the trace metal transport in Bang Pakong Estuary (Thailand) showed that lead was usually detached during estuarine transport.

Ingestion and inhalation are two major routes which lead enter the human body. Absorption of lead through the skin is insignificant. Lead exists as vapors and aerosols in the air. Respiratory absorption of lead depends on the size of the inhaled particles. Inhalation is an important source of lead since its absorption is higher than that of other entry paths. Gastrointestinal absorption involves lead uptake from food and drinking water. About 5 - 10% of the lead ingested is absorbed into the body and the rest is excreted via the faeces (Berman, 1980). Merian (1991) stated that consumption of contaminated food is also an important source.

Long-term exposure to lead is dangerous as it is cumulative in humans. Blood lead levels have been used as a biological index to review the level of lead exposure (Hall, 1972). According to Harrison and Laxen (1981) in humans, lead has a half-life of approximately 20 days in blood and 600 to 3000 days in bone.

2.1.4 Cadmium

According to Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. (1997), cadmium is a soft silver-white metal that is usually found in combination with other elements. Cadmium compounds range in solubility in water from quite soluble to practically insoluble. Chemical symbol for cadmium is Cd and the atomic weight is 112.41 g/mol.

Staessen and Lauwerys (1993) mention that cadmium is a ubiquitous environmental pollutant that the biological functions in humans are unknown. Pinot (2000) stated that, it occurs in nature as a natural component of rock and sediment, soil and dust, air and water, and plant and animal tissues.

Pinot *et al.* (2000) also mention anthropogenic cadmium resulting from the industrial production of batteries, plastics, alloys, and synthetic materials is released into the environment in the form of atmospheric emissions (70%), liquid effluents, sludge, and solid waste.

Jarup *et al.* (1998) and Staessen *et al.* (1993) state that exposure to cadmium can have both indirect and direct effects on bone loss. Cadmium-induced bone damage may be mediated through the dysfunction of renal tubular cells caused by increased cadmium concentration in the kidneys

There is also evidence that cadmium acts directly on bone tissue. In the past, experimental data indicated that cadmium could affect bone mineralization without clear signs of renal effects (Berglund *et al.*, 2000; Pinot, 2000). Now it is more firmly established that cadmium exposure at levels that have not impaired renal function can target bone directly, causing early bone loss by decreasing bone formation and increasing bone resorption (Wilson and Bhattacharyya, 1997). The concentration of cadmium for the long term is much dangerous for health.

2.2 Biomonitoring

According to Rainbow (1995) individual bio-monitors respond differently to different sources of bio-available metal-for example, in solution, in sediment or in food. He stressed out an ideal bio-monitors should be sedentary, easy to identify, abundant, long lived, available for sampling throughout the year, large enough to provide sufficient tissue for (individual) analysis, resistant to handling stress caused by laboratory studies of metal kinetics and/or field transplantations, tolerant of exposure to environmental variations in physicochemical parameters such as salinity, and net accumulators of the metal in question with a simple correlation between metal concentration in tissues (body) and average ambient bio-available metal concentration over a recent time period. However he also stressed out that not all organisms fulfill these criteria, nor indeed do all species commonly employed as bio-monitors fulfill all the criteria and most critically, a bio-monitor should be a net accumulator of the relevant metal, preferably a strong net accumulator thus minimizing any danger of significant contamination of a sample during laboratory handling.

Therefore according to Rainbow (1995) knowledge of the biology of the infaunal animal of choice is clearly a prerequisite to an understanding of its position in a suite of bio-monitors. He explains that a key feature to the choice of bio-monitors in any suite is a knowledge of their biology, for example method of feeding, extent of production of respiratory or irrigatory currents, life history and breeding season, length of life, age structure of population, etc. He further explains that an understanding of such aspects of the biology of bio-monitors allows the identification of a source of metal contamination.

2.3 Bioconcentration factor

Concentration factors (CF) for various metals in biological materials have been determined by numerous authors (Goldberg, 1965; Bojanowski, 1973; Cherry and Shannon, 1974). The values of concentration factor relate the concentration of metal in the organisms, which may have been derived by uptake from water, suspended matter, sediments and food, to that of the surrounding environment. Knowledge of concentration factor values makes it possible to estimate the relative ability of an organism to concentrate metals from the medium in which it lives (Szefer, 1991).

2.4 The taxonomy of *Meretrix meretrix*

The taxonomy *Meretrix meretrix* as cited by Yokoyama (1926) from Linnaeus (1758) are as follows:

Kingdom : Animalia

Phylum : Mollusca

Class : Bivalvia

Subclass : Heterodonta

Super family : Veneroida

Family : Veneroidea

Genus : *Meretrix*

Species : *meretrix*

Figure 2.1: *Meretrix meretrix*

2.5 Morphology and Biology of *Meretrix meretrix*

Meretrix meretrix is colourful with a smooth shell. The shell is thin and the outer sides of the shell have polar colours which start from white to brown and darker brown. The inner shells are white in colour.

Vo (2000) stated that, according to the biological characteristic, the mature *Meretrix meretrix* that can be catch is around 34-35 mm which it have reproduce for the first time. *Meretrix meretrix* have an infauna characteristic that embeds itself below the muddy area while the siphon is being used as a feeder (Rudi, 2000). According to Böhlmark (2003)

Meretrix meretrix is filter feeders that accumulate heavy metal and are therefore good indicators of contamination. In previous study, Wang *et al*. (2005) mention that *Meretrix meretrix* can practically accumulate heavy metals such as Cd, Cu, Pb and Zn and have potential as bioindicator for those metals.

2.6 Habitat Distribution

According to Carpenter and Neim (1998), *Meretrix meretrix* usually found in intertidal and sublitoral zone that is normally contain the muddy and sand substrate, which is around the West Indo-Pacific. Normally it can be found near the estuary with a sloping topography within 20 meter depth.

There are several factors that contribute to the distribution of this species which are the temperature, depth, conductivity, dissolve oxygen (DO), salinity, pH and the texture of the substrate. Table below explain the physical and chemistry factor in Teluk Miskam Panimbang, which it prove that this factors contribute the distribution of *Meretrix meretrix*.

Table 2.1: The Physical and Chemistry Distribution Factor of *Meretrix meretrix* in Teluk Miskam Panimbang (Rudi, 2000).

No.	Parameter	
1	Temperature (°C)	28-30
2	DO (mg/L)	4.76-5.95
3	salinity (%)	10-32
4	pH	6-7
5	Substrate Texture	Muddy, sandy

Rudi (2000) also state that *Meretrix meretrix* love the smooth texture substrate (high contain of dust and mud). This is because the species have an infauna characteristic that embeds itself below the muddy area while the siphon is being used as a feeder.

Meretrix meretrix was chosen for four reasons. *Meretrix meretrix* is more accessible in the region and also bigger in size, facilitating collection and analysis (minimum dry weight 0.2 g). Its size is up to 90 mm in shell length and it is easily distinguished from other species. There are earlier studies on this species and other *Meretrix* species (*M. lusoria*); (Hung *et al*., 2000).

CHAPTER 3

METHODOLOGY

3.1 Study area

Tanjung Kidurong lagoon was selected as a study area. The location area chosen have potential pollution risks. It is likely to be exposed to pollution because there are a great amount of activities and development going on within the area.

Figure below shows the sampling area.

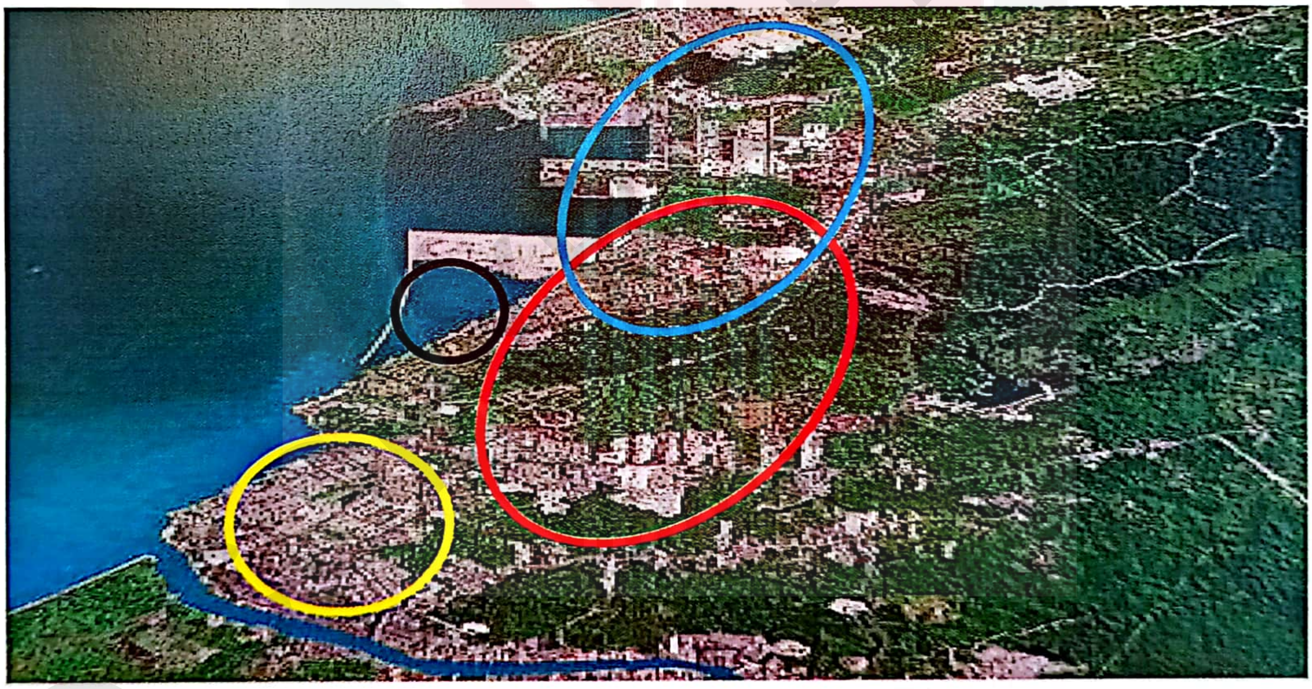


Figure 3.1: Location of sampling site, Tanjung Kidurong beach, Bintulu.

Black circle: Sampling Area　　Blue circle: Industrial Area

Red circle: Residential Area　　Yellow circle: Town Area

3.2 Period of study and sample collection

Studies were conducted in three consecutive months starting from September to November 2007. Two main samples that were randomly collected along 100 meter transect line from Tanjung Kidurong beach; *Meretrix meretrix* and sediments. The physicochemical characteristic of the surrounding water conditions was also be measured at the site (in-situ). Mussels were randomly taken by excavating directly into the sediments along the Tanjung Kidurong beach using a small hoe or hand shovel and placed in to plastic bag. Than the sample were taken back to the lab and stored in fridge.

3.3 Materials and methods

3.3.1 Acid wash

Firstly, equipment was washed using detergent to wash away any leftover stains and residues from previous digestion. Then the equipment was rinsed with distilled water and soaked in HCL 5% for at least 24 hours. After that, the equipment was washed and rinsed using distilled water and eventually dried in the oven at 60 – 80°C until dried.

3.3.2 Acid digestion preparation (sediment and tissue)

3.3.2.1 Sediment samples

In the laboratory, sediments were placed on aluminum foils and dried in the oven for 60˚C for at least 72 hours until a constant dry weight. Later, the dried sediments were crushed by using a pestle and mortal into powder form and sifted through a 63 µm stainless steel aperture. To produce homogeneity, the sieve was shaken vigorously (Yap *et al.*, 2002). Then 0.5-1 gram of dried sieved homogenized sediments was weighed and put into digestion tubes. The dried sample then was digested in a combination of 10 ml concentrated HNO_3 (AnalarR grade; BDH 69%) and $HClO_4$ (AnalarR grade; BDH 60%) in the ratio 4:1. Then the sediment samples was put in a hot-block digester at low temperature (40°C) for 1 hour and then fully digested at 140 °C for at least 3 hours (Yap *et al.*, 2002). After that, it was diluted until 40 ml with distilled water. After dilution, the samples were filtered through Whatman No. 1 filter paper using a funnel into the acid-washed pill box. Then metal contents in the prepared samples were determined using an air-acetylene flame atomic adsorption spectrophotometer (AAS).

3.3.2.2 Flesh

Meretrix meretrix obtained from sampling site was rinsed with distilled water to remove any excess contamination. The flesh of *Meretrix meretrix* was carefully removed from its shell. The flesh was placed in aluminum foil and dried in the oven at 60-80°C until constant weight was gained. About 0.5-1 g of dried soft tissues were weighed and placed in acid washed test digestion tubes. Three replicates of each flesh tissue was digested in 5 ml concentrated nitric acid (BDH; 69%) (Yap *et al.*, 2003). The digestion procedure is the same as the sediment digestion procedure. Digestion is considered finish when the last solution is cleared and brownish fume no longer be released from digestion tubes (Shibli, 2003). Then the sample was diluted with double distilled water until 20 ml. After dilution, the samples were filtered through Whatman No. 1 filter paper using a funnel into the acid-washed pill box (Nurulrashidah, 2006).

3.3.3 Determination of In-Situ Water Physicochemical Parameter

Water parameters from surrounding environments such as pH, salinity, temperature, and dissolve oxygen also were measured using hydrolab equipment. A total of five readings (N=5) for each physicochemical parameter is taken in-situ at each of the sampling locations.

3.3.4 Determination of Heavy Metals (Zn, Cu, Pb and Cd)

In this study, Zn (Zinc), Cu (Copper), Pb (Plumbum), and Cd (Cadmium) are analyzed. Determination of heavy metals concentration from prepared samples of acid digestion is done by using the air-acetylene flame ASS, an inorganic analytic instrument designed by Perkin-ElmerTM, model AAnalyst800.

Standard working solutions was prepared from 1000mg/L stock solution supplied by Merck Titrisol for each metals studied for AAS calibration. The concentration of metal was done by multiplying the readings obtained from AAS with the dilution factor and was divided with sample weight (Shibli, 2003). The formula is shown as below:

$$\underset{(\mu g/g)}{\text{Heavy metal concentration in gram sample}} = \frac{C \times D.F}{W}$$

Where,

C = Concentration from AAS in (mg/L)

D.F = Dilution factor (total volume of sample solution) (ml)

W = Sample mass (dry weight) (g)

3.3.5 Working chart flow

The chart flow below illustrates in general the working procedure involved during lab work analysis Yap *et al.* (2003):

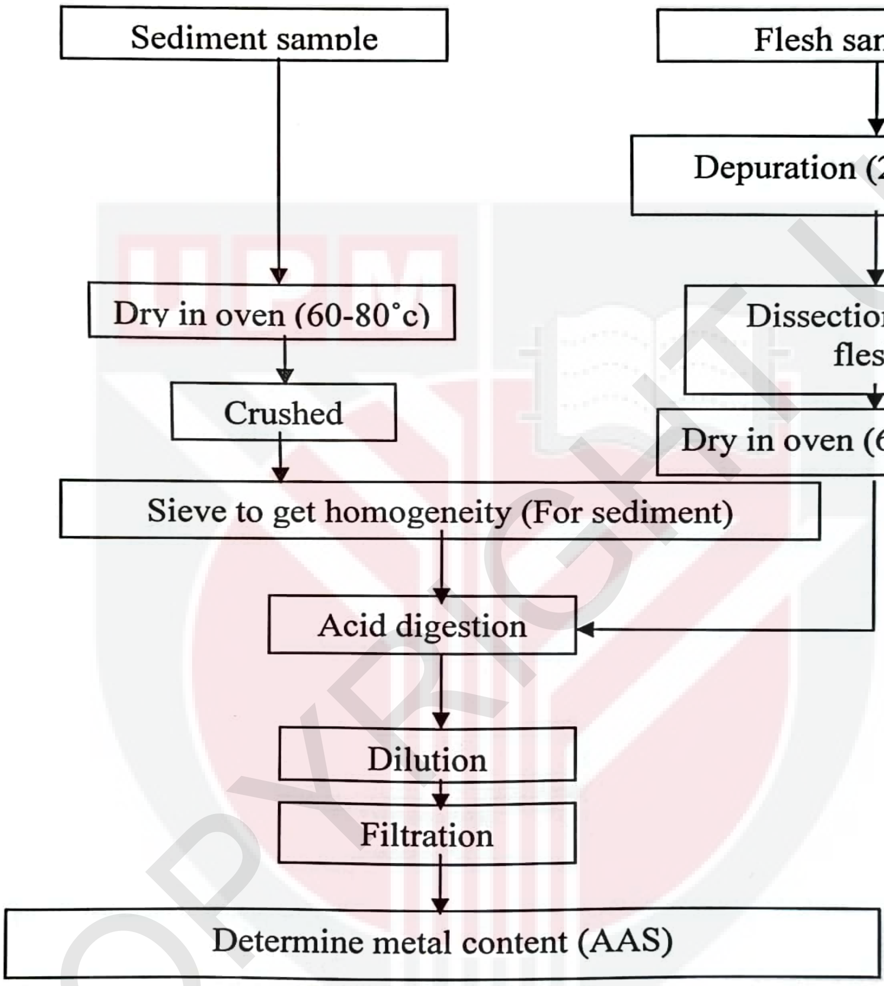


Figure 3.2: Schematic diagram of methodology

3.3.6 Statistical analysis

Independent T-test and one way ANOVA was used to find the concentration differences of heavy metals in *Meretrix meretrix* according size and concentration difference of heavy metals in *Meretrix meretrix* according to temporal basis. Statistical software, SPSS 14.0 for windows was used for data analytical purposes.

CHAPTER 4

RESULTS

4.1 Zn, Cu, Pb and Cd concentration in flesh of *Meretrix meretrix*.

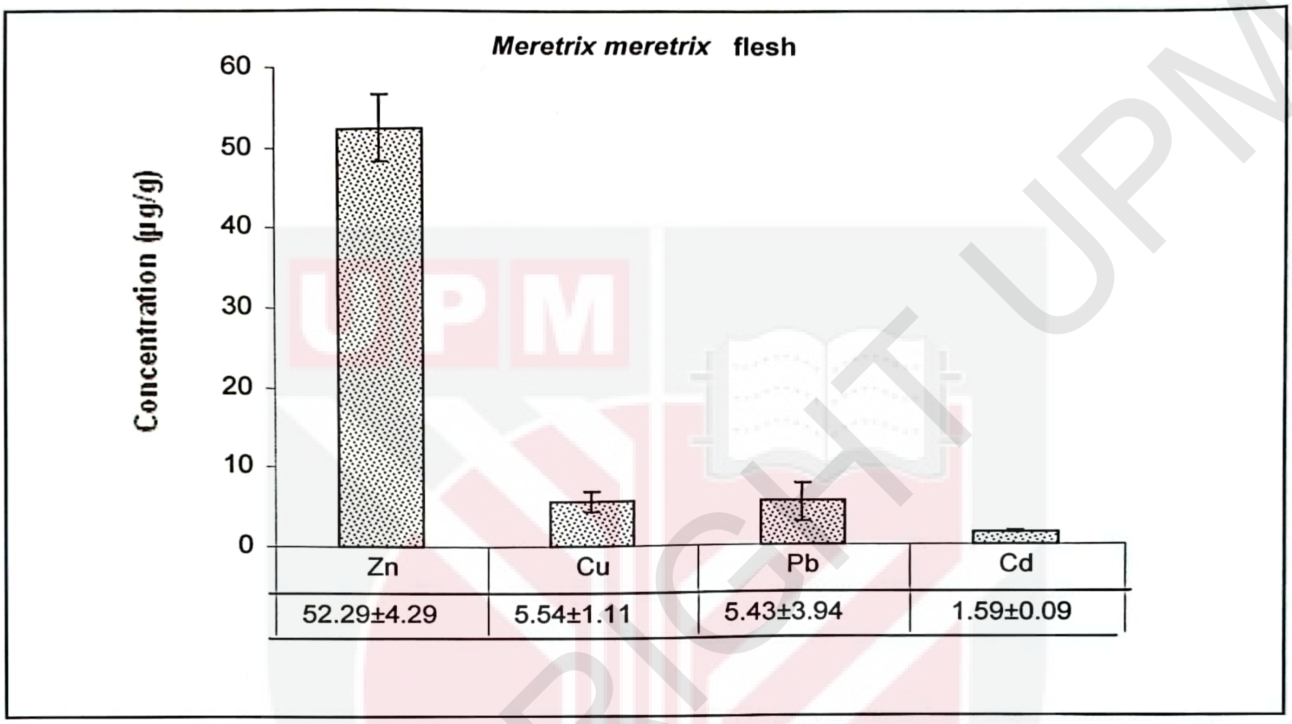


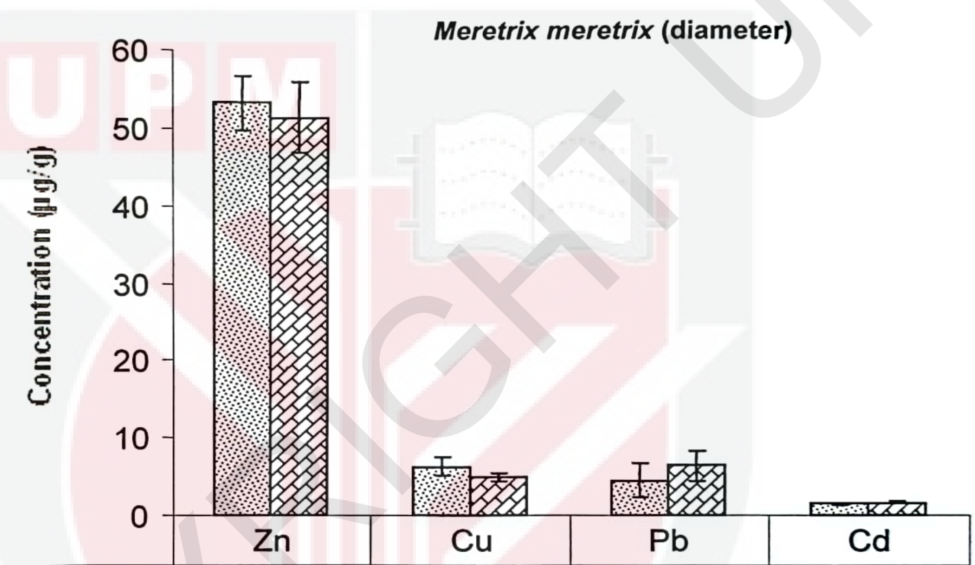
Figure 4.1: Heavy metals (Zn, Cu, Pb and Cd) concentration (Mean ± SD) in *Meretrix meretrix* flesh samples taken from Tanjung Kidurong Beach (September – November) (N = 24) (20 – 80 mm).

The mean heavy metals concentration of Zn, Cu, Pb and Cd in *Meretrix meretrix* flesh taken from Tanjung Kidurong beach is presented in figure 4.1. The highest heavy metal concentration with mean value of 52.29 µg/g was reported for Zn. This was followed by Cu, Pb and Cd which reported the mean concentration value of 5.54 µg/g, 5.43 µg/g and 1.59 µg/g respectively.

The descending order of heavy metal accumulation in *Meretrix meretrix* flesh was as follow:

Meretrix meretrix: Zn > Cu > Pb > Cd

4.2 Zn, Cu, Pb and Cd concentration in flesh of *Meretrix meretrix* according diameter (20 – 40 mm) and (41 – 80 mm).



	Zn	Cu	Pb	Cd
Meretrix meretrix (41-80) mm	53.29±3.48	6.25±1.21	4.47±2.28	1.51±0.13
Meretrix meretrix (20-40) mm	51.30±4.52	4.83±0.47	6.40±1.96	1.66±0.08

Figure 4.2: Heavy metals (Zn, Cu, Pb and Cd) concentration (Mean ± SD) in *Meretrix meretrix* according to diameter ranging between (20 – 40 mm) and (41 – 80 mm) sampled in Tanjung Kidurong Beach (September – November).

In other separate study, the heavy metals (Zn, Cu, Pb and Cd) concentration in *Meretrix meretrix* flesh samples were divided into two size diameter ranging between 41 – 80 mm and 20 – 40 mm which is presented in figure 4.2. Heavy metals concentration in flesh of *Meretrix meretrix* measuring between 41 – 80 mm reported the mean value of 53.29

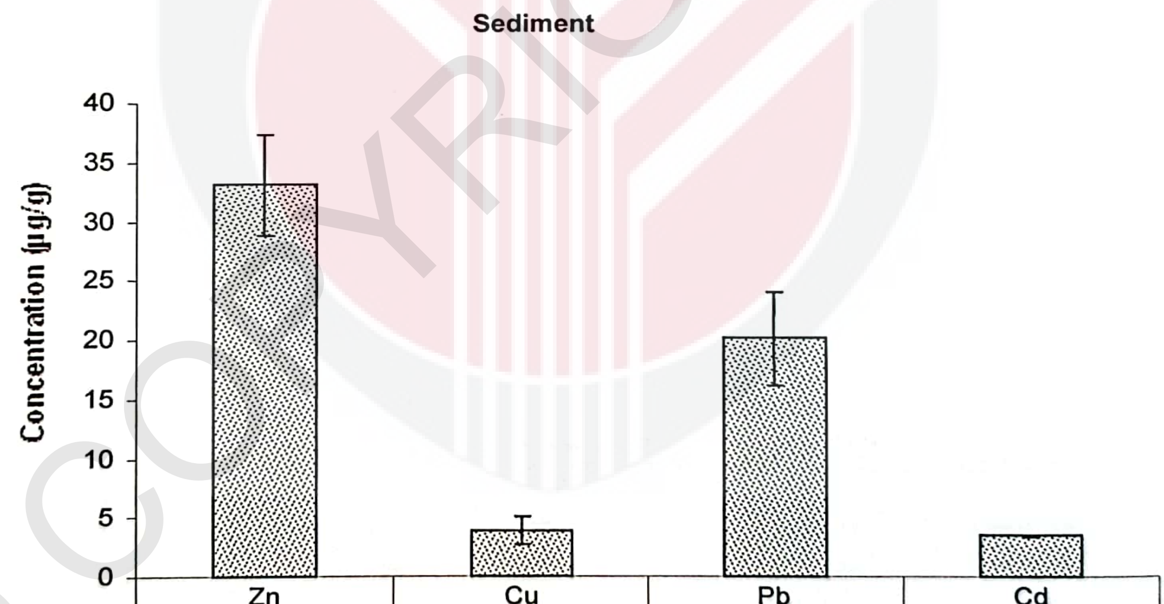
μg/g, 6.25 μg/g, and 4.47 μg/g and1.51 μg/g for Zn, Cu, Pb and Cd respectively. Meanwhile, heavy metals concentration in flesh samples of *Meretrix meretrix* measuring between 20 – 41 mm reported the mean concentration value of 51.30 μg/g, 6.40 μg/g, 4.83, and 1.66 μg/g for Zn, Pb, Cu and Cd respectively.

The descending order of heavy metals accumulation in *Meretrix meretrix* flesh samples according to their diameter was as follow:

Meretrix meretrix (20 – 40 mm): Zn > Cu > Pb > Cd

Meretrix meretrix (41– 80 mm): Zn > Pb > Cu > Cd

4.3 Zn, Cu, Pb and Cd concentration in sediments of Tanjung Kidurong beach.



Zn	Cu	Pb	Cd
33.04±4.29	3.87±1.16	20.12±2.30	3.47±0.13

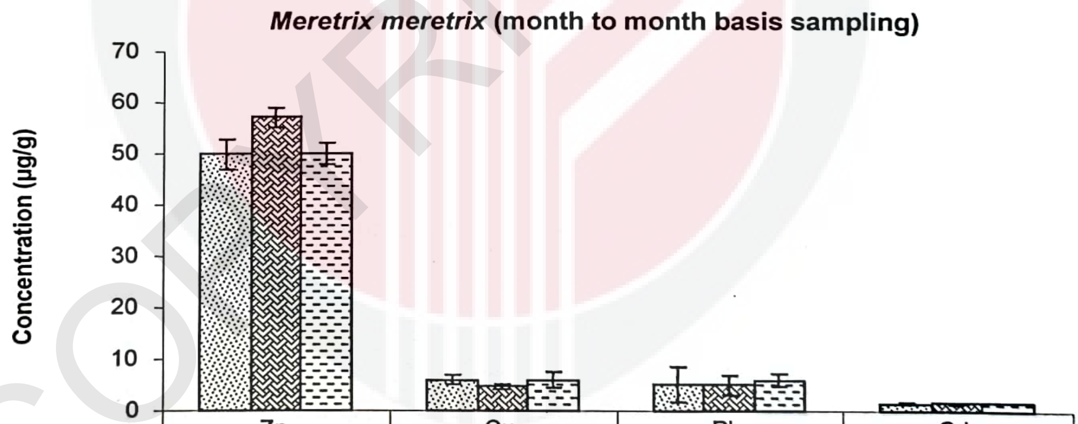
Figure 4.3: Heavy metals (Zn, Cu, Pb and Cd) concentration (Mean ± SD) in sediments samples taken from Tanjung Kidurong Beach (September – November) (N=24)

Figure 4.3 shows the mean heavy metals (Zn, Cu, Pb and Cd) concentration in sediment. Samples taken from Tanjung Kidurong beach. Zinc recorded the highest heavy metals concentration with the mean value of 33.04 μg/g. This was then followed by Pb, Cu and Cd which measured the mean concentration value of 20.12 μg/g. 3.87 μg/g and of 3.47 μg/g respectively.

The heavy metals (Zn, Cu, Pb and Cd) accumulation trend in sediments of Tanjung Kidurong Beach can be arranged as follows:

Sediment: Zn>Pb>Cu>Cd

4.4 Temporal variations (September – November) of heavy metals (Zn, Cu, Pb and Cd) concentration in *Meretrix meretrix*.



	Zn	Cu	Pb	Cd
first sampling	49.83±2.75	5.92±0.81	5.24±3.44	1.54±0.18
second sampling	57.07±1.80	4.71±0.63	5.05±1.77	1.58±0.08
third sampling	49.99±2.12	6.00±1.45	6.021.32	1.65±0.09

Figure 4.4: Heavy metals (Zn, Cu, Pb and Cd) concentration (Mean ± SD) in *Meretrix meretrix* samples taken from Tanjung Kidurong Beach (September – November) according month to month basis sampling.

Heavy metal concentration mean in *Meretrix meretrix* according month to month basis showed in figure 4.4. The mean heavy metals concentration in the sediment taken from September – November range 49.83 µg/g – 57.07 µg/g for Zn, 4.71 µg/g – 6.00 µg/g for Cu, 5.04 µg/g – 6.02 µg/g for Pb and 1.54 µg/g – 1.65 µg/g for Cd.

4.5 Water physichochemical parameter and substrate texture in sampling area (Tanjung Kidurong Beach)

Table 4.1: Water physicochemical parameter and substrate texture at sampling site (Tanjung Kidurong Beach).

No.	Parameter	
1	Temperature (°C)	29
2	DO (mg/L)	5.48
3	Salinity (%)	22%
4	pH	10.23
5	Substrate Texture	Muddy, sandy

Table 4.1 shows water physicochemical parameter and substrate texture at sampling site (Tanjung Kidurong) recorded temperature with 29°C, DO with 5.48 mg/L, salinity with 22% and pH with 10.23.

CHAPTER 5

DISCUSSION

5.1 Heavy metals (Zn, Cu, Pb and Cd) concentration in flesh of *Meretrix meretrix* in Tanjung Kidurong.

Bintulu that going to be a develop area in the state of Sarawak is known for its high shipping activities associated with the presence of domestic port, commercial agriculture such as oil palm industries, and other heavy industrial activities related, which may in term also contribute to these sources of heavy metals. As stated by Macfarlane and Burchett (2001), pollutants, ultimately derived from a growing number of diverse anthropogenic sources such as industrial effluents and wastes, urban runoff, sewage treatment plants, boating activities, agricultural fungicide runoff, domestic garbage dumps, and mining operations, have progressively affected more and more different ecosystems. According to Guns *et al.* (1999), amongst these human activities have caused lead (Pb), zinc (Zn), copper (Cu), cadmium (Cd), and others released into the environment in high amount, higher than the natural release, bringing the toxic effects to living organisms. Heavy metal also can get their way into aquatic systems, rivers, lakes or oceans through atmospheric fallout, dumping wastes, accidental leaks, runoff of terrestrial systems industrial, domestic effluents and geological weathering (Eisler, 1981). Other than that it can also be assumed that the presence of heavy metals concentration in *Meretrix meretrix* may have also derived from natural inputs from the environment such as weathering.

The descending orders of heavy metal accumulation in *Meretrix meretrix* flesh were as follow:

Meretrix meretrix: Zn > Cu > Pb > Cd

The concentration of Zn was the highest followed by Cu, Pb and Cd. This shows Zn and Cu is an essential nutrient that plays an important role in the structure of proteins and cell membranes. Zn and Cu was an essential metal involved in a wide variety of metabolic functions (Hamer, 1980; Páez-Osuna *et al*., 1995). Cu, is an important and essential microelement acting as a respiratory pigment of marine invertebrates and which could be metabolically regulated by mollusks (Förstner and Wittmann, 1983) while Zn is an essential nutrient that plays an important role in the structure of proteins and cell membranes (O'Dell, 2000).

However the high uptake of Zn in *Meretrix meretrix* studied may have come from oil palm plantations and industrial activities, such as the effluent from palm oil factories which may contribute to the high Zn presence in this area (Bintulu coastal area) as stated by Gurmit *et al.* (1999) about the high presence of these metals in POME (Palm Oil Mill Effluent) released from growing oil palm activities.

5.2 Heavy metals (Zn, Cu, Pb and Cd) concentration in flesh of *Meretrix meretrix* according to the diameter sizes.

Table 5.1: Mean concentrations (μg/g dry weight ±S.D) of heavy metals (Zn, Cu, Pb and Cd) in different diameter of sizes of *Meretrix meretrix.*

Metals	Diameter: 41 – 80 mm	Diameter: 20 – 40 mm
Zn	$53.29^{a}\pm3.48$	$51.30^{a}\pm4.52$
Cu	$6.25^{a}\pm1.21$	$4.83^{b}\pm0.47$
Pb	$4.47^{a}\pm2.28$	$6.40^{b}\pm1.96$
Cd	$1.51^{a}\pm0.13$	$1.66^{b}\pm0.08$

Means with same superscript in the different column means no significant difference at $p<0.05$

Lobel *et al.* (1991) and Yap *et al.* (2003) found that body size of mollusc is important factors which affect the concentration of heavy metals. Through the rates of uptake and excretion, the body size factor can be determined in order to know it can affect the concentration of heavy metals in mollusc (Phillips *et al.*, 1993). Table 5.1 showed the comparison of mean between both sizes. The mean concentration for both sizes were significantly different ($P<0.05$) for Cu, Pb and Cd with exception of Zn.

The new tissues also could be formed at a greater rate than metals transported into the tissues to establish a steady state concentration (Vinikour *et al.*, 1980) meanwhile Otchere (2003) state that in bioaccumulation in bivalves, the uptake of the metal is higher in the smaller individual than in the larger ones generally resulting in "growth dilution" effect. This study shows a lower concentration of metals (Pb and Cd) in bigger size *Meretrix meretrix.* Although, bigger size of *Meretrix meretrix* accumulates more Cu because, Cu is an essential metal involved in a wide variety of metabolic functions

(Hamer, 1980; Páez-Osuna *et al.*, 1995), so it is necessary for *Meretrix meretrix* accumulate Cu for its growing.

5.3 Comparison of heavy metal concentration in *Meretrix meretrix* with other related bivalve studies.

Table 5.2: Comparison of reported concentration (μg/gdw) of Zn, Cu, Pb and Cd in bivalve from various regional studies with the present result and those from other studies done

Studies	Zn	Cu	Pb	Cd	References
***Perna viridis*, west coast of Peninsular Malaysia**	75.1 - 129	7.76 - 20.1	2.51 - 8.76	0.68 - 1.25	Yap *et al.*,(2003)
***Perna perna*, Rio de Janeiro, Brazil**	44	6.1	0.4	0.3	Ferreira *et al.*, 2004
***Meretrix meretrix*, Maputo bay, Mozambique**	127	212	0.23	0.79	Böhlmark, 2003
***Meretrix meretrix*, T.kidurong beach Malaysia**	46.15-85.11	NA	6.6-30.42	0.48-1.23	Nurulrashidah, 2006
***Meretrix meretrix*, T.kidurong beach Malaysia**	52.29	5.54	5.43	1.59	This study

NA=not available

The comparisons of heavy metal concentration in the flesh of *Meretrix meretrix* with other studies are showed in Table 5.2. All the data of heavy metal from current study showed slightly higher than the previous study conducted by Yap *et al.* (2003) and Ferreira *et al.* (2004). Different area of study gave different concentration of heavy metals. It attributed mainly to the geographical condition and localizes input of anthropogenic activities. According to Böhlmark (2003) in Maputo bay, Mozambique showed a rapid urbanization and the industrial pressure on the coastal areas is continually increasing. Industries such as fisheries, maritime cultivation, transport and tourism together with deficient wastewater treatment are a threat to the marine life (Böhlmark, 2003).

The metal uptake also may differ in relation to ecological needs, metabolism, and the contamination gradients of water, food, and sediment, as well as other factors such as salinity, temperature, and interacting agents (Pagenkopf, 1983; Goyer, 1991; Canli and Furness, 1995).

5.4 Standards and guidelines for human consumption

One of the objectives of this study was to compare the concentrations of heavy metals in *Meretrix meretrix* with safety guideline. The heavy metals concentration levels are extremely important issue with relations to human consumption. This is due to the possibility of high heavy metals concentration concentrated within the flesh of *Meretirx meretrix* studied. High concentration of heavy metals in the edible flesh may pose a threat to humans if consumption exceeds threshold levels.

Referring to permissible limit set in Malaysian Food Regulation (1985), the concentration of heavy metals for *Meretrix meretrix* was still lower except for Pb and Cd. The limits for

Zn, Cu, Pb and Cd were 100 µg/g, 40.00 µg/g, 2.00 µg/g and 1.00 µg/g respectively. However the limits provided by the Ministry of Health Malaysia (2002) were presented as wet weight basis, whereas this study was presented in dry weight basis. So therefore there is a possibility that the concentration levels in Pb and Cd for this study are still within the permissible limits. Conversion from dry weight to wet weight basis is needed to clarify and justify this founding and statement.

Table 5.3: Guidelines on heavy metals for food safety set by different countries.

Location	WB	Zn (µg/g)	Cu (µg/g)	Pb (µg/g)	Cd (µg/g)
1.Permissible limits set by Malaysian Food Regulation (1985)	Wet	100	30	2.00	1.00
3.International Council for the Exploration of the Sea (ICES, 1988) for status: 'increased contamination'	Dry	NA	NA	3.00	1.80
4.Maximum permissible levels established by Brazilian Ministry of Health (ABIA, 1991)	Dry	250.00	150.00	10.00	5.00
5.Permissible limit set by Ministry of Public Health, Thailand (MPHT, 1986)	Dry	667.00	133.00	6.67	NA
6.Food and Drug Administration of the United States (USFDA, 1990)	Dry	NA	NA	11.50	25.00
7.Australian Legal Requirements (NHMRC, 1987)	Dry	750.00	350.00	NA	10.00
9.Metal levels of *Meretrix meretrix* from the Tanjung Kidurong Beach (This study)	Dry	52.29	5.54	5.43	1.59

NA=not available

Since all the concentrations of the mussels collected from the Tanjung Kidurong are still within the permissible limits set by most of the countries (dry weight basis)(Table 5.3), this indicated that consumption of mussels from Tanjung Kidurong is unlikely to cause health problem in the short-term period or in small amount of consumption.

5.5 The comparison of heavy metal concentration (Zn, Cu, Pb and Cd) in sediments from other studies

According to Lennart (1996), the sediment is a complex structure with a considerable heterogeneity in its physical, chemical and biological composition. The importance of biological processes for the function of a sediment ecosystem is unequivocal and a large number of sediment processes are, on a superficial level, dependent on biological activity. Sediment is also a matrix of materials, which is comprised of detritus, inorganic and organic particles and is relatively heterogeneous in terms of its physical, chemical and biological characteristics (Sarkar *et al.*, 2004). According to Yap *et al.* (2002), sediment is believed to be the metal repository and only a minor fraction of materials escapes into the coastal waters.

Sediments are known to accumulate pollutants from terrestrial sources and coastal waters (Muller and Forstner, 1975), and can also be used as an indicator to monitor metal pollution in the biosphere and the effects of anthropogenic events in the environment (Guy *et al.,* 1978). It is widely accepted that sediments can be used to monitor metal pollutions in the biosphere and the effects of anthropogenic events in the environment. It is also widely believed that the sediments provide the main sink for heavy metals in the aquatic environment (Guy *et al.*, 1978). This is because metals accumulated by the suspended matter or trapped by the bottom of the sediment (Dauvalter, 1998), that cause by strong attraction from metals and the particles in sediments.

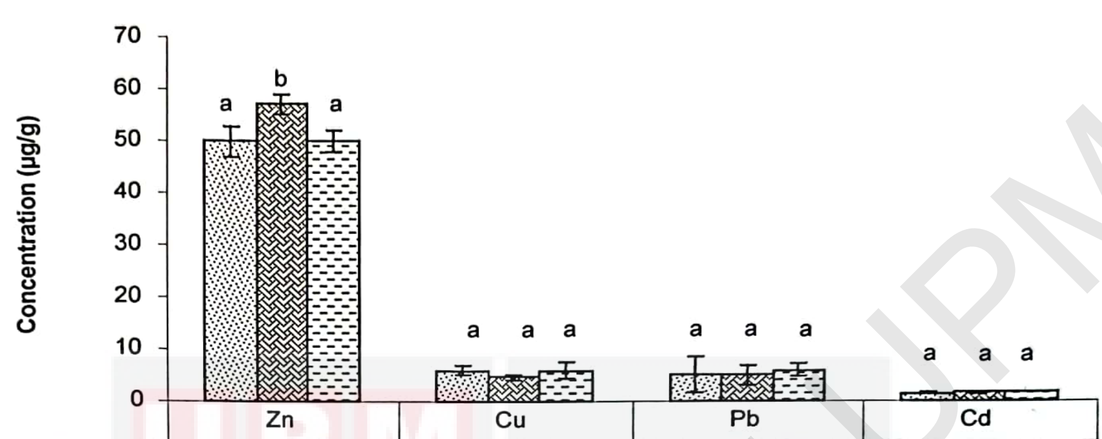
Table 5.4: Comparison of total metal contents (μg/g dw) of marine surface sediment from various geographical regions with the present study.

Area	Zn	Cu	Pb	Cd	References
Johor Straits	68.5-231	NA	26.4-69.9	0.110-0.360	Wood *et al.*, 1997
Coast of Penang, Malaysia.	73-110	9-14	18-44	1-7	Seng *et al.*, 19871
Tanjung Kidurong, Bintulu	39-91	7-13	20-33	1-5	Ismail, 1993
Tanjung Kidurong Beach	23.99-33.61	NA	17.9-39.62	0.75-1.2	Nurulrashidah, 2006
Tanjung Kidurong Beach	33.04	3.87	20.12	3.47	This study

NA=not available

The comparisons of heavy metal concentration in the sediment with other studies are showed in Table 5.4. The ranges for Cd are higher than the previous study reported but the range for Zn, Cu and Pb are lower than the previous study. As a study conducted by Nurulrashidah (2006), the range from current study was slightly lower for Zn. This could be related to the discharge of effluent from the nearby domestic and industrial input. Tanjung Kidurong is near to fisherman quay and some petro-chemical plants and port activity in the vicinity that may also be suspected as anthropogenic sources in the area.

5.6 Temporal variations (September – November) of heavy metals (Zn, Cu, Pb and Cd) concentration in *Meretrix meretrix*.



	Zn	Cu	Pb	Cd
September	49.83±2.75	5.92±0.81	5.24±3.44	1.54±0.18
October	57.07±1.80	4.71±0.63	5.05±1.77	1.58±0.08
November	49.99±2.12	6.00±1.45	6.021.32	1.65±0.09

Figure 5.1: Heavy metals (Zn, Cu, Pb and Cd) concentration (Mean ± SD) in *Meretrix meretrix* samples taken from Tanjung Kidurong Beach (September – November) according month to month basis sampling.

There are no significant temporal variations (P< 0.05) were registered in *Meretrix meretrix* (Figure 5.1) in all heavy metal elements, except for Zn. This behavior is probably indicating that these elements, that are reaching the coastal environment, are from the same source, and follow a similar distribution pattern. Although, study from Ferreira *et al.* (2004) on *Perna perna* showed significant temporal variation due to their site have humid floodplain areas enriched with organic and vegetable material in different degradation stages with concomitant heavy metal liberation.

5.7 Water physicochemical parameter and substrate texture comparison in sampling area (Tanjung Kidurong Beach) with study from Rudi (2000).

Table 5.5: Water Physicochemical parameter and substrate texture comparison with study from Rudi (2000).

No.	Parameter	This study	Rudi, 2000
1	Temperature (°C)	29	28-30
2	DO (mg/L)	5.48	4.76-5.95
3	Salinity (%)	22%	10-32
4	pH	10.23	6-7
5	Substrate Texture	Muddy, sandy	Muddy, sandy

The comparison of physicochemical parameter from this study is still in range compare to study from Rudy except for pH that is higher. The pH is higher maybe happen because of equipment failure while sampling. However if that not the case the following two statement can explain. Ho *et al.* (1999) mentioned that toxicity of heavy metals can mess with the pH of the seawater. Yokoyama *et al.* (2006) stated that pH cannot be easily determined for a certain location in the open sea because of the water flow. Water physicochemical is important that influence in distribution of *Meretrix meretrix* (Rudi, 2000). Besides, substrate texture also plays an important role because *Meretrix meretrix* is infauna behave organism that hide in the mud at shallow territorial water (Rudi, 2000).

CHAPTER 6

Conclusion

Based on the heavy metal concentration found in the soft tissues of *Meretrix meretrix*, the following conclusion can be drawn. First, different size of *Meretrix meretrix* collected from Tanjung Kidurong has different levels of metal accumulation. The concentrations of metals (Pb and Cd) are higher in the smaller diameter sizes. Second, heavy metal concentration in the mussels collected from Tanjung Kidurong are still within the permissible limits set by most of the countries, these show that consumption of mussels from Tanjung Kidurong is unlikely to Cause Health problem in the short-term period.

REFFERENCES

Adriano, D.C. 1986. Trace element in terrestrial environment. Springer Verlag. New York, pp 533

Agency for Toxic Substances and Disease Registry (ATSDR). 1997. Draft Toxicological Profile for Cadmium, Public Health Service, U.S. Department of Health & Human Services, Atlanta, Georgia.

Akta Makanan. 1983, dan Peraturan Makanan. 1985. *Warta Kerajaan Malaysia 29.*

Berglund, M., Akesson, A., Bjellerup, P., Vahter, M. 2000. Metalbone interactions, *Toxicol*. **112-113**:219-225.

Berman, E. 1980. Toxic Metals And Their Analysis. Heyden, London, Philadelphia, Rheine. 239 pp.

Böhlmark, J. 2003. *Meretrix meretrix* as an Indicator of Heavy Metal Contamination in Maputo Bay, Department of Earth Sciences, Uppsala University, Sweden

Bojanowski, R. 1973. The occurrence of major and minor chemical elements in the more common Baltic seaweed. *Ocean logia*. **2**: 81-152.

Canli, M., Furness, R.W. (1995). Mercury and cadmium uptake from seawater and from food by the Norway lobster *Nephrops norvegicus*. *Environmental Toxicology Chemistry*. **14**: 819–828.

Cappo, M., Alongi, D.M., Williams, D, and Duke, N. 1995. A review and synthesis of Australian Fisheries Habitat Research: Major threats, issues and gaps in knowledge of coastal and marine fisheries habitats. Fisheries Research and Development Corportation. Http://www.aims.gov.au/pages/research/afhr/afhr-00.html. Cited on 14 July 2007.

Carpenter, K.E. and Neim, V. H. 1998. FAO Species Identification Guide for Fisheries Purposes: The Living Marine Resources of the Western Central Pacific, Volume 1. Seaweeds, Coral, Bivalves and Gastropods. Rome, FAO. 689p.

Cherry, R.D. and Shannon, L.V. 1974. The alpha radioactivity of marine organisms. At. Energy Rev. IAEA, Vienna, **12**: 3-45.

Dauvalter, V.A., 1998. Heavy metals in the bottom sediments of the Inari-Pasvik lake-river system. *Water Resour*. **25**: 451-457

Davies-Colley R.J., Nelson P.O., Williamson K.J. 1984. Copper and cadmium uptake by estuarine sedimentary phases. *Environment Science Technology* **18**:491-499.

Eisler, R. (1997). *Copper Hazards to Fish.* Wildlife, and Invertebrates: A Synoptic Review. U.S. Geological Survey, Biological Science Reproduction. BSR-1997-0002, Washington, DC.

Ferreira G.A., André Luís dos Santos Machado A.L.S., and Zalmon R. I. 2004. Temporal and Spatial Variation on Heavy Metal Concentrations in the bivalve *Perna perna* (LINNAEUS, 1758) on the Northern Coast of Rio de Janeiro State, Brazil.

Förstner, U., Wittman, G.T.W. 1981. *Metal pollution in the aquatic environment*, 2nd ed. Springer-velmag, pp 486.

Gaskin, D.E. 1986. Kidnely and water metabolism. *In* M.M.Bryden & R.Harrison, Research on dolphins (pp.129-148). Oxford: Clarendon Press.

Giusti, L., Yang, Y.L., and Hewitt C.N. 1993. The solubility and partitioning of atmospherically derived trace metals in artificial and natural waters: A review. *Atmos Environ.* **27A (10)**:1567-1578.

Goldberg, E.D. 1965. Minor elements in seawater. In: J.P. Riley and G. Skirrow (Eds), Chemical Oceanography, Vol. 1. Academic Press, London and New York, pp. 163 196.

Goldberg, E.D. 1975. The mussel watch – A first step in global marine monitoring. *Marine Pollution Bulletin* **6**: 111.

Goldberg, E.D. 1986. The mussel watch concept. *Environ Monit Assess* **7**:91-103

Goyer, R.A. 1991. *Toxic effects of metals*. In: Amdur, M.O., Doul, J., Klaassen, C.D. (Eds.), Casarette and Doull's Toxicology: Basic Science of Poisons, 4th Edition. Pergamon Press, Oxford.

Guns, M., Hoeyweghen, P.V., Vyncke, W. and Hillewaert, H. 1999. Trace metals in selected benthic invertebrates from Belgian Coastal Waters (1981–1996). *Marine Pollution Bulletin* **38 (12)**: 1184 – 1193.

Guy, R.D., Chakrabarti, C.L. and McBain, D.C. 1978. An evaluation of extraction techniques for the fraction of copper and lead in model sediment system. *Water Research* **12**:21-24

Hall, S.K. 1972. Pollution and poisoning. *Environ. Sci. Technol.* **6**:31-35.

Harrison, R.H. and Laxen, D.P.H.. 1981. Lead Pollution Causes and Control. Chapman and Hall Ltd., London and New York. 168 pp.

Handy, R. D. (1993). The effect of accute exposure to dietary Cd and Cu on organ toxicant concentrations in rainbow trout, *(Salmo gairdneri)*. *Aquatic Toxicology* **21**: 1–14.

Hamer, D. H. 1980, Metallothionein. *Ann. Rev. Biochem.* **55**: 913-951.

Hamilton, E.I. 1987. Heavy Metal Concentrations in Coastal Seawater and Sediments off Prai Industrial Estate, Penang, Malaysia. *Marine Pollution Bulletin* **18(11)**:611-612.

Ho K.T., Kuhn A., Pelletier M.C., Hendricks T.L., Helmstetter A. (1999) pH dependent toxicity of five metals to three marine organisms. National Health and Ecological Effects Research Laboratory, U.S. Environmental Protection Agency, Narragansett, RI, USA. *Environmental Toxicology* **14(2)**, 235-240.

Huanxin W., Lejun Z. & Presley B.J. 2000. Bioaccumulation of heavy metals in oyster (*Crassostrea virginica*) tissue and shell. *Environmental Geology* **39(11)**: 1216-1226.

Hung T.C., Meng P.J., Han B.C., Chuang A., Chuang C. 2000. Trace metals in different species of mollusca, water and sediments from Taiwan coastal areas. Chemosphere 44: 833-841.

Ismail, A. 1993. Heavy metal concentration in Sediments off Bintulu, Malaysia. Marine Pollution Bulletin, Volume 26 (12), pp. 706 707.

Ismail, A., Jusoh, N.R., and Ghani, A.I. 1995. Trace Metal Concentrations in Marine Prawns off the Malaysian Coast. *Marine Pollution Bulletin* **3(11-3)**: 108-110, 1995.

Jarup, L., Berglund, M., Elinder C.G., Nordberg, G., Vahter, M. 1998. Health effects of cadmium exposure: a review of the literature and a risk estimate. Scand J Work *Environ Health* **24(suppl 1)**:7-51.

Kennish, M.J. 1997. *Practical Handbook of Estuarine and Marine Pollution*. CRC Press, Boca Raton, USA.

King, J.C., Keen C.L., Shils, M., Olson, J.A., Shike, M., Ross, A.C. 1999. Nutrition in health and disease. 9th ed. Baltimore: Williams & Wilkins; 223-239.

Kromhout, D., Bosschieter, E.B. and Lezenne, C.C. 1985. The inverse relationship between fish consumption and 20-year mortality from coronary heart disease. *New England Journal of Medicine* **312** : 1205–1209.

Lennart, T. (1996). *Microbial assays in soils* In: Joseph, T., Gabriel B., Dominique, R. (ed). Soil ecotoxicology. Lewis Publishers. New York. pp 208-209.

Liston, P. and Maher, W. 1986. Trace metal export in urban runoff and its biological significance. *Bull. Environ. Contam. Toxicol.* **36**:900-905.

Macfarlane, G.R. and Burchett, M.D. (2001). *Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the grey mangrove, Avicennia marina (Forsk.) Vierh. Marine Pollution Bulletin*. **42**: 233–40.

Merian, E. 1991. Metals and Their Compounds in the Environment. VCH Publishers, Inc., New York, (U.S.A.). pp. 972-1014.

Ministry of Health Malaysia. (2002). *The Malaysian Food Act 1983 and Food Regulation 1985*. Annual Fisheries Statistics 2002 Vol.1. Department of Fisheries Malaysia.

Muller, G. and U. Forstner, 1975. Heavy metals in sediment of the Rhinea and ElbeEstuaries: Mobilization or mixing effect. *Environm. Geol.* **1**:33-39

Nieboer, E. and Richardson, D. H. S. 1980. The replacement of the nondescript term "heavy metals" by a biologically and chemically significant classification of metal ions. *Environmental Pollution, ser. B.* **1**: 3-26

Nieboer, E., Richardson, D.H.S. 1980b. Lichens as monitors of atmospheric deposition. In: Eisenreich, S.J. (Ed.), Atmospheric Pollutants in Natural Waters. Ann Arbor Science, Ann Arbor, pp. 339-388.

Nriagu, J.O. 1978. Lead in soils, sediments and major rock types. pp. 15-72. In: The Biogeochemistry of Lead in the Environment. Part A. Ecological Cycles. Elsevier/North-Holland, Amsterdam

Nriagu, F.O. 1979. Global inventory of natural and anthropogenic emission of the trace metal to the atmosphere. *Nature* **279**:409-411

Nurulrashidah, N. 2006. Heavy Metal (Zn, Pb and Cd) Concentration in *Meretrix meretrix* in Bintulu. Bachelor degree's thesis, Faculty of Agriculture and Food Sciences, University Putra Malaysia Bintulu Campus.

O'Dell, B.L. 2000. Role of zinc in plasma membrane function. *J Nutr.*; 130(5S Suppl):1432S-1436S.

Páez-Osuna, P., Frias-Espericueta, M. G., Osunalópez, J. I., 1995, Trace metal concentrations in relation to season and gonadal maturation in the oyster *Crassostrea iridescens. Mar. Environ. Res.* ***40*****(1)**: 19-31.

Pagenkopf, G.K. (1983). Gill surface interaction model for trace metal toxicity to fish. Role of complexation, pH, water hardness. *Environmental Science Technology*. **17 (6)**: 342–347.

Perwak, J., Bysshe, S., Goyer, M. 1980. An exposure and risk assessment for copper. Washington, DC: EPA. EPA-440/4-81-015.

Phillips, D.J.H., Segar, D.A. (1986). Use of bioindicator organism to monitoring contaminants: program design imperatives. *Marine Pollution Bulltine*. **17**: 10-17

Pinot, F., Kreps, S.E., Bachetet, M., Hainaut, P., Bakonyi, M., Polla, B.S. 2000. Cadmium in the environment: sources, mechanisms of biotoxicity, and biomarkers. *Rev Environ Health* **15(3)**:299-323.

Prasad, A.S., 1998. Zinc deficiency in humans: a neglected problem. *J Am Coll*; **17(6)**:542-543.

Rainbow, P.S. 1995. Biomonitoring of heavy metal availability in the marine environment. *Marine Pollution Bulletin* **31(4-12)**: 183-192

Ramos, L., Fernaudez, M.A., Gonzalez, M.J.and Hernandez, L.M. 1999. Heavy metal pollution in water, sediments and earthworms from the Ebro River, Spain.*Bull. Environ.Contam. Toxicol*. **63**:305-311.

Reid, S. D., and McDonald, D. G. (1988). Effects of cadmium, copper, and low pH on ion tuxes in the rainbow trout, *Salmo gairdneri*. *Can. J. Fish Aquat. Sci.* **45**: 244–253.

Roberts, J.R. 1999. Metal toxicity in children. In Training Manual on Pediatric Environmental Health: Putting It into Practice 1999 Jun. Emeryville, CA: Children's Environmental Health Network.

Rudi, E. 2000. Ekologi dan Makanan Kerang *M. meretrix* Linnaeus 1758.disampaikan dalam Seminar Hasil Penelitian Dosen FMIPA Unsyiah. Banda Aceh, Jun, 2000.

Sarkar, S.K., Bilinski, S.F., Bhattacharya, A., Saha, M., Bilinski, H. (2004). Level of elements in the surficial estuarine sediments of the Hugli River, northeast India and their environmental implications. *Environment International*. **30**: 1089-1098.

Sellers, C.M., Jr., Heath, A.G., and Gass, M.L. (1975). The effect of sublethal concentrations of copper and zinc on ventilatory activity, blood oxygen and pH in rainbow trout (*Salmo gairdneri*).*Water Res.* **9**: 401–498.

Seng, C. E., Lira, P. E., Ang, T. T. (1987). Heavy metal concentrations in coastal seawater and sediments off Prai Industrial Estate, Penang, Malaysia. *Mar. Pollut. Bull.* **18**: 611-612.

Shibli, S.N.M. (2003). Heavy metals concentration(Fe, Cr, Zn, Cu, Cd) in landfill, its uptake and effect on macronutrient (N, P, K) uptake and growth of Acacia mangium. M.Sc. Thesis. University Putra Malaysia, Malaysia.

Staessen, J., and Lauwerys, R. 1993. Health effects of environmental exposure to cadmium in a population study. *J Hum Hypertens* **7**:195-199.

Szefer, P. 1991. *The Science of the Total Environment,* 101 (1991) 201-215 201 Elsevier Science Publishers B.V., Amsterdam.

Thorn, K., Cerrato, R.M., Rivers M.L. 1995. Elemental Distributions in Marine Bivalve Shells as Measured by Synchrotron X-Ray-Fluorescence. *Biological Bulletin* **188(1)**: 57-67.

Truong-Tran A.Q., Ho, L.H., Chai. F., Zalewski P.D. 2000. Cellular zinc fluxes and the regulation of apoptosis/gene-directed cell death. *J Nutr.* **130(5S Suppl)**:1459S-1466S.

Underwood, E.J. 1986. Trace Elements in Human and Animal Nutrition. 5th ed., Academic Press, New York.

Vinikour W.S., Goldstein R.M., Anderson A.R.V. 1980. Bioconcentration patterns of zinc, copper, cadmium and lead in selected fish species from the Fox River, Illinois. *Bull Environ Contam Toxicol* **[25:727]**:734.

Vo, S.T. 2000. Status and Solutions for Farming and Management of the Hard Clam *Meretrix lyrata* at Go Cong Dong, Tien Giang Province, Vietnam. *Phuket Marine Biological Center Special Publication* **21 (1)**: 167-170

Wang, Y., Liang, L., Shi, J., Jiang, G. 2005. Study on the contamination of heavy metals and their correlations in mollusks collected from coastal sites along the Chinese Bohai Sea. *Environmental International*. **31**: 1103-1113

Wilson, A.K., Bhattacharyya, M.H. 1997. Effects of cadmium on bone: an in vivo model for the early response. *Toxicol Appl Pharmacol* **145**:68-73.

Windom, H.L., R.G. Smith, Hungspreugs M., Dharmavanij, S. and Wattayakorn, G. 1988. Trace metal transport in a tropical estuary. *Mar. Chem.* **24**: 293-305.

Yap, C.K., Ismail, A., Tan, S.G., Omar, H. 2002. Correlations between speciation of Cd, Cu, Pb and Zn in sediment and their concentrations in total soft tissue of green-lipped mussel Perna viridis from the west coast of Peninsular Malaysia. *Environment International* **28** :117–126.

Yap, C.K., Ismail, A., Omar, H., Tan, S.G. 2003. Accumulation, depuration and distribution of cadmium and zinc in the green lipped mussel *Perna viridis* (Linnaeus) under laboratory conditions. *Hydrobiology* **498**:151–160.

Yap, C.K., Ismail, A., Tan, S.G. 2004. Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel Perna viridis (Linnaeus) collected from some wild and aquacultural sites in the west coast of Peninsular Malaysia. *Food Chemistry* **84:**569–575

Yasoshima, M., and Takano, B. 2001. *Bradybaena similaris* (Ferrusac) shell as a biomonitor of copper, cadmium, and zinc. *Bulletin of Environmental Contamination and Toxicology* **66(2)**: 239-248

Yokoyama, 1926. Neogene shells from Kôzuke and other Provinces. Jour. Fac. Sci. Imp. Univ. Tokyo, sec. 2, vol. 1, pt. 7, pp. 229-233, pl. 29.

Yokoyama, H., Inoue, M. and Abo, K. 2006. Macrobenthos Current velocity and topographic factors as indicator to assess the assimilative capacity of fish farm.

Zhang, S., Dong, W., Zhang, L., Chen, X. 1989. Geochemical characteristics of heavy metals in the Xiangjiang River, China. *Hydrobiologia.* **177**: 253-262

APPENDIX

SAMPLE MEASUREMENT

Figure A 1: Sample measurement

APPENDIX B

Figure B1: The flesh of Meretrix meretrix

Figure B2: Sieve sediment(63μm)

APPENDIX B

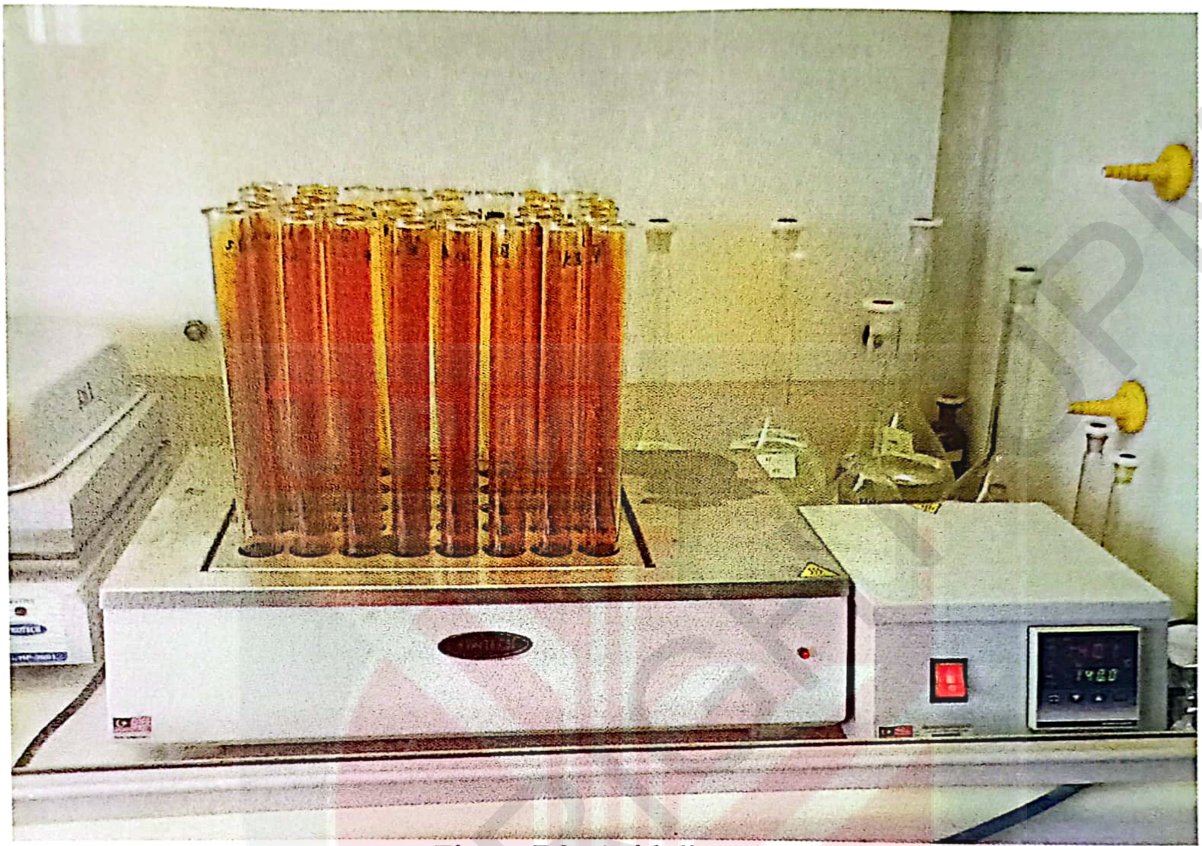


Figure B3: Acid digestion

APPENDIX C

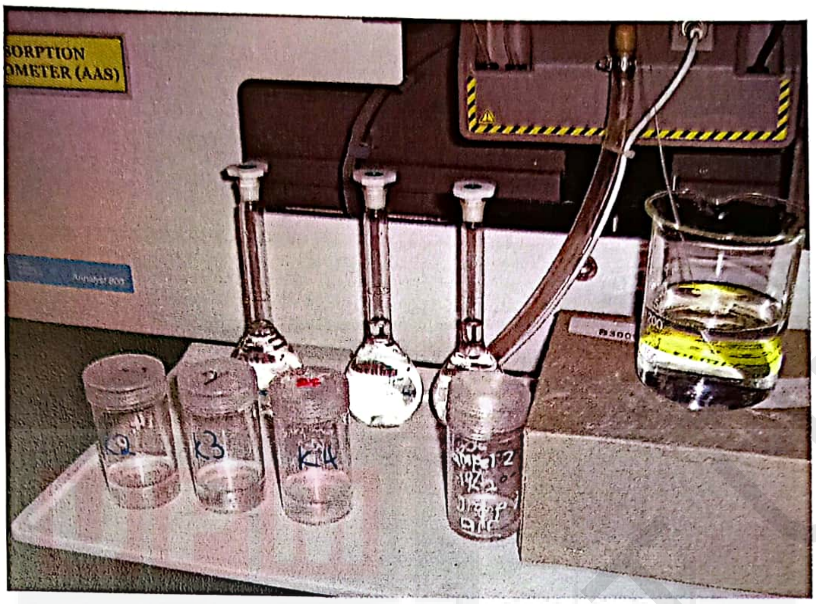


Figure C1: Determine the metal concentration using air-acetylene Perkin-Elmer™ flame atomic absorption spectrophotometer(AAS)

APPENDIX D

Table D1: One-way ANOVA results for heavy metals concentration in *Meretrix meretrix* according temporal variation..

Multiple Comparisons

Dependent Variable: concentration

metals	(I) month	(J) month	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
zn	september	october	-7.24241(*)	1.13217	.000	-10.0961	-4.3887
		november	-.16756	1.13217	.988	-3.0213	2.6862
	october	september	7.24241(*)	1.13217	.000	4.3887	10.0961
		november	7.07485(*)	1.13217	.000	4.2211	9.9286
	november	september	.16756	1.13217	.988	-2.6862	3.0213
		october	-7.07485(*)	1.13217	.000	-9.9286	-4.2211
cu	september	october	1.21244	.51538	.070	-.0866	2.5115
		november	-.08310	.51538	.986	-1.3822	1.2160
	october	september	-1.21244	.51538	.070	-2.5115	.0866
		november	-1.29554	.51538	.051	-2.5946	.0035
	november	september	.08310	.51538	.986	-1.2160	1.3822
		october	1.29554	.51538	.051	-.0035	2.5946
cd	september	october	-.03832	.06405	.823	-.1998	.1231
		november	-.11437	.06405	.199	-.2758	.0471
	october	september	.03832	.06405	.823	-.1231	.1998
		november	-.07605	.06405	.473	-.2375	.0854
	november	september	.11437	.06405	.199	-.0471	.2758
		october	.07605	.06405	.473	-.0854	.2375
pb	september	october	.19940	1.18233	.984	-2.7807	3.1795
		november	-.77738	1.18233	.790	-3.7575	2.2028
	october	september	-.19940	1.18233	.984	-3.1795	2.7807
		november	-.97678	1.18233	.691	-3.9569	2.0034
	november	september	.77738	1.18233	.790	-2.2028	3.7575
		october	.97678	1.18233	.691	-2.0034	3.9569

* The mean difference is significant at the .05 level.

APPENDIX D1

Table D2: Independent Sample Test results for Zn concentration in Meretrix meretrix according to two size ranges between 41 – 80 mm and 20 – 40 mm.

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
nilaimetals	Equal variances assumed	.982	.332	1.210	22	.239	1.99131	1.64525	-1.42074	5.40335
	Equal variances not assumed			1.210	20.651	.240	1.99131	1.64525	-1.43371	5.41633

a. metals = Zn

Table D3: Independent Sample Test results for Cu concentration in Meretrix meretrix according to two size ranges between 41 – 80 mm and 20 – 40 mm.

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
nilaimetals	Equal variances assumed	8.122	.009	3.795	22	.001	1.42333	.37508	.64546	2.20121
	Equal variances not assumed			3.795	14.164	.002	1.42333	.37508	.61973	2.22694

a. metals = Cu

Table D4: Independent Sample Test results for Pb concentration in Meretrix meretrix according to two size ranges between 41 – 80 mm and 20 – 40 mm.

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
nilaimetals	Equal variances assumed	1.214	.282	-2.230	22	.036	-1.93383	.86711	-3.73210	-.13556
	Equal variances not assumed			-2.230	21.521	.036	-1.93383	.86711	-3.73443	-.13324

a. metals = Pb

APPENDIX D2

Table D5: Independent Sample Test results for Cd concentration in Meretrix meretrix according to two size ranges between 41 – 80 mm and 20 – 40 mm.

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
nilaimetals	Equal variances assumed	1.018	.324	-3.406	22	.003	-.15151	.04448	-.24375	-.05927
	Equal variances not assumed			-3.406	18.757	.003	-.15151	.04448	-.24468	-.05833

a. metals = Cd

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "HEAVY METAL (Zn, Cu, Pb and Cd) CONCENTRATION OF *Meretrix meretrix* IN BINTULU" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in the form approved by the Faculty.

IBRAHIM BIN AZIZAN

Date: