

**DETERMINATION OF HEAVY METALS IN RAZOR CLAM (SOLENA SP.) AT  
MOYAN AND HULU LANGAT**

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## DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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# Determination of Heavy Metals in Razor Clam (*Solen* sp) at Moyan and Hulu Langat

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## ABSTRACT

Razor clam (*Solen* sp) was used to determine the levels of heavy metals (Cd, Mn, Fe, Cr and Hg). The samples were collected at three different stations at Moyan and also from Hulu Langat. The determination of heavy metals in sediments and different parts of razor clam (shell, tissue, foot, siphon and gill) was carried out using Flame Atomic Absorption Spectrophotometer (FAAS) and Flow Injection Mercury Spectrometer (FIMS). Fe was found to be the most abundant element in sediments, tissue, foot, siphon and gill parts of razor clam while Mn occurred in highest concentration in shell. Hg was found in lowest concentration in sediments and also in all parts of razor clam. The accumulation of heavy metals in Moyan and Hulu Langat varied between different parts. There were significant correlations for Cd in sediment and Cd in shell, tissue, foot, and siphon ( $r > 0.76$ ,  $P < 0.05$ ). Concentration of Mn in tissues significantly correlated with Mn in sediment ( $r = 0.72$ ,  $P = 0.05$ ).

Key words: Heavy metals, sediments, razor clam

## ABSTRAK

*Ambal (Solen sp.) digunakan untuk menentukan kandungan logam berat (Cd, Mn, Cr, Fe dan Hg). Sampel diperolehi dari tiga lokasi di Moyan dan juga dari Hulu Langat, Selangor. Penentuan kandungan logam berat dilakukan dengan menggunakan Spektroskopi Serapan Atom Nyala (FAAS) dan Spektroskopi Merkuri Analisis Suntikan (FIMS). Fe merupakan logam yang hadir dalam kepekatan tertinggi dalam sedimen, tisu, kaki, sifon dan hati ambal manakala kepekatan Mn pula paling tinggi dalam cengkerang. Kandungan Hg didapati paling rendah dalam sedimen dan semua bahagian ambal yang dianalisis. Kepekatan logam-logam berat dalam bahagian-bahagian ambal yang di analisis menunjukkan variasi di Moyan dan Hulu Langat. Terdapat korelasi yang signifikan untuk Cd dalam cengkerang, tisu, kaki dan sifon dengan Cd dalam sedimen ( $r > 0.76$ ,  $P < 0.05$ ). Kepekatan Mn dalam tisu menunjukkan korelasi yang signifikan dengan Mn dalam sedimen ( $r = 0.72$ ,  $P = 0.05$ )*

*Kata Kunci: Logam berat, sedimen, ambal*

# CHAPTER ONE

## INTRODUCTION

### 1.0 Background

Heavy metals are one type of harmful pollutants to living things such as to biota in the marine environment (Lavilla *et al.*, 2008). They are toxic to plants and animals when presence in large concentration. However, when heavy metals presence in small amounts, they are essential for plant and animal nutrition. For examples, boron, copper and iron are required by plants and animals. Different heavy metals come from different sources (Lepp, 1981). In aquatic environment, heavy metals mainly from naturally occurring geochemical materials (Lau *et al.*, 1998). Some of trace elements can cause toxicity to species by inhibit their growth (Langston and Bebianno, 1998).

Various aquatic organisms are widely used as biomonitors of metal contamination in water and sediments (Martin and Coughtrey, 1982). To be good biomonitor, the species should be abundant in the study area, easy to identify and can be sampled at all times, and also have enough tissues for analysis. Bivalve mollusks are abundant in many terrestrial and aquatic ecosystems (Lau *et al.*, 1998). They are aquatic organism that has been developing rapidly in many temperate countries due to their potential as source of income. The examples of bivalves are oyster and mussel. Razor clam is one example of bivalve mollusc species. Superfamily of razor clam is Solenacea. Species that categorized in this family are *Ensis arcuatus*, *Ensis siliqua* and *Solen marginatus* (Dariba *et al.*, 2006). In Sarawak, *Solen* species is commonly found such as “ ambal biasa”, “ambal riong” and “ ambal jernang”. Local people refer razor clam as “ambal”. Razor clam can be found in the intertidal

sandy beaches of Samarahan Division and other location where ambal can be found is at Muara Tebas (Pang, 1993).

Sediment contamination by trace metals is one of the environmental problem that causing impact to aquatic environment. Sediments act as sink or source of contaminants to the water (Magalhães *et al.*, 2007). In water, sediments are transported together with heavy metals to certain places (Poot *et al.*, 2007). Heavy metals adsorbed on fine silt or clay, then used by marine life or deposited in sediments (Elston *et al.*, 2005). Some of the pollutants are accumulated in the deeper sediment layers. Fine-grained sediments have large surface to mass ratios. Thus, they can adsorb and transport many contaminants when flowing in aquatic system (Lick *et al.*, 1992). The binding form of copper in sediment always related to sulphide and organic content. Zinc bound to iron and manganese while lead distributed over the different labile and non-labile binding fraction. When redox potential of anaerobic sediment occurs, 7-37% sediment releases those heavy metals. Heavy metals adsorbed onto clays and sand and can easily release through ion exchange. For example, by lowering of sediment pH may give rise to mobilization of heavy metals bound to carbonate (Kelderman and Osman, 2007).

Trace metals that accumulate in sediments are not necessarily available for uptake into biota. A proportion of the metal may be bound to in dissolved complexes or sediment surfaces. Alterations in physiochemical parameters of the environment can influence the relative proportions of the metals that can be taken. The examples of alterations that can occur are alterations in pH and salinity (Langston and Bebianno, 1998).

## **1.1 Statements of Problem**

Razor clam has tendency to accumulate pollutants such as heavy metals due to agricultural activities and other activities such as fisheries. If the levels of heavy metals are high, razor clam will not be suitable for consumption as human health will be affected. Sediment contaminated by heavy metals also poses effects to marine organism.

## **1.2 Objectives**

The main purpose of this study was to determine the heavy metals (Fe, Cd, Cr, Mn and Hg ) levels in the tissue, shell, gill, foot, and siphon of razor clam and also in sediments collected from Moyan. This study was also carried out to determine the relationship between metals concentration in razor clam with sediment using correlation study. Statistical analyses were carried out to determine if there is any significant difference in concentration of metals between the different parts of razor clam.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Heavy Metals

Heavy metals can be defined as a group of metals and semimetals (metalloids) related with contamination and toxicity. Term of “heavy” implies for high density while “metal” terms refers to pure element or an alloy of metallic elements. They have densities above 7 g/cm<sup>3</sup>. Metals that classified into this group have higher atomic weight (Duffus, 2002). In Periodic Table, the elements next to diagonal line from boron (B) to tellurium (Te) are known as metalloids. They have properties of metals and non metals (Kotz *et al.*, 2006).

Trace metals are heavy metals that present in low concentration or amount. The examples of heavy metals are iron (Fe), copper (Cu), cadmium (Cd), chromium (Cr), manganese (Mn), nickel (Ni) and lead (Pb) The transition elements with incompletely filled d-orbitals are heavy metals. There are 53 heavy metals including Arsenic. The ability of heavy metals cations form complex compound depends on d-orbitals (Prasad *et al.*, 2006). Heavy metals can be distinguished according to their tendency to form stable complexes like chelates. The presence of low concentration of trace elements causes shifts in the composition of dominant species such as phytoplankton cause toxicity to organisms. As a result, species exhibit differential sensitivity to toxic substance due to rate of uptake of heavy metals and essential for those elements. For example, about 0.05 µg l<sup>-1</sup> of cadmium detected in *Skeletonema costatum* sp (Langston and Bebianno, 1998).

Generally, there are many sources of heavy metals in environment. They can come from geochemical materials that produced through human activities (Martin and Coughtrey, 1982). The geochemical processes such as mineral dissolution, ion desorption, complexation, speciation, adsorption, precipitation and transport influence the toxicity. When rain water infiltrates through watershed soils, heavy metals are released into surface water as a result of mineral dissolution and desorption. Heavy metals in water are adsorbed by colloidal particles and are precipitated into sediment as solid phases (Prasad *et al.*, 2006).

An anthropogenic activity is another source of heavy metals (Nayar *et al.*, 2004). Some of the substances used containing heavy metals. When these heavy metals flow into river, they cause contamination to aquatic organism and also sediments. Fossil fuel, wood combustion and also industrial process are the main anthropogenic sources of metals to the atmosphere. It is believed that, burning fossil fuels is contributed to release Be, Co, Hg, Mo, Ni, Sb, Se, Sn, V, Cr, Cu, Mn and Zn content. Zinc is released from vehicle tyres while copper from brake lining, manganese from moving parts and gasoline additives (Pereira *et al.*, 2007). Pesticides are used to control the pests, fungal diseases and weeds crops. Heavy metals from pesticides are dissolved and bound-to-sediment transported chemical (Bermu' dez-Couso *et al.*, 2007).

### **2.1.1 Iron (Fe)**

Iron is fourth most common element in the earth and categorized as second most abundant metal after aluminium. This heavy metal is chemically active. It readily dissolves in dilute mineral acids (Cotton *et al.*, 1987). It can present as haemetite ( $\text{Fe}_2\text{O}_3$ ), magnetite ( $\text{Fe}_3\text{O}_4$ ) and also limonite ( $\text{FeO}(\text{OH})$ ). Iron commonly occur in form of oxidation states  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  while

$\text{Fe}^{4+}$  and  $\text{Fe}^{6+}$  are rarely found, especially in many organic and organometallic compounds containing iron. Iron may occur in sediments in certain amounts from downstream of discharges of ferruginous mine water. It also can occur in the tissue of all living organisms. In high concentrations, it can growth disorders in plants while in fish, concentrations of iron in the range of 1-2 mg Fe/L are toxic. However, higher levels than this can be tolerated, depends on species of fish. It can reach an average value of 2 mg/L (Dojlido and Best, 1993).

### **2.1.2 Copper (Cu)**

Copper is not abundant (Cotton *et al.*, 1987). However, it is possible to give chronic effect to aquatic life and also causes sediments contamination when present in high concentration. If present in low concentration, it can be used for living things. Copper commonly discharge from point sources such as industrial waste that containing copper (Kennish, 2000). Copper is used as catalyst in the chemical industry, electrochemical industry and also in the production of piping. It can exist in the form of  $\text{Cu}^0$ ,  $\text{Cu}^{1+}$ ,  $\text{Cu}^{2+}$  and  $\text{Cu}^{3+}$ , but the common form is  $\text{Cu}^{2+}$ . Copper can occur in sediments when copper complexes enters the sea, the copper become more biologically available after breakdown of complexes and adsorbed onto particulate matter, deposited in the sediment (Dojlido and Best, 1993).

### **2.1.3 Cadmium (Cd)**

Cadmium is soluble in water but not soluble in bases. Its abundance in nature is relatively low (Cotton *et al.*, 1987). Cadmium is present in rock and soils at concentration of 1 ppm or less. It is mostly mobile in the soil or sediment (Lepp, 1981). As copper, cadmium can cause chronic effect to aquatic life. The effect includes lack of tissue repair and tissue inflammation. It also

contributes to sediments contamination and commonly discharges from point source (Kennish, 2000).

Cadmium does not occur in solution for long in natural water. It is precipitated as carbonate or adsorbed onto particulate matter and deposited into bottom sediments. The concentration of cadmium in sediments varies from 0.1 to 3000 mg/kg dry weight. After cadmium incorporated into sediment, it does not redissolve in neutral or alkaline conditions. It is because, in the anoxic conditions in the sediments, cadmium sulphate is formed. Cadmium sulphate is insoluble form of cadmium. The adsorption of cadmium by sediments increases with an increase in pH. At pH above 7, all ions of the metal will be adsorbed. Cadmium is readily accumulates in aquatic organisms and sediments (Dojlido and Best, 1993).

#### **2.1.4 Chromium (Cr)**

Chromium is a d-block transition metal of Group VIB. Atomic number of chromium is 24 and atomic weight is 51.996. Commonly, it is used in alloy steel and chrome plating. The color of chromium metal is grey and brittle. It also can be highly polished. It is resistant to attack by oxidation. This property makes the chromium suitable to be used in alloys. The hardness and resistance of alloys increases with presence of chromium. It produced from ore chromite (Alloway, 1995). It commonly occur as the ore chromite ( $\text{FeCr}_2\text{O}_4$ ). About 30% of chromium contain in this form. All chromium compounds are coloured and can exists as  $\text{Cr}^{3+}$  or  $\text{Cr}^{6+}$  molecules (Dojlido and Best, 1993).

### 2.1.5 Manganese (Mn)

Manganese is relatively abundant and quite electropositive. It readily dissolves in non-oxidized acids (Cotton *et al.*, 1987). It can exist in oxidation states from Mn (II) to Mn (VII). Manganese (II) salts are mostly water soluble. It is essential for living things uses, but it can become toxic when present in higher concentration (Alloway, 1995). Manganese can be found in combination with iron as in biotite mica. Manganese is used in the steel industry for making alloys and also can be found in fertilizers. The source of manganese from mines and industries that used that elements, enter the surface of water and deposited into sediments. Manganese can exist in oxidation states ranging from -3 to +7. High doses of manganese are toxic to living organisms. At low pH, the toxicity is greater (Dojlido and Best, 1993).

### 2.1.6 Nickel (Ni)

Nickel is ductile metal and found with combination of arsenic, antimony and sulfur (Cotton *et al.*, 1987). In Periodic Table, it is categorized in group VIII. Its atomic number is 28, and its atomic weight is 58.71. Nickel colour is whitish silver, hard but brittle, polishable, and conducts heat and electricity. It is extracted from sulphide and oxide ores (Alloway, 1995). Its principal ores are pentlandite ((FeNi)<sub>9</sub>S<sub>8</sub>), millerite (NiS) and garnierite and only occurs as the natural metal in meteorites. Nickel is used in the chemical industries such as production of alloys and also nickel plating for corrosion resistance. It also can present in crude oil in varies concentrations and the burning of petroleum products. Oxidation states which nickel can exists are ranging from -1 to +4, but +2 is commonly occurred in aqueous chemistry. Nickel ion forms stable complexes with both organic and inorganic ligands, then adsorbed onto particulate matter.

It less toxic than other heavy metals such as mercury, copper and cadmium (Dojlido and Best, 1993).

### **2.1.7 Lead (Pb)**

Lead is one of rare element in the earth. It is obtained by reduction of oxide or sulfide with carbon. It can dissolve in acid (Cotton *et al.*, 1987). It is a member of group IVB. Its stable oxidation states are Pb (II) and Pb (IV). Lead have greater ionic size more strongly absorbed than copper ( Alloway, 1995) . It affects the aquatic life when present in high concentration. In lower concentration, it used as nutrient in animals or plants (Lepp, 1981). Lead is used in building construction and also for storage tank lining. The pH 5-7 with presence of clay particles most of the lead is adsorbed and removed from the water column in sedimentary material. The source of lead in sediments is washoff from surface enters the surface of water and incorporated into sediments. The factors determine the adsorptions of lead onto sedimentary materials are particle size and organic content of material. Lead totally adsorbed onto sediment when soluble organic complexes in the water column are absent and pH levels is over 6 (Dojlido and Best, 1993).

### **2.1.8 Mercury (Hg)**

Mercury present as liquid at room temperature. It has melting point -38.88 0C and specific gravity of 13.55. Mercury is very heavy metal. Metallic mercury is very volatile, and at normal atmospheric temperature and pressure when air saturated with mercury vapour may contain 13.2 µg Hg/m<sup>3</sup> (Dojlido and Best, 1993).

## 2.2 Razor Clam

Razor clams move by their foot into the sand below their shell. The primary food source of clam is plankton. Razor clam that have total shell length greater than 5.6, indicate that they had attained maturity and undergo various stages of the gonad-maturation and spawning cycle. They reach sexual maturity. The age of razor clam based on the shell growth pattern are more feasible for accessing the stock of razor clam (Pang, 1993).

Another type of razor clam species is *Siliqua patula* that is an important bivalve mollusk harvested throughout its range by commercial and sport fisheries. It can be found at west coast of North America. The species commonly found are *Pacific Siliqua patula* and the northern or Arctic razor clam which is *Siliqua alta*. The Arctic razor clam is found in southern Cook Inlet westward to the Bering Sea and Siberia while the Pacific razor clam is more widely distributed at area such as California. *Siliqua patula* have long, narrow shell and may attain a length greater than seven inches (<http://www.w3c.org/TR/1999>).

## 2.3 Sediments

Sediment can be defined as particulate matter that can be transported by fluid flow. It is deposited as layer of solid particles on the bed or bottom of water. Clay microstructure of sediment is referring to fabric and physico-chemistry of fine-grained sediment. The physico-chemistry relates to interparticle forces of the sediment (Curry *et al.*, 2007). The ability of metals in sediment provides the opportunity for aquatic animals to biomagnify the metals. These metals then remobilised through the food chain. The bioavailability of metals in the sediments depends on the hardness and pH value of the surrounding water (Lau *et al.*, 1998). Although aquatic sediment

contain complex mixtures of contaminant such as heavy metals, but their influences to natural communities are not understand very well.

Toxicant may have more direct impact on microbial communities in sediment. The factor such as nature of the sediment and seawater characteristic affect metal bioavailability (Beyrem *et al.*, 2007). Bioavailability and toxicant contaminants depends on their partitioning between the sediment, pore water and overlying water. It also depends on sediment organic carbon content (Di Toro *et al.*, 1991). More metals are found as free ions in pore water. It is because the microcosms are closed to the system (Austen and McEvoy, 1997).

Heavy metals pose a serious risk to the health of an ecosystem, due to their short and long term toxicity. After heavy metals released into water system, they generally show more tendency to bind to suspended matter. Through sedimentation, they accumulate in aquatic sediments (Kelderman and Osman, 2007). Dissolved trace elements are more mobile in sandy soils compared with other soils because of high diffusion and connection water fluxes (Prasad *et al.*, 2006).

## **2.4 Heavy Metals Accumulation in Bivalves**

In aquatic biota, bioaccumulation potential expressed using ratios of chemical concentrations in organism tissue relative to chemical exposure concentration such as bioconcentration factors (BCFs) (DeForest, 2007). Bioaccumulation is accumulation of heavy metals by organisms. Uptake of heavy metals can occur through sorption of metals by microbial biomass and its byproducts and also physiological uptake of trace elements by organisms through

metabolically active and passive process (Prasad *et al.*, 2006). Accumulation of metals not only depends on the level of environment contamination but also other factor such as salinity, chemical species and interaction with other pollutants (Lopez-Artiguez *et al.*, 1989). Aquatic system has high potential to accumulate the contaminants because contaminants move quickly through aquatic system (Huang *et al.*, 2007). The most abundant contaminants in aquatic environment are heavy metals such as copper, cadmium, nickel, lead and zinc (Hagopian-Schlekat *et al.*, 2001).

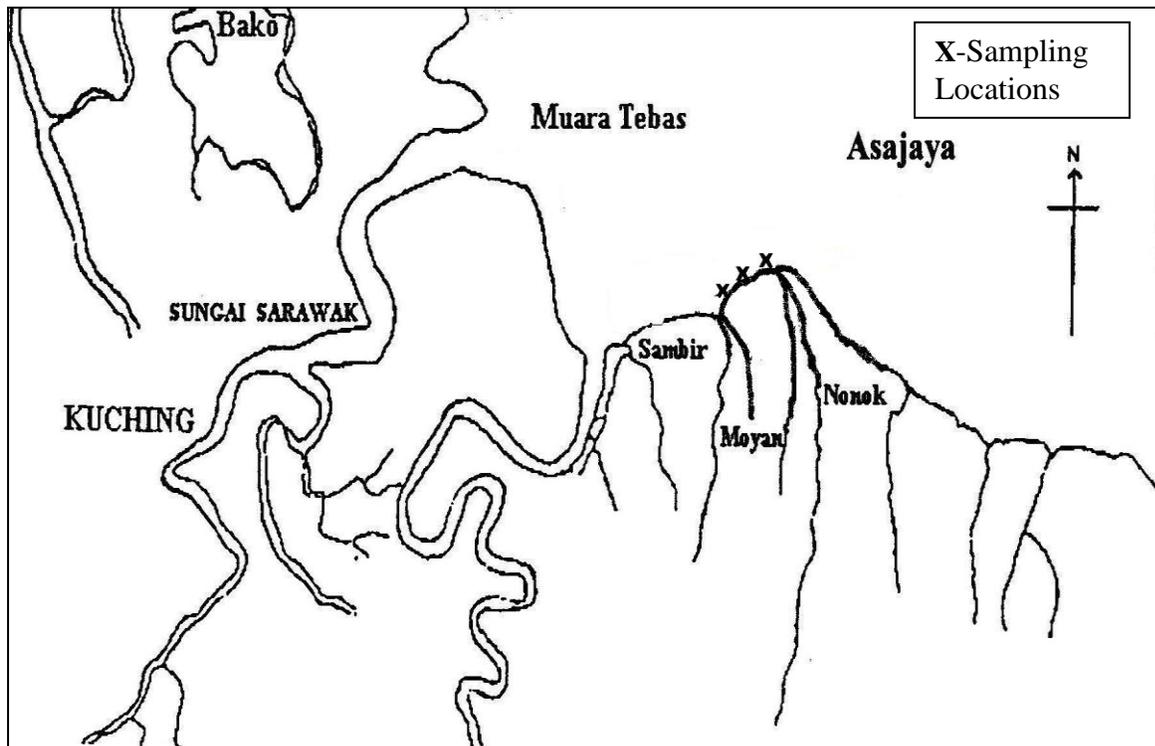
Marine bivalve such as oysters have ability to accumulate cadmium in soft tissues from water and sediments when exposure in high concentration levels of metals. The intracellular organ became target of metal toxicity is mitochondria because it very sensitive to metal exposures (Sokolova *et al.*, 2005). According to Gnassia-Barelli *et al.* (1995), heavy metals such as copper, when accumulate in marine invertebrates can disturb calcium homeostasis of that organisms. Mussels assimilate trace metals from their food and ingestion of inorganic particulate material (Conti and Cecchetti, 2003).

Bivalve *Pinctada radiata* and *Brachidontes pharaonis* showed that highest accumulation of Fe in shells. Accumulation of copper is lowest for *B. pharaonis* and cadmium concentration is lowest in *P. radiata*. The study revealed that accumulations in *B. pharaonis* were higher than *P. radiata* due to heavy metals have more tendencies to accumulate in small organisms compared to larger organisms and also due to seasonal factor (Goksu *et al.*, 2003). The research done in *Donax trunculus* at different contaminated sites in the gulf of Annaba show that different concentration of heavy metals are recorded at different season. The highest value of zinc had

been recorded in winter while in summer; other heavy metals like copper and lead were highest (Beldi *et al.*, 2006). Heavy metal commonly uptake through water, food and sediment. However, this process may depend on the ecological needs and also metabolism of animal beside the concentrations of heavy metals in these sources (Roesijiadi and Robinson, 1994).

**CHAPTER THREE**  
**MATERIALS AND METHODS**

**3.1 Sampling Locations**



**Figure 3.1: Map of sampling location at Moyan**

Razor clam and sediment samples were collected at Moyan beach at three locations namely are low tide station (LT), middle tide station (MT) and high tide (HT) as shown in Figure 3.1. Moyan is located at Asajaya area. The major activities of local people at Moyan are agriculture and fisheries. Samplings were carried out in the months of September and November 2007. Some razor clam samples (ambal riong) were also collected from Hulu Langat, Selangor

(Figure 3.2) on month of June 2007. The major industrial activity at Hulu Langat sampling area is iron industry.



**Figure 3.2: Map of sampling location**

## 3.2 Sample Collection

### 3.2.1 Razor Clam samples

Razor clam samples were collected by using long, elongated, slender stick of four feet in length a mixture of limestone powder, ashes and salts. Razor clam with shell lengths between 5 cm and 8 cm ( $n= 80$ ). Vernier caliper model CD-15CP was used to measure the shell length of razor clam. After collection, razor clam samples were washed with seawater to remove sediments. Then razor clam samples were kept into sealed glass containers. After that, the samples were kept in ice box and transported to the laboratory (Swaileh *et al.*, 1994).

### **3.2.2 Sediment Samples**

Sediment samples were collected from the area inhabited by razor clam. The samples were collected from each sampling station using the bottom grab sampler from top 20 cm of the bottom sediment. The sediment samples were kept in polyethylene bags and then were transported to the laboratory (Lau *et al.*, 1998).

## **3.3 Chemical Analysis**

### **3.3.1 Razor Clam Analysis**

At the laboratory, razor clam samples were stored in freezer at temperature  $-20^{\circ}\text{C}$ . The samples were thawed and washed with tap water and deionized water to remove unwanted materials. Razor clams were dissected for gill, foot, siphon, tissue and shell (Figure 3.3). The samples were pooled and homogenized. Then, the samples were transferred into glass flask and oven-dried at  $70^{\circ}\text{C}$  for 48 hours. After that, samples were ground to fine powder using pestle and mortar. The samples were stored in sealed plastic bags. These sealed plastic bags were kept in dessicator until further analysis to dry the moisture from surrounding.