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COMPARISON OF HEAVY METALS IN RAZOR CLAMS (*SOLEN* SP .) OF ASAJAYA LAUT AND MUARA TEBAS

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Bachelor of Science with Honours
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This project is submitted in partial fulfillment of the requirements for the degree of
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DECLARATION

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

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Comparison of heavy metals in razor clams (*Solen* sp) of Asajaya Laut and Muara Tebas

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Abstract

Pb, Fe, Zn, Cu, Cd and Mn contents in razor clams (*Solen sp*) and sediments were measured using Flame Atomic Absorption Spectrophotometer (FAAS). Razor clams from both sampling sites showed the similar patterns in accumulating heavy metals. Tissues of razor clams recorded highest concentrations of Fe and Zn, while shells accumulated highest concentration of Pb and Mn. The concentrations of Cu and Cd were low in razor clams from both sampling sites. Based on FAO limits, Pb concentration in shells of razor clams was more than the designated limit at both sampling sites. The heavy metal concentrations in sediments were also measured and it was higher than razor clams. This study revealed that sediments from Muara Tebas were slightly polluted with Pb. There were significant correlations between Cu concentrations in tissues of razor clams with the sediments ($r = 0.7459$) at Asajaya Laut.

Key words: Razor Clams, Heavy metal, Pollution, Flame Atomic Absorption Spectrophotometer.

Abstrak

Kepekatan Pb, Fe, Zn, Cu, Cd dan Mn didalam ambal (Solen sp) dan sedimen dari Asajaya dan Muara Tebas telah di analisis dengan menggunakan Spektroskopi Serapan Atom Nyala (FAAS). Ambal dari kedua-dua tempat menunjukkan tren yang sama dari segi kepekatan logam berat. Bahagian tisu ambal merekodkan kepekatan Fe dan Zn yang paling tinggi, manakala cengkerang menunjukkan kepekatan Pb dan Mn yang paling tinggi. Kepekatan Cu dan Cd yang diperolehi dalam sampel ambal didapati sangat rendah dari kedua-dua tempat pensampelan. Berdasarkan kepada had yang ditetapkan oleh FAO, kepekatan Pb didalam cengkerang ambal melebihi had yang dibenarkan bagi kedua-dua tempat. Kepekatan logam berat didalam sedimen turut dianalisa dan ia adalah tinggi berbanding kepekatan dalam ambal. Kajian ini menunjukkan bahawa sedimen di Muara Tebas sedikit tercemar dengan Pb. Tedapat korelasi yang signifikan di antara kepekatan logam Cu dalam tisu ambal dengan sedimen (r $= 0.7459$ *) di Asajaya Laut.*

Kata Kunci: Ambal, Logam berat, Pencemaran, Spektroskopi Serapan Atom Nyala

CHAPTER ONE

INTRODUCTION

Much attention has been paid to heavy metal contamination in water environment and their potential hazard to organisms and human beings (Liang *et al.*, 2004). Mollusks are well-known to accumulate heavy metals (Lau et al., 1998) and have been widely used as bioindicator for monitoring heavy metal contaminations in aquatic environment (Otchere, 2003 and Wagner and Boman, 2004). In addition to using bivalves as bioindicator organisms of coastal contamination, it is also recognized that they can be important links for contaminants between sediments and higher organisms, including man, that consume them (Cheggour *et al.*, 2005). Bioindicator or biomonitoring describes species that accumulate trace metals or other substances in their tissues and therefore can be used to monitor the bioavailability of these substances in a particular environment (Wagner and Boman, 2004).

Bivalves can accumulate metals from ambient seawater and ingested food metal uptake from the dissolved phase because these animals pump a substantial quantity of water through their gills (Chong and Wang, 2001) and clams primarily gain nutrition from deposit-feeding and filter-feed (Cheggour *et al.*, 2005). The main site of heavy metals accumulation in clam is found to be kidney and the minor site is the muscle. The kidney and muscle could be considered as long-term storage sites (chronic pollution), whereas the mantle and especially the gills reflect a short-term contamination (Duquesne and Coll, 1995). The metal concentrations in bivalve tissues are site-specific and related to local environment conditions such as sediment type, temperature, salinity, and ambient metal concentrations and loadings (Cheggour *et al.*, 2005).

Sediments are important sinks of metals and may also serve as an enriched source of metal for benthic bivalve organisms (Cheggour *et al.*, 2005). Sediments can bind and accumulate a wide variety of metals and organic compounds (Wagner and Boman, 2004). Heavy metals discharged from industrial or sewage effluents or from atmospheric deposition may be rapidly removed from the water column and transported to bottom sediment. Consequently, metal concentrations in sediments are often several orders of magnitude higher than those in ambient seawater (Chong and Wang, 2000).

Various species of edible bivalve mollusks such as clams, oyster and cockles are found on the mangrove mudflats and intertidal sandy beaches in Peninsula Malaysia as well as Sarawak. Razor clam (*Solen* sp) or locally known as "ambal" is found abundantly in the intertidal sandy beaches in the Kuching and Samarahan Division of Sarawak. There are three different species of Ambal in the genus *Solen* that commonly found in Sarawak. There are locally known as 'Ambal Biasa', 'Ambal Riong', and 'Ambal Jernang' (Pang, 1992). The traditional technique to catch this 'Ambal' is using a mixture of lime stone powder, ashes and salts. The piece of sharpened wooden stick on one end of about four feet in length is the usual equipment to catch the razor clams. The paste mixture of lime stone powder, ashes and salts are put into a small container, which has been attached to the stick. A short piece of coconut leave stalk is used to take small amount of lime slat mixture before immediately put into the burrow and in contact with the clam within. The presence of clam can be recognized in the sandy substrate from tiny hole on the sandy surface. The tiny hole is similar with the shape of it shell valve. Besides that, identification of clam location in the sandy substrate can be done by tapping the stick on the sandy surfaces, the clam will retract their siphons back into the burrows. There is another technique to catch larger and bigger clams (5.1cm - 11.2cm) that usually found at low tide areas such as "ambal Riong". After recognizing the tiny hole on the sandy surface, a spoon is used to dig the hole before tapping the stick content small amount of limesalt mixture. This is because the use of wooden stick is no longer effective to catch larger and longer clams. Basically, the collectors only wear sock because they are not allowed to put on any foot wear when collecting clams.

Razor clams have been traditionally collected for human consumption over the past decades and razor clams have grown tremendously as one of the famous seafood item. Despite their popularity in the market, little information is available on the suitability of the razor clams for human consumption. It is important to monitoring the aquatic environment to ensure public health safety because most people depend on sea for their daily activities and edible species like clams serve as important food sources. The point sources that might contribute to heavy metals pollution in the study areas are industries effluents from industries that are located along Sungai Sarawak, runoff water, agricultural activities and populations. The worldwide transport and petroleum spills also lead to contamination of the sea. The hypothesis of this research is that the razor clams could be used as bioindicator for heavy metals to test the pollution level in Kuching Bay. There were a few comprehensive study has been undertaken in Sarawak to examine the magnitude of heavy metal in razor clam from Asajaya Laut and Muara Tebas.

The objectives of this study was to determine and to compare the heavy metals content namely Cd, Cu, Fe, Mn, Zn, Pb in shell and tissue of razor clam and sediment samples at Asajaya Laut and Muara Tebas. This research also attempted to investigate whether the razor clams accumulate metals relative to sediment and if there are any correlation between metal concentrations in shell or tissue samples with sediment samples. The results obtained will be compared with international standards for metals in mollusks/shellfish compiled by the Food and Agricultural Organization (FAO) of the United Nations (Wagner and Boman, 2004).

differently according to the site conditions and the specific metal. The variation of metal concentrations in sediment may also arise as a result of changes in anthropogenic or natural input and even seasonal cycles in post-depositional remobilization of Fe, Mn and sulphide phase (diagenetic activity). The metals consist of Cd, Cu, Pb and Zn in surface sediments and bivalves *Cerostoderma edule* from two lagoon systems from the Moroccan Atlantic coastline were subjected to seasonal variation. Sediment metal concentrations were highest in the wet season (Cheggour *et al.*, 2001).

The grain size and both organic matter and mud content affected the concentrations of contaminant in sediment. The smaller grain size of sediment has greater ratio of surface area to volume and for this reason they have higher ability to bind the metals more strongly. Besides that, the absorption of metals will increase with the amount of total organic matter in the sediment (Ahn *et al.*, 1996). However, the concentrations of metals in clam were generally less variable than those found for sediment.

There were no significant correlations between metal concentration in the clams and those in the sediments (Trocine and Trefry, 1996). The concentrations of contaminants in sediments do not reflect the absolute contaminant abundance at a certain point but rather a function of the relative fluxes of contaminants and suspended particulates in the system. Hydrophobic chemicals are known to associate with sediment particles including those from both suspended particulate matter and bottom deposits. The bioavailability of sediment-bound contaminants is difficult to expect because it's complex and variable composition of natural particles. Sediment-bound contaminants cause a particular risk to whole aquatic ecosystem, especially when the particles are ingested by benthic organisms because hydrophobic and persistent environmental chemicals tends to concentrate on sediment particles, from both suspended matter and bottom deposits (Guerrero *et al.*, 2003).

Sandy sediments and Asiatic clams were collected at seven sites along 150 km of the Rio de la Plata coast to assess the magnitude of trace metal pollution in the area. The metal concentration of Cr, Zn, and Mn in sediment was in range of 16 μg/g – 27 μg/g, 26 μg/g– 99 μg/g and 221 μg/g – 489 μg/g respectively. While the concentration of Cd, Ni, Cr, Mn, Cu, and Zn in bivalves was in the range of 0.5 μg/g – 1.9 μg/g, 1.3μg/g – 6.4 μg/g, 1.3 μg/g – 11 μg/g, 15 μg/g – 81 μg/g, 28 μg/g - 89 μg/g and 118μg/g – 316 μg/g respectively. Cu levels in Asiatic clams are among the highest based on the data above. The sediments showed a less clear pattern possibly due to their coarse nature (>98% sand) and higher proportion of mineral – associated residual metals. The clams showed a complex pattern due to the variability introduced by size-related factors and the natural dynamics of suspended particulate matter in the estuary. The metals concentration in sediments was higher than bivalves (Bilos *et al.*, 1998).

The concentrations of metals such as Cd, Cu, Ni, Mn, Fe, and Zn in four Moroccan estuarine sediments and bivalves were determined. Metal concentrations in sediment are high except for Cd. The metals may have multiple origins: urban, industrial and agricultural with atmospheric deposition a likely additional source for Cd and Cu. Metal burden in the *Scrobicularia plana* are also generally high and are related to sediment

CHAPTER TWO

LITERATURE REVIEW

The metal concentrations in bivalve tissues usually related to local environmental conditions such as sediment type, temperature, salinity, and ambient metal concentrations and loadings (Cheggour *et al.*, 2005). Heavy metals may enter aquatic ecosystems through natural processes such as soil leaching, rock erosion, and volcanic activity. However, there were another numerous sources that difficult to identify consist of originates from industrial activity, domestic effluents, and sludge from treatment plants (Rietzler *et al.*, 2001).

Bivalves such as mussels, clams and oysters are known to have high capacity for metal concentration, making them potentially very dangerous for consumers. The potential hazards of metals have long been recognized. Most metals are toxic to humans even though some may be beneficial at low concentrations. Metal concentrations and accumulation in bivalves are influenced by metal bioavailability, season of sampling, hydrodynamics of environment, size, sex, changes in tissue composition and reproductive cycle (Otchere, 2003).

The variation in fine-sediment metal concentrations depends on the type of season (wet and dry). This variability of metal concentration could be an expression of the natural sediments characteristics over the two seasons. These characteristics may be manifested level, especially for Mn and Fe and to a lesser extent Cu. Besides that, the metal concentrations in tissues of *Scrobicularia plana* were lower than those in surrounding sediment. Metals which are absorbed to surficial sediments that are ingested by bivalves may be assimilated, with efficiencies dependent on the sediment type, the animal species and its physiology, and the metal itself, metals that help comprise the sediment grain itself are not subjected to assimilation and hence are not available to benthic organisms (Cheggour *et al.*, 2005).

Study done at Sarawak environment revealed that mollusk species, *Brotia costula* and *Clithon* sp was contaminated with As in their tissues due to the gold mine effluents at Sungai Bau, a tributary of sungai Sarawak Kanan. The two mollusk species also accumulated few other pollutants namely Cu, Fe, Se and Zn (Lau *et al.*, 1998). Another study was done on heavy metals accumulation in the tissues of hydrothermal vent clam *Vesicomya gigas* from Guaymas basin Gulf of California. The highest amount of Cd (113.2 μg/g), Fe (403.2 μg/g), Hg (4.96 μg/g), Mn (18 μg/g) and Zn (844.8 μg/g) were detected in the gills whereas Cu (29.7 μg/g) and Pb (3.67 μg/g) occurred mainly in the mantle (Inzunza *et al.*, 2003).

The bivalve species, *Ruditape philippinarum* and gastropod species, *Rapana venosa* along the Chinese Bohai Sea have the potentiality as biomarkers. *Crassostrea talienwhanensis* possessed a much greater ability for bioaccumulation of Cu and Zn while *Rapana venosa* manifested the most bioaccumulation capacity of Cd. The concentration of Cu, Cd and Zn in some of the samples exceeded the maximum permissible levels established by WHO (Liang *et al.*, 2003). Bivalve species *Pletholophus swinhoei*, (*Unionidae* family) of freshwater bivalves, was evaluated as biomonitor for trace element pollution at two rural sites, An Thin and Duy Minh in the northern part of Vietnam. Significantly higher concentrations of Ba (140 μg/g), Be (0.03 μg/g), Br (5.9 μg/g), Cr (0.36 μg/g), Fe (1900 μg/g), Mn (1600 μg/g), Ni (0.88 μg/g), P (10800 μg/g) and Sr (27.3 μg/g) was measured in An Thin, whereas the only element with a significant higher concentration in Duy Minh was As (4.9 μg/g). Mussels of the *Unionidae* family contained heavy metals but the concentrations were below the legal limits for metals in mollusks compiled by Food and Agricultural Organization of the United Nation (Wagner and Boman, 2004).

The concentration of Fe, Cd, Zn, Mn, Cu, Ni, and Pb in clams at Echlo Island, Northern Territory, Australia was 94.84 μg/g - 419 μg/g, 6.0 μg/g - 20.3 μg/g, 1.09 μg/g - 6.28 μg/g, 2515 μg/g – 6256 μg/g, 0.47 μg/g - 3.18 μg/g, 1.71 μg/g - 5.64 μg/g, 0.45 μg/g - 2.17 μg/g respectively. Accumulation of Mn in razor clams was highest in spite of low concentration in the surrounding sediment. Therefore, it could be used as a bioindicator of Mn in tropical environment (Peerzada and Pakkiyaretham, 1992).

The concentrations of heavy metals (Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn) were measured in the bivalves (*Modiolus auriculatus* and *Donax trunculus*) collected from the Egyptian coasts of Mediterranean sea and *Brachlodonates* sp from the Egyptian coasts of Red sea. The average concentrations of the heavy metals analyzed exhibited the following decreasing order: Fe > Zn > Cu > Mn > Ni. Co > Pb > Cd for both Mediterranean sea and

Red sea. The analyses of Cd, Co, Ni, Pb, and Zn showed higher average concentration for samples collected from Red sea than that of collected from Mediterranean sea, while Fe, Cu and Mn showed the reverse result (Sikaily *et al.*, 2004).

Research done by Duquesne and Coll (1995) demonstrated that the main site of heavy metals accumulation in clam was found to be kidney and minor site was the muscle. The concentration of Cd, Cu, and Zn found in the kidney ranged from 62 μg/g – 138 μg/g, 2.1μg/g - 7.3 μg/g and 30 μg/g - 450 μg/g respectively. Cd concentration in the gills and mantle of clam increased significantly from all three sites, whereas they decreased in kidney and muscle. The variations are possibly due to the fact that kidney and muscle represent long-term storage sites of the metals while gills and mantle by contrast reflect shorter-term effects. The variation of Cd levels across sites and in the different tissues could also be related to the bioavailability of the metal. The bioavailability of trace metals is a key factor determining tissue metal concentrations. Most of the metals bound to particulate material are ingested through the digestive tract and stored in the kidney. Thus, the gills have been identified as the most important tissue for soluble-metal uptake. The kidney is the major site for metal storage based on the kidney of *T. crocea* that has the highest level of Zn and Cd, the greatest metal accumulation rates, and the highest intra-specific variability. However, the variability of the levels of metals in the gills was the lowest. The metal uptake rates in muscle of clams were the lowest. The accumulation rates of mussels were 4.8μg/g and 1.7 μg/g per day for mantle and muscle respectively.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling locations

This study was carried out at beaches located at Asajaya Laut and Muara Tebas during low tide (Figure 3.1). Samplings were carried out on September and November 2004 at Asajaya Laut, while on December 2004 and January 2005 at Muara Tebas. The sampling stations were classified into 3 major positions namely low tide station (S1), middle tide stations (S2, S3) and upper tide stations (S4, S5).

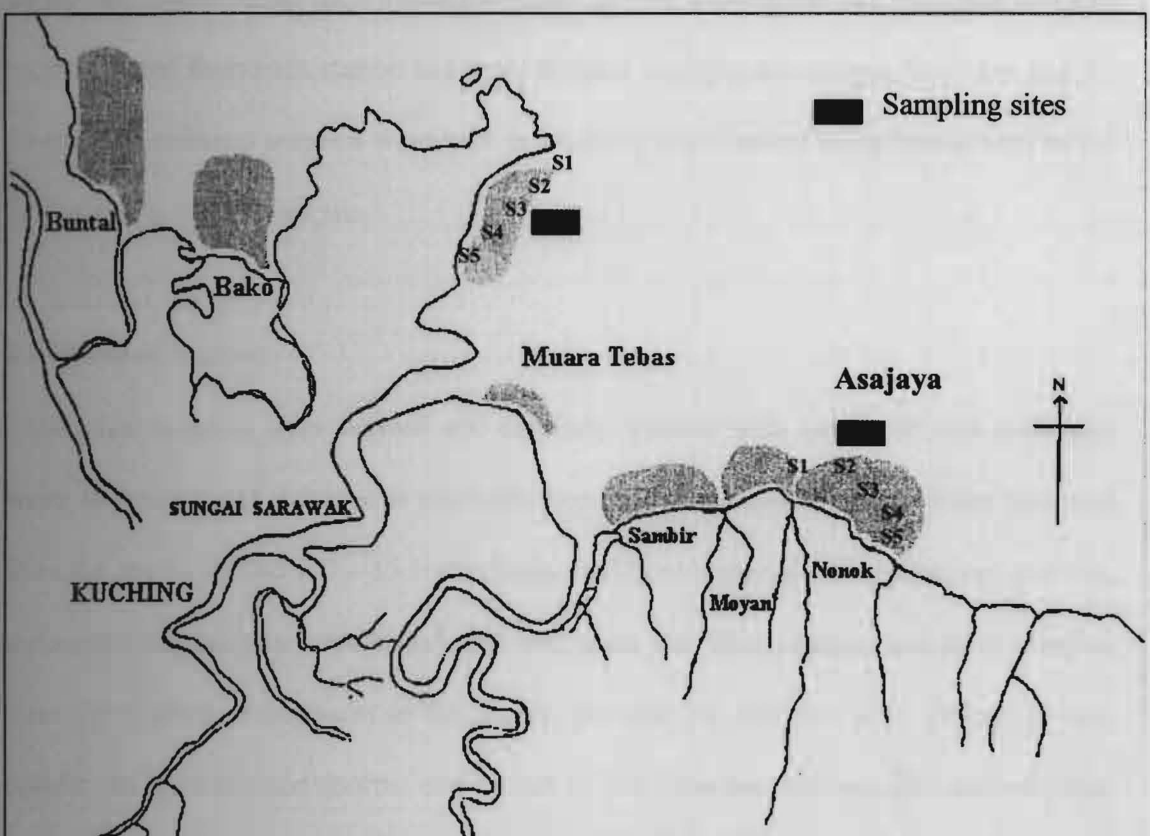

Figure 3.1: Map of the sampling areas

3.2 Sample collection

The razor clams were collected using long, elongated, slender stick of four feet in length a mixture of limestone powder, ashes and salt. Razor clam samples ranging between 3.0 and 10.0 cm in length were collected at both sites. Immediately after collection, the samples were washed with seawater to remove sediment before being kept in the labeled glass jar. The samples were kept in ice freezing box and transported to the laboratory.

Sediment samples were collected from each sampling stations around the area inhabited by the razor clams using the PVC tubes. Three to four random surface sediment samples were collected from each station and were divided into two sub-ranges, 0 - 5 cm and 5 - 10 cm. The sediment samples were kept in labeled plastic before being transported to the laboratory for further analysis.

3.3 Chemical analysis

Razor clam samples were thawed and carefully washed with tap water and deionized water to remove any extraneous material. For each station, soft tissues were removed from the shells, pooled (10 - 15 individuals) and homogenized. After cleaning process, the razor clam samples were transferred into glass jar. Then, tissues and shell samples were freeze-dried. Subsequent to the drying process, the samples were ground to fine powder using pestle and mortar and stored in polyethylene bottles. The polyethylene bottles containing samples were kept in a desiccator to eliminate excess moisture (Bahemuka and Mubofu, 1999). The composite homogenates were splitted into three sub-samples for replicate analyses.

Acid wet digestion method modified from Lau *et al* (1998) was employed to the razor clam samples. One gram of tissue sample was weighed and digested with 6 ml of concentrated nitric acid (63%) and 1 ml of 30 % hydrogen peroxide using hot plate digester. While for the shell samples, one gram of shell samples was digested with the mixture of 2 ml of concentrated nitric acid (63%), 5 ml of concentrated hydrochloric acid (37%) and 1 ml of 30 % hydrogen peroxide. The samples were dissolved for one hour and then digested for another one hour. The digested samples were filtered and diluted to 50 ml with deionized water. The concentrated hydrochloric acid was used for digestion to dissolved metals in the solution but not completely. Thus, nitric acid was used to dissolve all the remaining metals because it is the good oxidizing agent (Vandecasteele and Block, 1993). The levels of heavy metals in the digested were determined with a Perkin – Elmer 3110 Flame Atomic Absorption Spectrophotometer (FAAS).

Oven-dried sediment samples were ground to the size of 50 mesh. One gram of sediment samples was digested for three hours with freshly prepared mixture 1:3 of 20 ml of nitric acid and hydrochloric acid using hot plate digester. The digested content was filtered using 0.45 μm filter paper and diluted to 50 ml in volumetric flasks with deionized water. The samples were transferred into polyethylene bottles and preserved in the refrigerator prior to analysis using FAAS.

Fresh working standard solutions were prepared using AAS stock solution (1000 ppm). Blank samples were also prepared. All results are expressed as μg/g dry weight. Table 3.1

represents the parameters of the analytical instrument used for the metals detection in this study.

Table 3.1: Wavelengths, sensitivity of FAAS and detection limits

Element	Wavelength (nm)	Sensitivity (mg/L)	Detection limits (mg/L)	Range of standard solutions (ppm)
Cd	228.8	0.02	0.0005	0.5 – 2.0
Cu	324.7	0.03	0.001	0.5 – 2.0
Fe	248.3	0.04	0.003	1 – 4
Mn	279.5	0.03	0.001	1 – 4
Zn	213.9	0.011	0.0008	0.5 – 2.0
Pb	283.3	0.19	0.01	0.5 - 2.0

3.4 Statistical analysis

One-way analysis of Variance (ANOVA) is used to test whether the data means of the groups are different. Thus, one-way ANOVA was applied in this study to test the differences between razor clam metal concentrations at different sampling sites and sampling period, and also metal concentration in sediments at two different depths. Regression analysis (using Microsoft Excel 2000) was carried out to determine correlation coefficient (r) between the concentrations of metals in sediments with tissues and shells of razor clams.