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Determination of selected metallic elements in marsh clam, *Polymesoda expansa*, collected from Tanjung Lumpur Mangrove Forest, Kuantan, Pahang

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Abstract

Presence of metallic elements in organisms is one of the important topics in environmental pollution, toxicology and food safety. It has been extensively studied because of the impact of these elements on the ecosystem and health of the seafood consumers. This study provides information on the concentration of some essential metals (copper, Cu; zinc, Zn) and non-essential metals (cadmium, Cd; lead, Pb) in the commonly consumed marsh clam, *Polymesoda expansa*, sampled randomly from Tanjung Lumpur mangrove forest, Kuantan, Pahang. It also focuses on the human health risk assessment in terms of toxicity of these metallic elements. Closed acid digestion method was used to digest the samples and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used to measure the metal contents in the samples. The mean concentration of each metal in the tissues was recorded on a dry weight basis. The average values were: Cu, 15.5 µg/g.; Zn, 296 µg/g ; Pb, 2.31 µg/g and Cd, 1.04 µg/g . These values are within the permissible limits set by Malaysian Food Regulation (1985) and the World Health Organization (1982). The pollution load index (PLI) value of 2.94 indicates that there is no serious metal pollution in this study area. The correlation coefficient matrix between four metallic elements shows positive value, suggesting that the selected metals may have originated from the same source and accumulated in the clam tissue. A positive correlation was noted between the size of the clam and concentrations of Cd, Zn and Cu whereas it was negative for Pb. The present finding shows that the marsh clams from Tanjung Lumpur are safe for human consumption. However, a proper and continuous assessment should be done in order to monitor any changes in the contents of metallic elements since Tanjung Lumpur is near the Kuantan city center and the mining activities at the upstream area might at some stage cause metal pollution.

Keywords: Heavy metals, Marsh clam, Tanjung Lumpur, Mangrove forest, Permissible limits.

Introduction

Metallic elements occur in the natural environment. They are very persistent, nonresponsive to bacterial attack, do not dissipate, and can cause deleterious effects by producing various reactions in animals and plants (Clark et al., 1989). There is a great deal of interest in studying the metallic elements in the environment and their entry into aquatic habitats. Metals can be divided into two types according to their needs in organisms. Cu, Zn, Fe and Mn are needed by organisms for physiological processes and this makes them essential (Sathawara et al., 2004). On the other hand, non-essential (or potentially toxic metals) do not play any role in organisms and they might pose threat to human health when consumed for a long period even in low concentrations (Gu et al., 2015). Examples of these metals are Cd Pb and Ni. This study focuses on the analysis of Cu, Zn, Cd and Pb in tissues of marsh clam (*Polymesoda expansa*) from Tanjung Lumpur mangrove forest, Kuantan. Marsh clam occurs widely in tropical countries, including Malaysia (Palomares, 2017). Locally known as "lokan", it is one of the sources of animal protein for the coastal communities (Rintelen, 2011). It is also considered a delicacy by the tourists (Edward et al., 2009). The clam is supplied by the fisher folk to eateries and seafood restaurants, and freshly caught specimens are also sold at the landing areas along the mangroves.

Tanjung Lumpur is a traditional fishermen village located at Sungai Kuantan river mouth surrounded by mangrove forest (Kamaruzzaman et al., 2010) where diverse species of flora and fauna can be found. According to Redzwan et al. (2014), recreational activities, fishing, boating and marketing are concentrated in this area. These activities may release pollutants either from point or nonpoint sources (Hossen et al., 2014) which might accumulate to toxic levels. Marsh clam, like other filter feeders, has the ability to accumulate metallic elements from surrounding (Zahir et al., 2011) and this can lead to biomagnification through food chain and threat to human health (Dabwan et al., 2016). Serious diseases such as kidney failure and Parkinson's disease have been linked to metal intoxication in human bodies (Hossen et al., 2014).

Despite considerable data on concentration of metallic elements in bivalves, little information is available on this aspect on marsh clam from Tanjung Lumpur, Sungai Kuantan which is a major landing area. This study is intended to provide additional information on the baseline data on the accumulation of metallic elements in this bivalve and determine the safety of this seafood species for human consumption.

Materials and Methods

Sample collection

The study area was Tanjung Lumpur, a mangrove area located at Sungai Kuantan, Pahang (Figure 1). It is located at the river mouth area of Kuantan estuary (Kamaruzzaman et al., 2010), where the freshwater from Sungai Kuantan mixes with seawater from South China Sea. Mangrove ecosystem of this area is an important habitat for a diverse variety of organisms, ranging from bacteria, actinomycetes (Abdul Malek et al., 2015) to macro organisms such as the marsh clam.

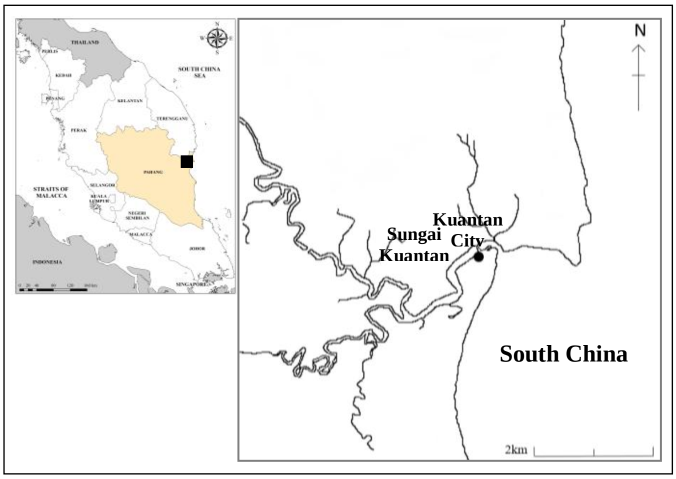

Figure 1. Map of Tanjung Lumpur mangrove forest, Kuantan

A total of 100 individuals of marsh clam, were collected randomly from the Tanjung Lumpur mangrove area in September 2016. Long metal stick was dug into the mud to detect the presence of the clam. When the metal stick established contact with the shell of the clam, a clicking sound was produced, showing the presence of clam. The clam was collected and kept at a low temperature in ice box and transported back to the laboratory for analysis.

Laboratory pre-analysis

The glassware and other facilities used for laboratory analysis were cleaned by 10% nitric acid, followed by rinsing with distilled water and drying. In the meantime, teflon beakers for closed acid digestion were heated using 67% nitric acid on a hot plate until the presence of bubbles was detected at the bottom of the beaker. After that, teflon beakers were rinsed with distilled water and dried in oven (Ong et al., 2017).

The clams sampled for observations were washed with water. Their shell and total length were recorded. Using ceramic knife, the samples were dissected to extract the tissue samples. Soft tissues were separated from the shell and weighted. The samples *in toto* were transferred into labelled petri dish, and dried in an oven at 60°C until constant weight was obtained. The dry samples were powdered using porcelain mortar and pestle.

Laboratory analysis

The analysis of metallic elements followed the procedures as described by Ong and Gan (2017). Dry powdered tissue sample weighing 0.05g was transferred into teflon beaker and mixed with 1.5mL Suprapur® 67% nitric acid for acid digestion process. Reagent blank and certified reference material (DOLT-4 Dogfish liver, National Research Council of Canada) were used simultaneously to control the accuracy of the procedure (Ong et al., 2016). The samples were kept in oven for 7 to 8 hours at 100°C to complete the digestion process. The acid-digested samples were transferred into centrifuge tubes and diluted to 10mL with deionized water. After processing, these samples were analyzed for the metallic element contents using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Data analysis

Data on metallic element concentrations were expressed in µg/g dry weight. However, in interpretation of human risk assessment, the data were expressed in µg/g wet weight. Statistical analysis of the data was performed using Microsoft Excel and SPSS software. The relationship between the size of marsh clam and metallic element concentration was established through correlation and regression analysis. Pollution load index (PLI) was determined by the method proposed by Angulo (1996) based on the formula:

$$\text{Pollution Load Index (PLI)} = \sqrt[n]{CF_1 \times CF_2 \times CF_3 \times \ldots \times CF_n}$$

where,

$$\text{Contamination factor (CF)} = \frac{\text{Concentration of metal}}{\text{Concentration of baseline data}}$$

For understanding the safety for human consumption of the marsh clam, provisional tolerable weekly intake was calculated to estimate the intake in an individual.

Results and Discussion

Table 1 summarizes the biological parameters of 100 individuals of marsh clam used in this study. In terms of size, the samples ranged between 53 to 73mm (average 62 mm) in length and 56 to 80 mm (average 67 mm) in width. For the wet weight, the tissue samples ranged between 5.6 to 17.8g with a mean of 9.7g. After drying the mean (dry) weight recorded was 1.3g in a range of 0.6 to 2.5g.

Table 1. Mean minimum and maximum fresh weight, dry weight, length and weight of the samples

	Fresh weight (g)	Dry weight (g)	Length (cm)	Width (cm)
Mean	9.7	1.3	6.1	6.7
Min	5.6	0.6	5.3	5.6
Max	17.8	2.5	7.3	8.0

Table 2 shows the percentage of recovery for Cu, Zn, Pb and Cd based on the certified reference material, with DOLT-4 dogfish liver as a guideline. All the metallic elements showed a good percentage recovery exceeding 90%. Therefore, it can be assumed that the analysis and methods used to determine the metal concentrations are reliable.

Table 2. Recovery test value for Cu, Zn, Pb and Cd

Metals	Certified value (µg/g)	Measured value (µg/g)	Recovery value (%)
Copper	31.2±1.1	29.0±1.6	94
Zinc	116±6.0	110±8.4	95
Lead	0.16±0.04	0.157±0.05	98
Cadmium	24.3±0.8	22.8±0.53	94

Table 3 shows the mean, minimum and maximum concentrations of metallic elements in the marsh clam from Tanjung Lumpur mangrove forest, Kuantan. The highest accumulation was recorded for Zn, followed by Cu, Pb, and the lowest for Cd. Both the essential metals, Zn and Cu, have a significantly higher concentration than the non-essential metals (Cd and Pb).

Table 3. Mean, minimum and maximum concentration of Cu, Zn, Pb and Cd (n=100)

Metals	Mean (µg/g dry wt.)	Min (µg/g dry wt.)	Max (µg/g dry wt.)
Cu	15.5±3.09	7.03	48.6
Zn	296±69.2	130	571
Pb	1.94±0.46	0.190	4.54
Cd	1.04±0.12	0.624	1.76

(n= number of sample)

A comparison of the concentrations of essential and non-essential minerals denotes the higher accumulation of the former in the clam. The possible explanation is because the marsh clams might need these essential metals for their living processes while the non-essential metals do not play any role in metabolism of the organism, and thus there is no preferential uptake for these substances (Hossen et al., 2015). Cu, for example, is needed for synthesis of haemoglobin (Sivaperumal et al., 2007) and deficiency of it might result in disruption in blood composition and nervous system (Dabbaghmanesh et al., 2011). Zn is important in metabolic activities of living organisms related to nutrition and biochemical pathways (Hossen et al., 2015). Both Cu and Zn can also be incorporated in blood pigments and proteolytic enzymes (Yap et al., 2009), causing their concentration to be higher compared to non-essential metals in the marsh clam's tissues.

Among the metallic elements studied, Zn has the highest accumulation value compared to Cu, Pb and Cd. Zinc that accumulated in marsh clam may come from its application as an anticorrosive agent (Natalia and Roberto, 2014) and antifouling product (Konstantinou and Albanis, 2004) that are used to paint the boats. Tanjung Lumpur is surrounded by fishing villages, and, therefore, it is anticipated that these pants are used in significant quantities. Besides that, there are also some restaurants serving customers with seafood and might discharge the leftover waste in the aquatic system nearby. All these possible sources might contain Zn that can leak into the aquatic habitat and accumulate in the water column and sediment. Marsh clams filter water to obtain food particles and this could facilitate entry of Zn in its body. The chemical property of Zn to oxidize easily helps in its easy absorption into soft tissues (Hossen et al., 2015).

Mean concentration of Cu in marsh clam reflects the second highest accumulation rate, after Zn, which is 15.5µg/g dry wt. Tanjung Lumpur is a traditional fishing village, so anti-fouling paint, fish landing facilities, boating activities and fuelling have been carried out for a long time there. These might have resulted in deposition of Cu in Sungai Kuantan and eventually, being accumulated in the tissues of marsh clam. Excessive exposure to Cu in human consumers can cause acute toxicity and health problems through impairment of the normal functioning of liver and kidney (Gorell et al., 1997).

In this study, mean concentration of Pb (1.94µg/g dry weight) is low compared to Cu and Zn. However, it is still higher when compared to Cd. Pb is an element which can produce toxic effects even in low concentration. Input of Pb into water may originate from burning of fossil fuels during boating activities (Bonvalot et al., 2016) and use of diesel fuel and leaded aviation gasoline (Jalal et al., 2009). Besides that, combustion of fossil fuels from vehicles nearby might also contribute to release of Pb into aquatic environment where it accumulates in the sediments and organisms. Continuous exposure of humans to Pb might cause mental retardation and kidney disease (WHO, 2000), and disruption of blood flow (Arnich et al., 2012).

Cadmium is a carcinogenic chemical (Rahman et al., 2012) which is linked to Parkinson's and Wilson's diseases in humans (Montgomery, 1995). Cadmium, unlike other metals, has a strong retention ability, allowing it to stay longer in the marsh clam tissues. Since the location of this study area is far from any agricultural or industrial site, the concentration of Cd in the marsh clam is low, within permissible limits. However, a continuous monitoring is necessary in view of highly toxic and carciogenic effects of this heavy metal.

The data obtained in this study was compared with the previous investigations on the same species but from different locations in Johor, Sarawak and Terengganu (Table 4). The pattern is basically similar where Zn element has the highest accumulation rate compared to Cu, Pb and Cd. Zinc and Cu concentrations in marsh clam from Kuantan are within the range as reported in those studies while Pb and Cd concentrations are lower. These differences are possibly due to the different anthropogenic activities in the sampling areas. Samples from Parit Jawa in Johor along the Malacca Straits had higher concentration compared to samples from the area located in the South China Sea region. This may be due to coastal region along Malacca Straits being relatively more industrialized and the intense shipping activities have led to pollution of the water. Generally, clam from Kemaman in Terengganu have higher metal content compared to Tanjung Lumpur, Kuantan and this is probably due to more industrial activities and the location of shipping port along the Kemaman river that might discharge these pollutants into the aquatic environment.

Edward et al. (2008) in their study found that the Cu concentration in marsh clam from Parit Jawa exceeded the maximum permissible limit established by Malaysian Food Regulation (1985). Their findings conclude that this is probably because of the presence of paddy fields that use pesticides containing Cu to protect the crop.

Table 4. Comparison of selected metallic elements concentration in marsh clam at different location

Location	Concentration (µg/g dry wt.)				References
	Copper	Zinc	Lead	Cadmium	
Parit Jawa, Johor	36.9	368	17.2	3.59	Edward et al. (2009)
Kuching, Sarawak	0.84	62.2	2.89	1.15	Yusoff and Long (2011)
Kemaman, Terengganu	15.9	56.6	6.9	2.3	Dabwan and Taufiq (2016)
Kuantan, Pahang	15.5	296	1.94	1.04	This study

Figure 2 shows the correlation between weight of marsh clam with the concentrations of Cu, Zn, Pb and Cd. Three of these metals (Cu, Zn and Cd) have a positive correlation with the size of the clam as is evident from the regression analysis, reflecting the changing nature of the bioaccumulation process in the marsh clam tissue (Kamaruzzaman et al., 2008). Besides, possible explanation of the pattern could be that as weight of the clam increases they consume more by the filter-feeding mode of life that in turn increases the metal intake (Fukunaga et al., 2011). Another reason might be the larger size of the clam providing more surface area in contact with the sediment, and the general environment, leading to more exposure (Chapman et al., 1998).

Lead has a negative correlation with the clam size. Kamaruzzaman et al. (2010) are of the view that Pb when accumulated in an organism's body up to certain extent, it tends to remain constant. This could reflect some regulatory mechanism but it is a topic for further research. Furthermore, smaller sized bivalve (younger specimens) might have a higher metabolism that makes them accumulate more metals than the bivalve of bigger size (Salami et al., 2008). In addition to the weight, there could be several other factors affecting accumulation of metals in the bivalves. In fact, metal accumulation depends on a complexity of factors which may be biological (species, age, genotype, phenotype, feeding habit, and reproductive state) as well as physico-chemical, (pH, temperature, organic content of substrate, rainfall and salinity (Boening, 1999).

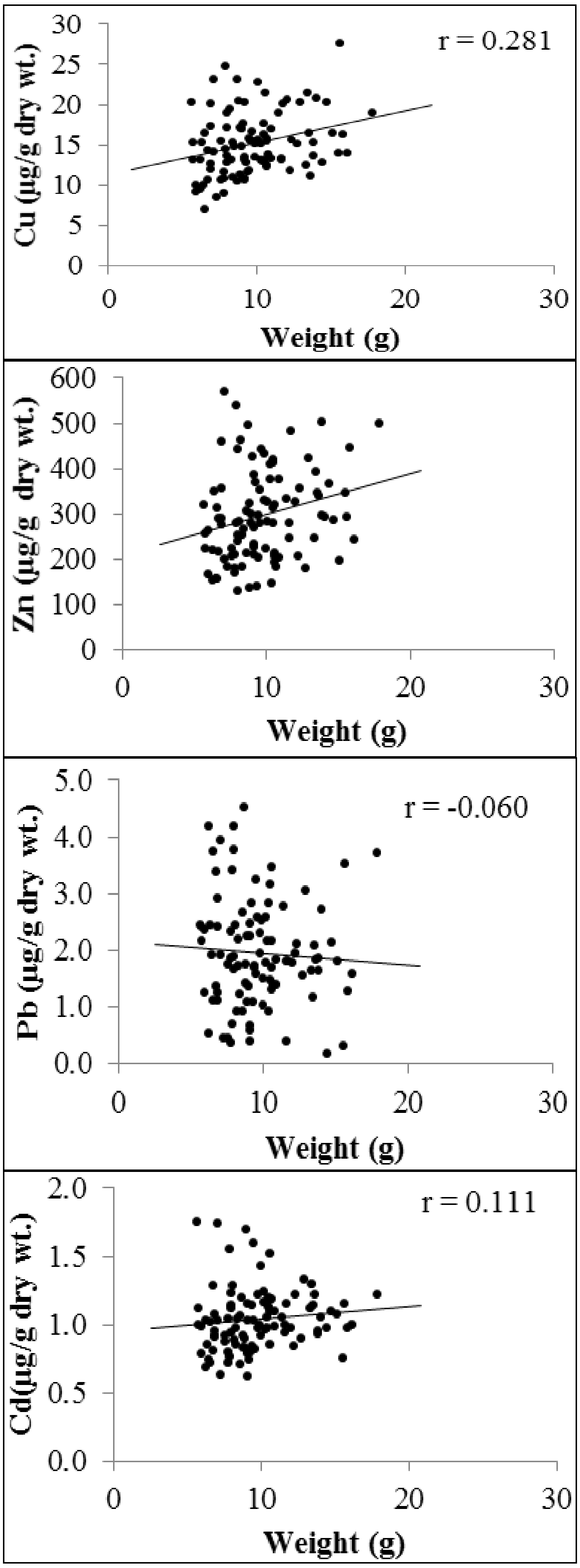

Figure 2. Correlation between concentration of metals studied and weight of in-toto tissue of marsh clam

Table 5 shows the inter-metal correlation between Cu, Zn, Pb and Cd in tissue of marsh clam at Tanjung Lumpur magrove area. Copper and Pb maintain a positive correlation, Cu-Zn, Cu-Cd, and Zn-Cd exhibit moderately positive correlation whereas Zn-Pb and Pb-Cd show a weak positive correlation. A positive correlation among metals signifies that the contamination of these metals might be from the same sources of pollution (Molawa et al., 2011). It can be assumed that all the metals studied might come from the boating and fishing activities nearby and also from the urban run-off. The sampling area of this study is close to a busy road and thus also receives Pb from vehicle emissions.

Table 5. Correlation among metals in the marsh clam

	Copper	Zinc	Lead
Zinc	0.488	-	-
Lead	0.722	0.139	-
Cadmium	0.429	0.492	0.100

According to Angulo (1996) PLI calculated value that reflects the level of contamination can be the basis of categorizing the sites. Thus, a site is considered highly contaminated if the PLI is equal to 100. More studies are needed to establish if PLI exceeding 50 requires remedial action and value below 50 is not any cause for concern. When heavy metal profile is examined it appears that Tanjung Lumpur mangrove forest has a low exposure to metal pollution and rather than any alarm for clam sourced from there, what is needed is a regular monitoring.

Table 6 shows that according to Malaysian Food Regulation (1985), Cu and Pb concentration in marsh clam from Tanjung Lumpur are below the permissible limit whereas Cd is slightly higher. As far as Zn is concerned, the concentration is higher than the Malaysian Food Regulation (1985) but it is still below the limit allowed by Ministry of Public Health Thailand (1986) and Australian Legal Requirement for Food Safety (1987). It is an essential metal that the body can tolerate in higher concentrations, but of course, within limits (Udechukwu et al., 2013). Excessive intake can cause bone problem. Therefore, further monitoring study needs to be carried out in order to make a better conclusion about the safety of eating marsh clam from Tanjung Lumpur, Kuantan.

Table 6. Concentration of permissible limits of heavy metals (μg/g wet wt.) set by different countries

Countries	Cu	Zn	Pb	Cd
Malaysian Food Regulation (1985)	30.0	100	2.00	1.00
Brazilian Ministry of Health (ABIA,1991)	150	250	10	5
Ministry of Public Health Thailand (MPHT,1986)	133	667	6.67	-
Australian Legal Requirements for Food Safety (NHMRC,1987)	350	750	-	10
This study	8.45	162	1.26	1.25

*Data on wet weight basis after recalculation according to the wet-dry weight ratio.

Provisional tolerable weekly intake (PTWI) is a tolerable intake standard set by Joint FAO/WHO Expert Committee on Food Additives (JECFA) when dealing with contaminant that hold threshold value to toxicity level (Herrman and Younes, 1999). According to World Health Organization (WHO, 1989), JECFA defined PTWI for indicating the quantity of a chemical that can be consumed in a week for whole life without posing any notable health risk. Table 7 shows the recommended value of intake for marsh clam from Tanjung Lumpur, calculated using the following formula:

$$PTWI\left(\frac{mg}{\text{kg bw}}\right) = \frac{amount\ of\ food\ ingested\ per\ week\ \left(\frac{kg}{week}\right)x\ metal\ concentration\ in\ the\ food\ ingested\ \left(\frac{mg}{kg}\right)}{kg\ body\ weight}$$

(Thapa et al., 2014)

The maximum weight of soft tissue that can be consumed was 0.37kg per week for an adult with the weight of 63kg (Table 7). This estimation is based on the Cd value calculated to determine if the metals exposure did not exceeded the PTWI value. According to this standard the maximum consumption weight of marsh clam from Tanjung Lumpur is 39 individuals per week for an adult with 63kg body weight.

Table 7. Estimated weekly intake of marsh clam according to PTWI value for an adult of 63kg body weight

	Cu	Zn	Pb	Cd
PTWI (mg/kg body weight)	3.5	7	0.025	0.007
PTWI (mg/kg for 63kg adult)	220.5	441	1.575	0.441
Maximum consumption (kg/week)	5.01	1.07	0.45	0.37
Maximum number marsh clam can be consumed (individual/week)	522	112	47	39

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