

Heavy Metals in Marsh Clam (*Polymesoda expansa*) as Bioindicators for Pollution in Industrial and Sand Mining Area of Kelantan River Basin, Malaysia

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Abstract

Industrial and sand mining activities have severely degraded the water quality of the Kelantan River, as well as the river ecosystem. However, there have been inadequate studies on the effect of industrial and sand mining on organisms in the Kelantan River. Therefore, this study assessed the concentrations of Pb, Cd, Zn and Cu in the bivalves of genus (*Polymesoda expansa*) marsh clams from the Kelantan River tributaries, namely the Geting River and the Semarak River, which are located near to an industrial and a sand mining area, respectively. More than 50 marsh clams were collected at these sites. Heavy metals in marsh clams were extracted using aqua regia methods and analyzed using a Flame Atomic Absorption Spectrometer (FAAS). The mean concentrations of Pb, Cd, Zn and Cu at the Semarak River were 4.61, 2.16, 194 and 6.20 mg/kg, respectively. Accordingly, the concentrations at the Geting River were 4.43, 2.50, 196 and 7.90 mg/kg, respectively. The pattern of metal concentrations within the whole tissue of marsh clams from the Geting River and the Semarak River were in the following order: Zn > Cu > Pb > Cd (concentration in mg/kg). The mean concentration of selected metals (Zn, Cd, Pb) in the studied samples exceeded the permissible limit set by the Malaysia Food Safety and Food Regulation 1985, the Food and Agricultural Organization, the World Health Organization, the Ministry of Health Thailand, and the Food and Drug Administration of United States (USFDA). Therefore, continuous monitoring of these bioindicators is essential to minimize the increasing health risk for aquatic life, as well as in food sources for human consumption, in the Kelantan River Basin.

Keywords: Marsh clams, Bioaccumulation, Heavy metals, Aquatic environment, Kelantan River

Introduction

Malaysia is a Southeast Asian country with high socioeconomic development. In recent years, the water environment in most areas of Malaysia has suffered a certain level of various types of pollution [1-3]. The Kelantan River, which is the major river in Peninsular Malaysia, has shown increasing levels of heavy metal pollution in recent years [4]. The Kelantan River has been profoundly affected by anthropogenic activities such as industrialization, agricultural practice and the discharging of domestic sewage. Sand mining in the Kelantan River has constantly degraded the river bed and has caused waterway siltation, thus deteriorating water quality, which has greatly depleted the fish and benthic organism populations [5]. Contamination of the river with various kinds of pollutants enriched with heavy metals caused by sand mining might be the main reason of these local biodiversity shifts. A small amount

of essential metals like Fe, Mn, Cu and Zn are vital to most living organisms for their growth [6,7] but will have a deleterious impact when concentrations of these metal pass the standard limits [8]. Excessive consumption of bivalves which have been bio-accumulated with nonessential metals such as Cd, As, Hg, Pb, Ni and Cr, which are toxic to organisms even at low concentrations, may imperil human health [9,10].

Bivalves are well known as filter feeders for suspended particles within water columns that may be contaminated with numerous contaminants including heavy metals obtained from either anthropogenic activities or natural emissions; this is becoming a source of heavy metal uptake in their bodies [11]. They tend to concentrate contaminants at a much higher level than the natural background concentrations in the environment [12]. Therefore, they are the most suitable bioindicators to observe heavy metal pollution in the aquatic ecosystem [13]. Marsh clams have been previously used as biomonitors to examine the ecological condition of a marine habitat [14].

The accumulated heavy metals in marsh clams can reach humans via the food chain and chronic illnesses probably can be induced in humans when these metal-polluted marsh clams are consumed. Therefore, it is crucial to determine the concentrations of heavy metals in marsh clams to assess the potential human health risks of marsh clam consumption. The permissible limits of each heavy metal in food have been set by the Malaysia Food Safety and Food Regulation 1985, the Food and Agricultural Organization, the World Health Organization, the Ministry of Health Thailand and the Food and Drug Administration of United States (USFDA) in order to determine whether marsh clams are edible. Hence, this study was conducted to evaluate the contamination levels of heavy metals in the Kelantan River using *Polymesoda expansa* as a bioindicator for 4 heavy metals (Cu, Zn, Cd and Pb).

This study provides necessary data for further research to monitor the levels of heavy metals in marsh clams in the Kelantan River tributaries, namely the Geting River and the Semarak River, which are located near to an industrial and a sand mining area, respectively. The information obtained from this study is new knowledge and is a preliminary study for the local and international scientific community. This new knowledge has contributed in terms of providing some useful detailed information about the level of contamination in the rivers around Kelantan, especially near to a sand mining area in Kelantan. Additionally, the information gained from this study might benefit policymakers when making judgments. Government agencies, for example, the Ministry of Health Malaysia, might use the data to calculate the health risks to humans of consuming marsh clams, as well as to suggest the consumption limits for marsh clams that are of high risk.

Materials and methods

Sample collection

Marsh clams in the range of 54 - 80.9 mm were collected in January - March 2018 from the mangrove area of the Geting River, which is close to the Tumpat industrial area near Kampung Geting in Kelantan at the coordinates E 102° 06' 22.248 N 06° 13' 35.893. Similarly, in Tok Bali Pasir Puteh, marsh clams were collected near to a sand mining area at the Semarak River, coordinates E 102° 30' 44.635 N 5° 51' 48.079. Both sampling locations of marsh clams are shown in **Figure 1**.

Sample preparation

The collected samples were stored in a laboratory refrigerator. The samples were divided into 3 different sizes, at ranges of 40 - 55, 56 - 75 and 76 - 90 mm. Clams get bigger as they get older. However, we could not predict the clams age based on size only. The edible parts of the samples were weighed, followed by undergoing a drying process. The dried samples were weighed and grounded into powder using a pestle and mortar. The homogenate samples were divided into 3 subsamples for the analyses of replicates. After preparation, the homogenate samples were stored at 20 °C in polypropylene containers until further analysis [15].

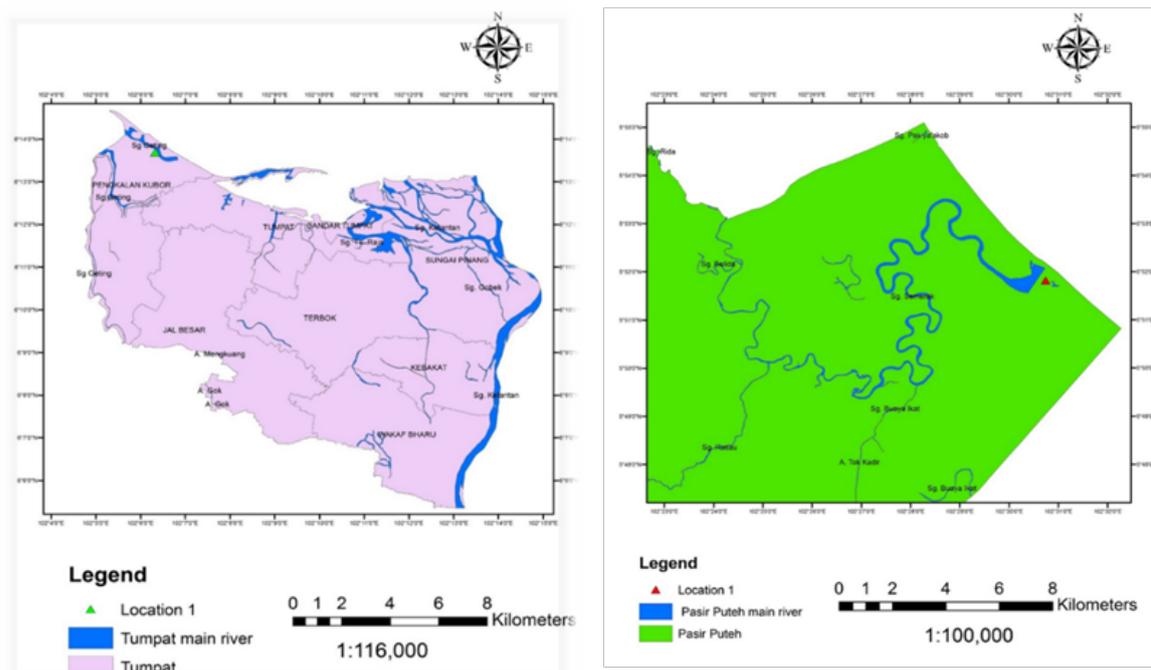


Figure 1 Locations of the Geting River, Tumpat Kelantan and the Semarak River, Pasir Puteh Kelantan, which are located near to an industrial and a sand mining area, respectively.

Analytical method

The homogenate samples were weighed into sizes of 2.5 g in petri dish. The analytical method involved an aqua regia digestion procedure consisting of digesting the samples on a hot plate with a mixture of HCl and HNO₃, as prescribed by Kmaruzzaman *et al.* [6] and Ebadi *et al.* [16]. The nitric acid reacted with concentrated HCl to form aqua regia; $3\text{HCl} + \text{HNO}_3 \rightarrow 2\text{H}_2\text{O} + \text{NOCl} + \text{Cl}_2$. After filtration, the samples were prepared for determination of Cd, Cu, Pb and Zn by using a Flame Atomic Absorption Spectrometer (F-AAS), model PinAAcle900f Perkin Elmer, with detection limits for Zn, Pb, Cd and Cu of Zn 0.75, 0.75, 0.9 and 1.5 ppm, respectively.

Result and Discussion

Heavy metals in marsh clams

The average concentrations of Cu, Zn, Pb and Cd in the tissue of marsh clams of both study areas, which were the Geting River and the Semarak River, are presented in **Figure 2**. The average concentrations of these metals were found in the following ranking: Zn > Cu > Pb > Cd. The metal levels in the marsh clam tissue of both places gave a similar ranking. The highest concentration was Zinc (Zn) and the lowest was Cadmium (Cd).

Based on **Figure 2**, the concentration of zinc (Zn) in the Geting River at Tumpat and the Semarak River at Tok Bali was significantly higher at 194 ± 0.09 and 196 ± 0.16 mg/kg, respectively. The Semarak River at Tok Bali showed the highest value of Zn concentration compared to the Geting River at Tumpat Kelantan, which might have been due to high mining activities, shipping, and fishing boat activities. Zn is an essential element for humans, but a deficiency, as well as an excess of its consumption by humans is harmful. Zn was found to be the highest concentration in aquatic organisms because zinc is extremely important for the immune system in organism bodies [17].

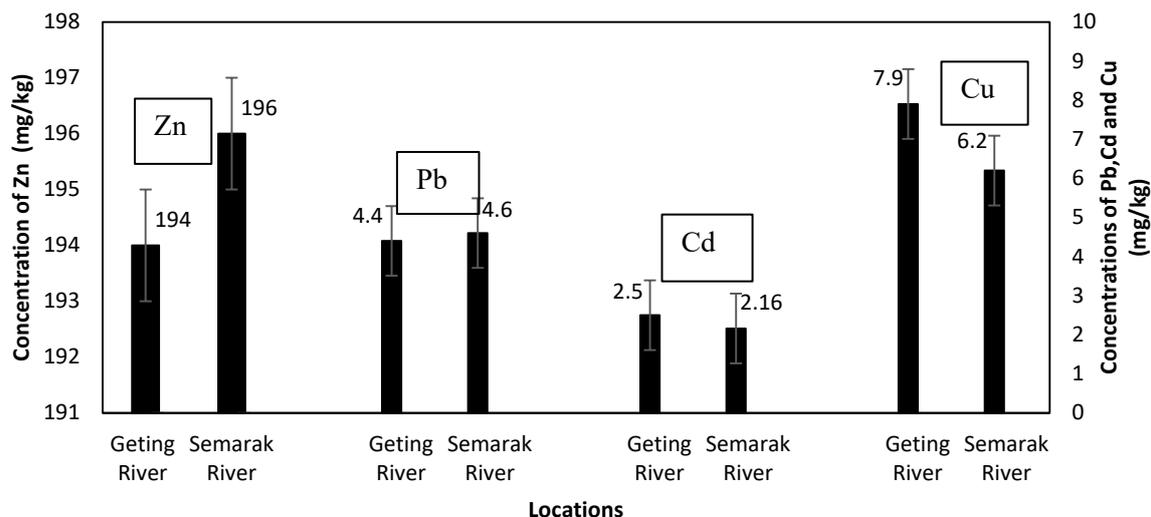


Figure 2 Concentrations of heavy metals (Zn, Pb, Cd and Cu) in Marsh Clams from the Geting River and the Semarak River, Kelantan.

Concentrations of lead (Pb) were detected in all marsh clams of both places. The average concentrations of lead in this species were 4.4 ± 0.01 and 4.6 ± 0.02 mg/kg at the Geting River and the Semarak River, respectively. These metals are toxic and are not metabolized in clams. Therefore, these metals are easily excreted from the flesh. Lead is a cumulative toxic trace metal and is one of the environmental contaminants which can promote serious damage to human health through damaging blood circulation, the central nervous system, the liver, and the kidneys [18,19].

Moreover, the concentrations of cadmium (Cd) in the marsh clams at the Semarak River and the Geting River were 2.2 and 2.5 mg/kg, respectively. Cd is an essential component for the manufacture of batteries, anti-corrosive coatings of metals and bearing alloys. Other studies have also found higher concentrations of heavy metals, i.e., Cd, in selected fish samples from the Kelantan River which were above the permitted levels set by regulations [20]. The concentrations of Cd in fish tissue from the Kelantan River were 0.076 mg/kg, as reported by Hashim *et al.* [20], lower than the value of Cd in marsh clams from this study. Concentrations of Cd in marsh clams were higher compared to those in fish because clams live in sediment, while fish live in water. Concentrations of heavy metals in sediment are higher compared to River water because sediment acts as an ultimate sink for inorganic and organic compounds including heavy metals, aliphatic hydrocarbons, and polycyclic aromatic hydrocarbons [1,3].

The concentrations of Copper (Cu) in the soft tissue of marsh clams were determined to be 7.9 ± 0.04 mg/kg from 2 different places at the Geting River, Tumpat Kelantan. However, in the Semarak River, the concentration of Cu was comparatively lower, at $6.2 \text{ mg/kg} \pm 0.05$, than the Getting River. The higher level of Cu might function as a biocide which is responsible for the protection of biofouling in marine vessels [21]. Copper is an essential trace metal for living organisms due to its vital role in allowing many critical enzymes to function properly [22].

Effects of size on the concentrations of heavy metals

Figure 3 show the sizes of marsh clams and the concentrations of heavy metals. Generally, the smaller size of marsh clams had higher metal concentrations than the bigger and medium sizes. For example, the concentrations of Cu in the Semarak River determined that the smaller size of marsh clams had higher concentrations than the medium and larger sizes of marsh clams. In addition, bioaccumulation of Cd was higher in the medium size, Cu was higher in the smaller size, and Pb was found in higher concentrations in the small size of marsh clams, indicating no consistency in bioaccumulation patterns.

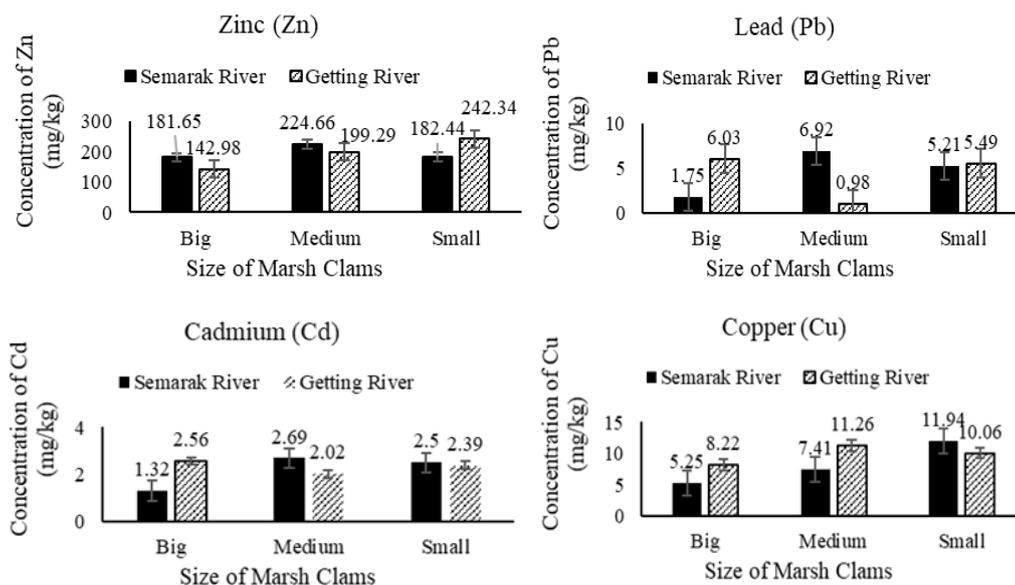


Figure 3 Variation between sizes of marsh clams and concentrations of heavy metals (Zn, Cd, Pb and Cu) in the Semarak and the Getting River, Kelantan.

Trace metal concentrations in clams depend on geographical, temporal, and biological factors [15]. Seasonal intense clam fishing is carried out at the Getting River and the Semarak River during the onset of rain in December. During this season there is considerable run-off from metal mining areas and from human activities. For example, the elevated concentrations of metals in the clams might originate partly from surface run-off in the Semarak River at Tok Bali, Kelantan. These findings tally with Tunisia, which has higher concentrations of heavy metals in clams during higher rainfall periods and in the period of most intense fishing activities [16].

In addition, the higher concentrations of heavy metals were mostly found in the small and medium sizes of marsh clams. This is because small shells have the ability to accumulate greater amounts of metals compared to big shells [23]. Allegedly, the bigger size of shells possesses a better ability to eliminate heavy metals. The rate of the metabolic process starts to slow down when it reaches the big-size stage. Therefore, the ability to accumulate metals also decreases when the marsh clam sizes are larger. Thus, the metal concentrations in the large-sized species were determined to be lower than that of the medium and small sizes. However, based on this study, the concentrations of metals in the samples was not directly proportionate to the sizes of the marsh clams.

The relationships between moisture content and the length of marsh clams were determined by using a regression test, as presented in **Figure 4**, and Pearson's correlation test. Subsamples of small, medium, and big individuals were distinguished with regard to size frequencies and shell characteristics. According to **Figure 4**, the moisture content of the marsh clams showed positive correlation with the length of the marsh clams, which indicated the size of marsh clams might influence the moisture content, especially for big and medium sizes. Based on Pearson's correlation, using a 2 tailed test, the results of the big size showed $r = 0.73$, $p < 0.05$ and for the medium size $r = 0.81$, $p < 0.05$ supported the influences of marsh clams size for water content, especially for the small and medium sizes. The higher moisture contents mean that marsh clams are sensitive to microbial spoilage, as well as meaning that the quality of marsh clams for longer preservation time will decrease the oxidative degradation of polyunsaturated fatty acids [24].

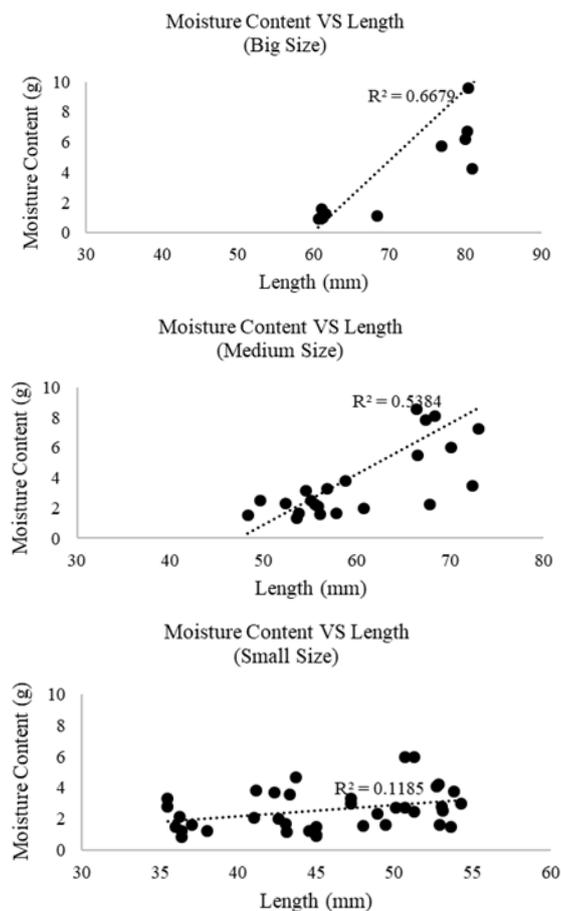


Figure 4 Correlation of moisture content and length of marsh clams in the Getting River and the Semarak River.

Pollution levels of *Polymesoda expansa* at the industrial and sand mining areas of Kelantan River

In developed countries, the limits of heavy metal concentrations in aquatic organism have already been set, in order to safeguard public health. Similarly, the Malaysian government has also set the maximum limits of contaminations for Zn, Pb, Cd and Cu based on the permissible limits recommended by the Malaysia Food Act, at 100.0, 2.0, 1.0 and 30.0 in the units of mg/kg, respectively [25]. In this study, Zn, Pb, Cd, and Cu concentrations were not only compared with the permissible limits set by the Malaysia Food Regulation but also the FAO/WHO, at 150.0, 0.5, 0.2 and 10.0 in the units of mg/kg respectively; the permissible limits set by the Ministry of Public Health of Thailand (MPHT) for Zn and Cd at 2.0 and 1.0 in the units of mg/kg, respectively; and the Food and Drug Administration of the United States (USFDA) for Zn, Pb, and Cd at 1.7, 10.0 and 3.7 in the units of mg/kg, respectively [26].

Based on **Figure 5**, only Cu concentrations in the marsh clams from the Semarak River at Tok Bali and the Geting River at Tumpat did not exceed the safety limit, of 30.0 mg/kg. The concentrations of Zn, Pb and Cd in the marsh clams from both the sites exceeded the permissible limits set by the Malaysia Food Regulation. The results were also compared with the regulations of other countries, such as with the Ministry of Public Health of Thailand (MPHT) and the Food and Drug Administration of the United States (USFDA). The results showed that only the concentrations of Cu were lower than the endorsed guidelines set by the Malaysia Food Regulation. The concentrations of Cd in the marsh clams from both places exceeded the permissible limits of the Malaysia Food Regulation, but the value did not exceed the safety limit from the USFDA, which by that standard meant the clams were safe for human consumption.

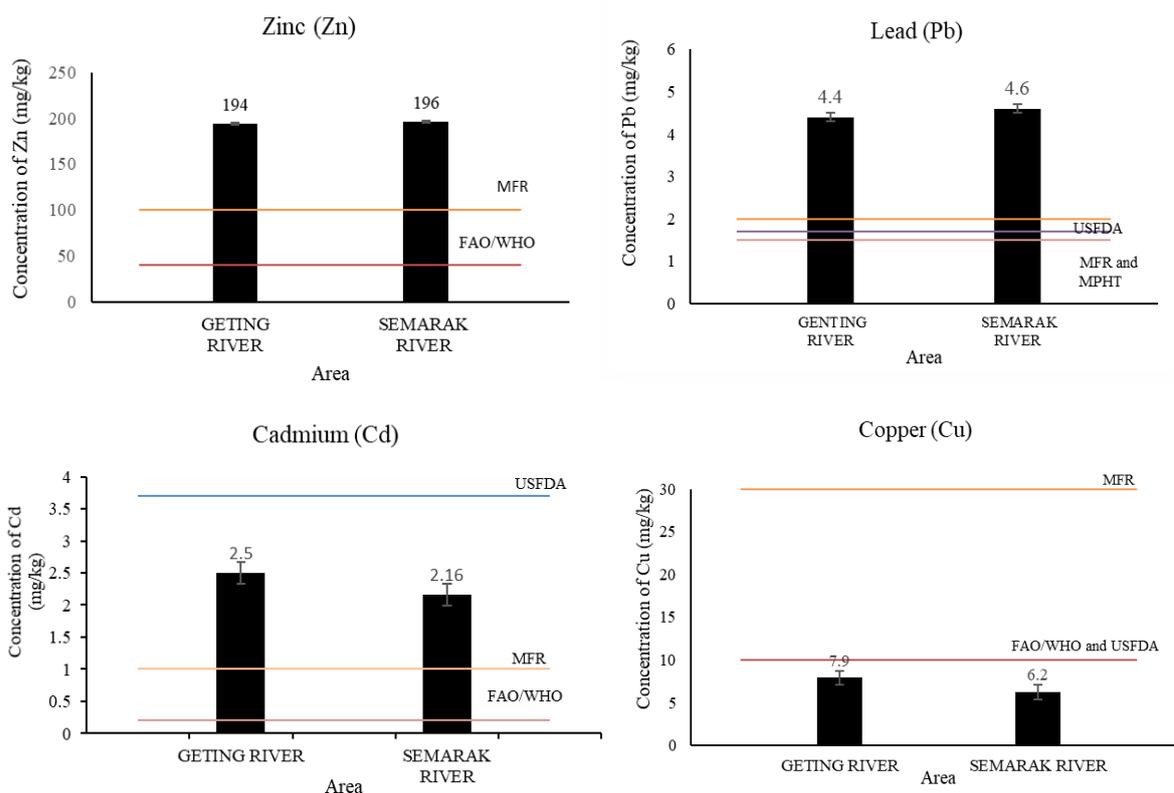


Figure 5 Zn, Pb, Cd and Cu concentrations in marsh clams with permissible limits from the Malaysia Food Safety and Food Regulation (MFR), the Food and Agricultural Organization (FAO), the World Health Organization (WHO), the Ministry of Public Health Thailand (MPHT), and the Food and Drug Administration of the United States (USFDA).

Conclusions

This study proved that heavy metals can be accumulated in the tissues of marsh clams. Metals transferred through aquatic food webs to marsh clams and humans are of environmental and human health concern. The concentrations of Zn, Pb and Cd in the marsh clams exceeded the maximum permissible levels as recommended by the Malaysia Food Regulation and the FAO/WHO. Therefore, precautions should be taken by humans during the consumption of these marsh clams, because long term consumption of these heavy metals via marsh clams could severely damage human health. In addition, it is very important to observe or monitor the concentrations of heavy metals in the Kelantan river near to industrial and sand mining areas in order to reduce the risk to human health.

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