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Concentration of Heavy Metals in Green–Lipped Mussel (*Pernaveridis*) from Muar Estuary, Johor

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ABSTRACT

A heavy metal pollution study was carried out in Muar Estuary, Johor by using green–lipped mussel (Pernaviridis) as a biomonitor. P. viridisfulfilled most of the important criteria as a biomonitoring agent for pollutant. It was a commercially important protein source in Malaysia, so in public health point of view, the concentration of pollutants found in their body was in concern. The concentration of selected heavy metals which included Cu, Zn, Cd, Pb, As and Hg in P. viridis were detected by using Inductively Coupled Plasma Mass Spectrometry (ICP–MS). The average concentration of Cu (17.86 ± 9.83 µg/g), Zn (66.16 ± 19.19 µg/g), Cd (0.36 ± 0.18 µg/g), Pb (0.74 ± 0.31 µg/g), As (7.59 ± µg/g) and Hg (0.12 ± 0.046 µg/g) were measured in dry weight. Levels of all metals studied do not exceed the permissible limit stated by Malaysia Food Regulation but level of As was beyond the limit stated by Food Regulation Singapore and Food Standard Australia. However, the PTWI calculated for Cu, Zn and Pb were below the value of PTWI established by Joint Expert Committee on Food Additives (JECFA), but PTWI for As was very close to the value stated. It poses a possibility of As poisoning by continuously consuming these green–lipped mussels. It was concluded that, P. viridis collected from Muar Estuary were safe to be consumed. Even though it does not pose a risk on acute toxicity but chronic toxicity might happened due to constantly consumption of this mussel.

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INTRODUCTION

Green–lipped mussel, Pernaviridis belongs to family Mytilidae. They prefer coastal estuarine area with depth less than 10m and were able to live up to 3 years [18]. They were distributed with a large geographical range in the coastal area of Indo–pacific region [8]. They can be found in intertidal and subtidal zones [24] and able to tolerate a huge range of salinity which is from 5.2ppt to 39.8ppt [21]. However, coastal estuarine areas are the place where P. viridis being cultivated [6].

According to Rajagopal *et al*. [21], marketable size for P. viridis was from 50mm to 60mm. It can growth up to 100mm, but their length of shell does not reflect the size of their meat [24]. Their growth rate can be 6 to 10mm per month [18] with an average of 83mm per year for their shell length (Rajagopal *et al*., 1998). However, an older mussel will have slower growth rate compared to a younger mussel due to the declining of metabolic rate. A matured male green–lipped mussel will have a milky–white color flesh while a matured female will have orange–red flesh [24].

As a suspension feeder they will filter tiny particles in the water column through their gills. They mainly feed on plankton and organic detritus [24]. P. viridis are sessile organism that found static on a hard substrate. Their sedentary lifestyle is a criteria to become a biomonitor which helps in determine the pollution status in their ambient water. Besides, they are commercially important as a protein source in Malaysia [30,8,24]. This species of mussel are recommended by many researcher as a good biomonitoring agent for heavy metal pollutions [14,6].

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Experimentation:

Muar estuary was situated at the southern part of Peninsular Malaysia which connected to the Strait of Malacca. This estuary was the sources of seafood such as fish, prawn, and oyster for local people. There were also some of the mangroves species inhabit beside the estuary. Over the year, the area beside Muar river estuary had been urbanized. Human activities including fishing, shipping activities were carried in this estuary. Beside, this river estuary was surrounded by town and industrial area which had the higher chances in receiving urban and industrial waste.

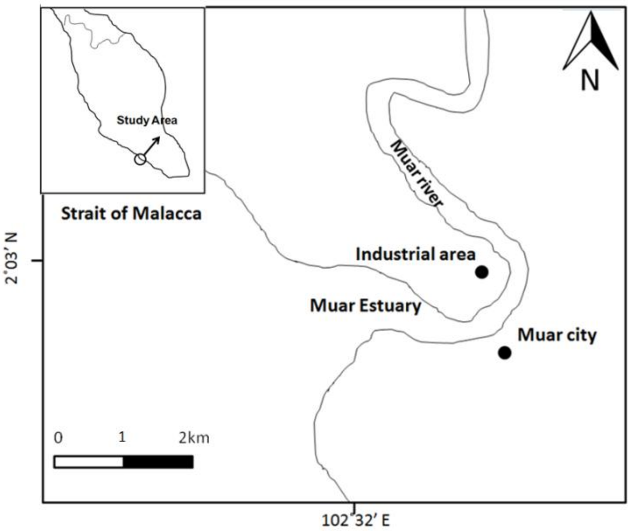

Fig. 1: Study area at Muar Estuary, Johor.

A total of 135 P. viridis were purchased from several local fishermen in Muar on 1st January 2015. After that, all the samples were stored in the ice box with low temperature. All the samples were then transferred to laboratory and stored in the freezer for further analysis.

All the samples were rinsed with water in order to remove all the sediment attached to it. Before dissection, the length of P. viridis was measured by using a vernier caliper and their weight was recorded. After that, P. viridis was dissected by using a dissecting set. The flesh of P. viridis was weight again before dried in the oven with 60˚C. Then dried samples were taken out once a constant weight was obtained and the dry weight was recorded. Dried samples were then being grinded with mortar and pestle to make it to finest size for acid digestion. Two replicates of standards (1946 dolt fish) and blanks were prepared in order to measure the accuracy of this experiment procedure [16].

0.05g of finely powdered sample was digested by 1.5mL of Suprapur nitric acid (HNO3) at 100˚C for 8 hours inside a sealed Teflon vessel. After cooling in room temperature, the digested sample solution on the teflon beaker was transferred into a centrifuge tube and diluted to 10mL with deionized water. The concentration of Cu, Zn, Cd, Pb, As and Hg in digested tissues were detected by using Inductively Coupled Plasma Mass Spectrometer (ICP–MS) [16].

The samples were sorted into two groups which is smaller size group (< 6cm) and bigger size group (> 6cm) (Putri *et al*, 2012). The concentration of selected heavy metals in the samples was measured in dry weight due to the differences of the moisture content in mussel tissues (Kamaruzzaman *et al*., 2011). The percentage recoveries for all heavy metals were calculated based on the certified value of metals concentration for standard reference material (SRM) (1946 dolt fish).

RESULTS AND DISCUSSION

A total 135 of green–lipped mussel (Pernaviridis) collected and analyzed. They were sorted into two groups which include small size group ranging from 34.8mm to 59.9mm and large size group ranging from 60.3mm to 76.6mm. The details of the length and weight for both of these groups were listed in Table 1.

Table 1: Length, whole mussel weight and muscle wet weight of P. viridis in small and large size group

Group of Size	Shell Length (mm) (min – max)	Whole Mussel Weight (g) (min – max)	Muscle Wet Weight (g) (min – max)	Total Number of Samples (n)
Small	34.8 – 59.9	2.09 – 10.31	0.51 – 4.54	83
Large	60.3 – 76.7	7.59 – 18.08	2.60 – 7.34	52
			Total	135

The concentration of the heavy metals was measured in ppm (µg/g dry weight). The mean concentration of Cu, Zn, Cd, Pb, As and Hg in P. viridis varies from the lowest (Hg: 0.12 µg/g) to the highest (Zn: 66.16 µg/g) in dry weight. The arrangement from the highest to lowest concentration of heavy metals was Zn > Cu > As >Pb> Cd > Hg. The details in the range and mean concentration for the selected heavy metals in P. viridis were listed in Table 2.

Table 2: The range and mean concentration (µg/g dw) of Cu, Zn, Cd, Pb, As and Hg for P. viridis

Heavy Metals	Range (µg/gdry weight)	Mean ± Standard Deviation (µg/gdry weight)
Cu	4.96 – 62.8	17.9 ± 9.83
Zn	30.2 – 130	66.2 ± 19.2
Cd	0.10 – 1.01	0.36 ± 0.18
Pb	0.24 – 1.56	0.74 ± 0.31
As	3.38 – 12.3	7.59 ± 1.65
Hg	0.03 – 0.28	0.12 ± 0.05

Heavy metal with the highest mean concentration was detected in Zn for both small (60.72 µg/g) and larger (74.83 µg/g) size groups of mussel (Table 3). Concentration of Zn range from 30.20µg/g to 117.00 µg/g for small group mussel and 42.20 µg/g to 129.60 µg/g for large group mussel. However, Hg has the lowest mean concentration, which is 0.12 µg/g for both small and large group of mussel. Range of Hg concentration for small and large group of mussel was 0.034 µg/g to 0.28 µg/g and 0.072 µg/g to 0.24 µg/g respectively. On the other hand, Cu was detected with second highest mean concentration in both groups of mussel among all heavy metals. The mean concentration of Cu was 16.77 µg/g and 19.61 µg/g for small and large size group of mussel respectively. Generally, the concentration of essential metals (Zn and Cu) was found higher than non–essential metals (Cd, Pb, As and Hg).

Pernaviridis inhabit coastal estuary area which had the higher chance to receive heavy metals due to a variety of anthropogenic sources. As a suspension feeder, they will absorb all essential and non–essential heavy metals that present in their food and their ambient environment (Rainbow, 2002). Heavy metals were considered as environmental toxic because they have the potential to bind with functional group of proteins hence hinder the normal functions of the protein, disrupt their structure, and inhibit the uptake of essential element to both marine organism and human.

Zinc was detected with the highest mean concentration in P. viridis with 66.16 µg/g dry weight (range from 30.20 to 129 µg/g dw) among all heavy metals. It is an essential element that was needed by organism for metabolic need and other enzymatic activities, so some amount of Zn will be store in their body [20,10]. This explains the higher amount of Zn in the sample.

Table 3: The range and mean concentration (µg/gdw) of Cu, Zn, Cd, Pb, As and Hg for both small and large size group of *P. viridis*

Group of Size	Total Number of Samples (n)	Heavy Metals	Range (µg/gdry weight)	Mean (µg/gdry weight)
Small	83	Cu	4.96 – 62.8	16.8
		Zn	30.2 – 117	60.7
		Cd	0.10 – 1.01	0.36
		Pb	0.24 – 1.56	0.71
		As	3.38 – 12.3	7.64
		Hg	0.03 – 0.28	0.12
Large	52	Cu	8.22 – 55.8	19.6
		Zn	42.2 – 130	74.8
		Cd	0.11 – 0.57	0.35
		Pb	0.25 – 1.42	0.79
		As	4.82 – 11.7	7.52
		Hg	0.07 – 0.24	0.12

Besides Zn, Cu concentration was the second highest detected in P. viridis with average of 17.86 µg/g dry weight. Cu was also categorized as an essential element for metabolic requirement, so certain amount of Cu will be stored in their body [20]. It was reported that Cu can be found in the antifouling paint for boat [26], since Muar Estuary is frequently travelled by boats. The source of this higher level of Cu might come from the antifouling paint for boat.

Arsenic was found with mean concentration of 7.59 µg/g dry weight. As present in seafood that was more commonly in organic form that is less toxic when compared to inorganic form [1]. Meanwhile, concentration of inorganic arsenic that found in fish and seafood are comparatively low which concentration will decrease with the increasing amount of total arsenic [1].

Lead was detected with concentration of 0.74 µg/g dry weight. Pb was a natural element that will be found in sea water, sediment and body of marine flora and fauna in low concentration [9]. It was reported that effluent from paint industries could be a possible source of Pb in river. [26]. Other possible common sources of Pb are from the atmosphere where it was burning of fossil fuel [13].

Cadmium concentration was ranging from 0.099 to 1.012 µg/g dw with average concentration of 0.36 µg/g dw. Cd concentration in shellfishes was found 10 times higher than in normal fishes [15]. It was reported that bivalves were not able to regulate Cd and tends to accumulate it in their body [12]. This was proved by experiment done by Yap *et al.* [29], result showed that P. viridis can accumulate up to 30 times higher concentration of Cd after expose to it. However it was stated that, Cd was present in food and environment with low concentration [7]. So, a low permissible concentration of Cd found in P. viridis is considered normal. Pb and Cd were non–essential element which means they do not have biological function to organism [17]. However a very low amount of them can be found in aquatic environment and the changes of their level was an indication of the increasing of anthropogenic activities [11].

The concentration of mercury was ranging from 0.034 to 0.28 µg/g dry weight with an average of 0.12 µg/g dry weight. Methylmecury was the organic form of mercury that commonly found through a food chain [4]. It was stated that, this form of mercury was largely found in seafood [5]. However, another study reported that, high concentration of inorganic mercury was not toxic to P. viridis because they were able to regulate Hg where significant amount of Hg was detected in the faeces of P. viridis [32].

When compared the concentration of non–essential (Cd, Pb, As and Hg) and essential element (Cu and Zn), essential elements had higher concentration than non–essential element in mussel. A same condition was reported by Kamaruzzaman *et al.* [10] which essential element had the higher concentration compared to non–essential element. According to Yap and Barwani (2012), Muar estuary had the potential on receiving waste discharge from household, shipping activities and other land used activities. This might be the sources of heavy metals detected in P. viridis. Other sources might be from the effluent outflow from industrial area. However, due to insufficient prove, we cannot determine where was the source of the heavy metals in the estuary.

Pollution Load Index (PLI) was an index established by Tomlinson (1980) to determine the bioavailability of metals in coastal area [3]. It was used to determine the pollution status of a study area. PLI with 100 indicating a critically polluted site and need an urgent study in order to minimize their pollution status while PLI value more that 50 showed that a detail study was needed to observe the polluted area [3]. The baseline concentration for Cu (6.31 mg/kg dw), Zn (53.82 mg/kg dw), Cd (0.25 mg/kg dw), Pb (1.27 mg/kg dw), As (1.00 mg/kg dw), and Hg (mg/kg dw) was used to calculate the cofactor for each of the metals [3,29]. PLI for Muar Estuary ranging from 9.1 to 35.5 with average number of 19.8.These value was below 50 which indicated that severe adjustment was not needed [3].

The permissible level of heavy metals stated by different countries was listed in Table 4. Based on the concentration of heavy metals in dry weight of mussel, the average concentration of copper, zinc, cadmium, lead and mercury detected in *P.viridis* was below the safety level stated, but the concentration of arsenic (7.59 µg/gdw) was exceeded the permissible level stated by Food Regulation Singapore and Food Standard Australia which is 1 µg/gdw. On the other hand, all metals concentrations do not exceed the permissible level stated by Malaysia Food Regulation based on the wet weight.

Table 4: Permissible level of heavy metals concentration (µg/g) for food listed by different countries

Guidelines	Weight Basis	Cu	Zn	Cd	Pb	As	Hg
Malaysia Food Regulation (1985)	Wet	30.0	100.0	1.0	2.0	500.0	–
Food Regulation Singapore (2006)	Dry	20.0	–	1.0	2.0	1.0	0.5
Food Standard Australia (2013)	Dry	–	–	2.0	2.0	1.0	0.5
Muar Estuary	Dry	17.9	66.2	0.4	0.7	7.6	0.1
	Wet	3.58	13.2	0.08	0.14	1.52	0.02

Exposure to Cu and Zn does not bring any adverse effects because it was needed by human body for enzymatic activities [20]. However exposure to a very high amount of Cu is a problem. When compared with Zn, Cu is five times more toxic than Zn. Chronic exposure to Cu can cause problem to liver, kidney and digestive system hence make people feel weak and sleepy while acute Cu exposure can cause death. However, cases of Cu toxicity were rarely been reported.

Excessive intake of Zn can cause effect in digestive system which leads to abdominal pain, vomiting and diarrhea [5]. However, acute exposure to Zn through foods is rare. It was also reported that, excessive Zn in body can cause anemia, Cu deficiency, impairment of immune system.

In addition, ingestion of high amount of Pb can bring adverse effect to human body especially children because of their higher metabolic rates. When Pb enter into our body, the main route for them is pass through our bloodstream and then to the bone and teeth. Generally it can affect almost all the organs and systems of human body, at the same time, hinder and replace the role of calcium. Chronic exposure to Pb can cause vomiting, abdominal pain and temporary loss in memories.

Even though the mean concentration of Cu, Zn, Pb and As exceeded the permissible level stated, the risk on getting heavy metals poisoning still base on the amount of mussel that being eaten [5]. In order to determine the potential health risk of consuming *P. viridis* collected from Muar estuary, weekly intake of this mussel by people was calculated. According to Yap and Tan [28] an estimated of 2.5g per day of green–lipped mussel was consumed by people in Malaysia which is equal to 0.0175kg per week. This value was used as the weekly intake for calculating the PTWI of selected heavy metals in *P. viridis*. The average of human body weight was 62kg as stated. The PTWI for Cu, Zn, Cd, Pb, As and Hg were calculated and listed in Table 5.

Based on the results, PTWI for all the selected heavy metals were far below the tolerable intake stated by FAO/WHO with the exception of As with PTWI calculated, 14.68 µg/kg. It was found that PTWI for As was very close to the value of PTWI established by FAO and WHO which is 15 µg/kg. This possesses a potential health risk on people who consumed these mussels continuously.

As can be separated into organic and inorganic form. It was reported that, most of the As compound found in seafood was in organic form which is not as much toxic as other form of As and it can be excreted by human body [23,1]. Inorganic As can cause problems to lung, vascular, and skin which than lead to cancer [23,1]. Acute exposure of inorganic As can bring toxicity to nervous systems [1].

Table 5: Provisional Tolerable Weekly Intake (PTWI) of Cu, Zn, Cd, Pb, As and Hg in *Pernaviridis.*

Heavy Metals	Mean Concentration (µg/kg wet weight)	PTWI established by FAO/WHO and EFSA (µg/kg)	PTWI Calculated (µg/kg per week)
Cu	122180	3500	34.49
Zn	460980	7000	130.12
Cd	2530	2.5	0.71
Pb	5140	25	1.45
As	52000	15	14.68
Hg	840	1.61	0.24

Even though most of the compound of As found in shellfish is in organic form, it was still some small portion of inorganic As detected [2]. So, it was still in concern because inorganic As is very toxic even in small amount. Based on previous study, there was no As concentration in *P. viridis* detected. So, a detail study was needed to find out the possible sources of As in Muar estuary and at the same time determine the portion of inorganic As in *P. viridis*.

Conclusion:

By comparing the mean concentration of metals between small and large size group mussel, essential element such as Cu and Zn were found in higher concentration in larger size group of mussel. However, Cd and As concentration were found higher in small size group of mussel with small differences. Besides, essential elements were positively correlated with shell length and dry flesh weight. When compare the mean concentration of this study with the permissible level of heavy metals stated by different country, the level of Cu, Zn, Pb and As do not exceed the permissible limits stated by Malaysia, Singapore and Australia. However, based on the Provisional Tolerable Weekly Intake (PTWI) calculated, As had the value of 14.68 µg kg–1per week which was very closed to the value of PTWI established by FAO and WHO. This indicates a potential risk of As toxicity in human body by consuming P. viridis. However in order to determine the possible sources of heavy metals, a detail study has to be done to find out the potential sources of metals in Muar Estuary. This study showed that, consumption on these P. viridis collected poses health risk to human; however any risk was depended on the amount of P.viridis being consumed.

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