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Bioconcentration of Aluminium and Copper in Giant Freshwater Prawns (Macrobrachium Rosenbergii) From Several Rivers in Kuantan, Pahang Malaysia

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Abstract**: Mining activities at the upstream of the Kuantan River basin can be resulted to adverse impacts for both human and aquatic lives. The heavy metal contamination of aquatic environment has gained the public attention. In fact, in recent decades, urban activities and industrial waste have led to an increase in contamination of heavy metals in the water bodies which may have direct effect on aquatic ecosystem. Therefore, this study aims to determine the level of Aluminium (Al) and Copper (Cu) in the muscle and gills of giant freshwater prawns (Macrobrachium rosenbergii) collected from five selected rivers in Kuantan, Pahang. Field sampling was conducted between September and December 2017. The giant freshwater prawns were digested and then analysed with inductively coupled plasma-mass spectrometry. The obtained results revealed that, Al levels in muscle of M.rosenbergii among all rivers occurred in the rank order: Pandan river>Riau river> Pinang river> Kuantan>Balok river, while in gills: Pandan river>Kuantan river>Balok river>Pinang river>Riau river. The concentration of Cu in muscle were in order of: Pandan river>Pinang river> Riau river> Balok river > Kuantan river, whereas Cu in gills were: Pandan river>Balok river>Kuantan river>Pinang river > Riau river. The highest content level of Al and Cu in both prawn's muscle (14.03 ± 3.55 and 12.28 ± 3.87) mg/kg and gills (7.06 ± 2.53 and 12.28 ± 3.87) mg/kg were caught from Pandan River. In risk and safety assessment, the metal concentrations in the edible muscle of prawn were found to be below the established limits by WHO (1982), MFA (1983, US FDA (2001), and JECFA (2000). This study suggests that the prawns caught from Kuantan river are safe for human consumption.

Keywords: Heavy metals, Aluminium, Copper, Macrobrachium rosenbergii

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I. INTRODUCTION

Bioconcentration of metal elements in crustaceans has been a controversy in Malaysia over the last two years due to largely unregulated open-cast bauxite mining gripping Pahang province and tonnes of bauxite were being transported out of the region. Crustaceans are able to accumulate chemicals in their tissues from the surrounding environment. Metal pollution in river system is a common environmental problem due to rapid industrialisation, mining activities, and agricultural waste. Enrichment of trace elements in water system can result to water being unsuitable for drinking as well as threat to human health.

Briefly, the longest river in Peninsular Malaysia, Kuantan River is located in the east coast of Peninsular Malaysia history shows that Kuantan river is important for recreation and ecology sustainability. It has a diverse species of fishes, which are regularly marketed for human consumption. Kuantan communities are directly or indirectly dependent for fishing in Kuantan River and its estuary. However, Kuantan River and its tributaries experience heavy metals pollution due to uncontrolled bauxite mining carried out in Bukit Goh which is located about 6.9 km to Kuantan River by road.

Metal elements are the major constituents of aquatic pollutants through their bioaccumulative and nonbiodegradable properties [1]. Humans may be contaminated by pollutants associated to aquatic systems by consumption of contaminated crustaceans [2]. Thus, bioconcentration of heavy metals in fish is important in fish species from bio-magnifications of metabolic capability, food chains and feeding habits [3].

Issue on the accumulation of metal in aquatic ecosystems such as river had been raised in Malaysia. The occurrence of this issue is due to the water pollution by heavy metals that are released from anthropogenic activities, such as bauxite mining.

In 2013, bauxite mining had started in Pahang in small-scale. Due to the high demand of bauxite, many irresponsible parties used unregulated and unscrupulous mining methods in bauxite mining in Pahang in order to generate more profit. Eventually, these irresponsible parties conducted bauxite washing illegally at nearby rivers leading to the occurrence of ‘red river’ with high level of heavy metals content [4].

The quality of water is determined by inland activities. However, comprehensive sampling and monitoring are necessary to confirm this matter. This study focuses to analyse the bioconcentration of aluminium and copper in the tissue of *Macrobrachium rosenbergii* collected from five different rivers in Pahang Rivers.

II. MATERIAL AND METHODOLOGIES

The sampling area of study area was located in Balok River, Pinang River, Pandan River, Kuantan River, and Riau River which are located near bauxite mining in Bukit Goh, Kuantan.

A total of 20 individuals of giant freshwater fish were used in this study. In terms of size, the samples ranged between 20 to 30cm in length and 3 to 3.8 cm width. For the wet weight, the tissue samples ranged between 9.8 to 19.0 g with a mean of 5.0 g. Dry weight recorded was 2.0 g in a range of 1.5 to 5.3 g.

Table 1: Sampling sites of *M.rosenbergii*

	Locations
Kuantan River	3°51'27.0"N 103°12'27.0"E
Riau River	3°51'53.9"N 103°13'30.5"E
Pinang River	3°50'26.5"N 103°15'29.5"E
Pandan River	3°77'72.7" N 103°18'67.1" E
Balok River	3°56'42.2"N 103°22'15.1"E

Sample collection

Field sampling was conducted during September and December 2017. Giant freshwater prawn (*Macrobrachium rosenbergii*) samples were collected from five rivers located near bauxite mining in Bukit Goh, Kuantan. The gill nets were set up for at each sampling station. Each net was inspected from morning to evening. The specimens were kept in an ice box, before transported to the laboratory for the analysis. The total fish length and width were recorded before having stored under -20 ºC until dissection was performed.

Dissection

The prawn samples were thawed at room temperature. Before dissection, the samples were cleaned several times with ultrapure water to remove foreign particles such as debris and other external adherent. The muscle and gills tissues had been removed by using stainless steel scalpels and knife. The samples were dried in an oven at 80 ˚C for overnight until samples reached constant weight. The range of dry weight of the samples was 10-15 g. On the next day, the samples dry weighted prior to analysis [5].

Digestion

Acid digestion method was used to digest the muscle samples based on the Association of Official Analytical Chemists [6]. The sample was digested with 10 ml of 69 % of nitric acid before being left overnight at room temperature. On the next day, the samples were digested on the block thermostat at 100 °C for 2 hours before cooling it down for 1 hour. After that, about 2 ml of 30 % hydrogen peroxide was added to the sample and heated for 1 hour until it formed a clear solution. Then, it was allowed to cool before solutions were filtered through 0.5 mm filter paper into 25 ml of volumetric flask. Lastly, deionised water was added into volumetric flask until the volume reached 25 ml. The concentration of heavy metals was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Metal calculations

The concentrations of gills and muscle were calculated by using the formula below [7]:

$$C = A \times B / M \quad (1)$$

C= Actual concentration (mg/kg)

A= Digested concentration (ICP) (mg/L)

B= Volume digested (L)

M= Weight of dried sample digested (kg)

Statistical analysis

One way analysis of variance (ANOVA) was used to indicate the significant differences of metal levels in *Macrobrachium rosenbergii* among different sites. All calculations and statistical analyses were performed using Microsoft Excel 2010.

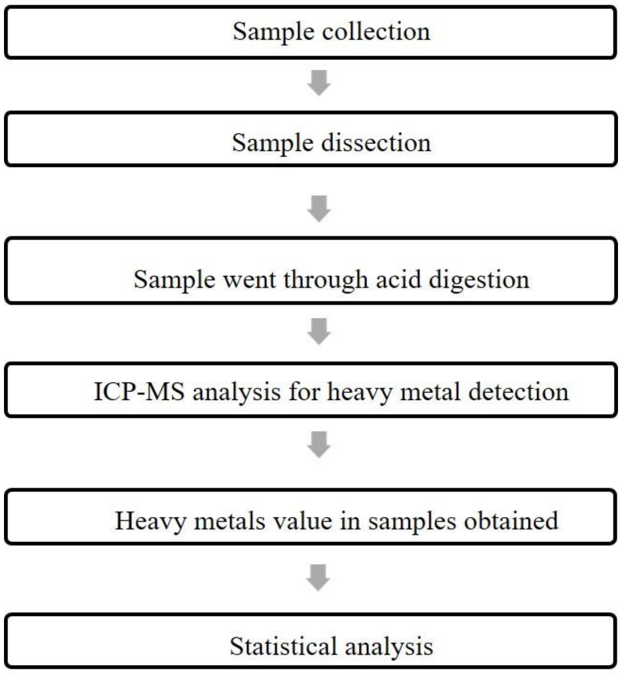

Fig. 1. *Research methodologies flowchart*

III. RESULT AND DISCUSSION

The sampling areas of study area namely Balok River Pinang River, Pandan River, Kuantan River, and Riau River are located near bauxite mining in Bukit Goh, Kuantan. A total of 20 individuals of giant freshwater fish were used in this study. In terms of size, the samples ranged between 20 to 30cm in length and 3 to 3.8 cm width.

For the wet weight, the tissue samples ranged between 9.8 to 19.0 g with a mean of 5.0 g. After drying, the mean (dry) weight recorded was 2.0 g in a range of 1.5 to 5.3 g.

Table 2: The concentration of heavy metals (mean ± SD) in dried basis of *M.rosenbergii*

	Mean heavy metal content mg/kg			
	Al		Cu	
	muscle	gills	muscle	gills
	Present study			
Kuantan River	1.79 ± 0.73	0.48 ± 1.00	2.00 ± 1.97	0.03 ± 1.00
Riau River	0.54 ± 0.13	6.33 ± 1.00	0.69 ± 0.26	2.49 ± 1.00
Pinang River	0.70 ± 0.16	1.20 ± 0.53	2.96 ± 2.06	1.57 ± 0.81
Pandan River	3.95 ± 2.58	14.03 ± 3.55	7.06 ± 2.53	12.28 ± 3.87
Balok River	0.38 ± 0.71	5.57 ± 1.00	0.71 ± 1.00	4.03 ± 1.00
	Previous study			
Kerang River, Sarawak. Idrus (2018) [9]	-	-	1.00	-
Kuala Selangor, Selangor Ashraf (2011) [10]	-	-	13.2	-

Concentrations of Al in muscle tissue of *M.rosenbergii* in Kuantan River, Riau River, Pinang River, Pandan River, and Balok River were (0.54 ± 0.13, 1.79 ± 0.73, 0.70 ± 0.16, 3.95 ± 2.58, , 0.38 ± 0.71) mg/kg and while in gills showed (6.33 ± 1.00, 0.48 ± 1.00, 1.20 ± 0.53, 14.03 ± 3.55 and 5.57 ± 1.00) mg/kg respectively. The highest concentration of Al in muscle (3.95 ± 2.58mg/kg) was found at Pandan River, while the lowest concentration of 0.38 ± 0.71 mg/kg was recorded at Balok River. In gills, the results also showed the highest Al concentration in Pandan River with 14.03 ± 3.55 mg/kg that was higher than the values recorded at other rivers. The lowest Al was found in Riau River with 0.48 ± 1.00mg/kg. Aluminium is reported as most abundant element in the earth's crust. When Al is present in high concentrations in the water, it was identified as a toxic agent to aquatic freshwater organisms. Al can cause effects on gill-breathing animals such as fish and invertebrates, and can lead to osmoregulatory failure. All samples contained acceptable levels of Al, but no comparison could be made as no safe level was defined for these elements.

Based on Table 2, the concentration of Cu in muscle of *M.rosenbergii* in Pandan River, Balok River, Kuantan River, Pinang River, and Riau River were recorded (0.69 ± 0.26, 2.00 ± 1.97, 2.96 ± 2.06, 7.06 ± 2.53, and 0.71 ± 1.00) mg/kg while in gills were recorded (2.49 ± 1.00, 0.03 ± 1.00, 1.57 ± 0.81, 12.28 ± 3.87,and 4.03 ± 1.00) mg/kg. The highest and lowest Cu levels in muscle were found 7.06 ± 2.53 mg/kg at Pinang River and 0.69 ± 0.26mg/kg in Pandan River, while the highest and lowest Cu levels in gills were recorded 12.28 ± 3.87 at Pinang River and 0.03 ± 1.00 at Kuantan River. Cu is an essential metal need in various metabolic activities and is well regulated in the human body [8]. However, high exposure of Cu may lead to anaemia, gastrointestinal problems, kidney and liver failure. The average Cu concentration found in the present study was lower than that reported for *M.rosenbergii* from Kerang River, Sarawak [9] and Kuala Selangor, Selangor [10]. The bioconcentration of Cu was observed in the organs mainly implicated in metal intoxication.

The muscle of *M.rosenbergii* from Balok River had the lowest Al content (0.38 mg/kg) and Kuantan River had the lowest Cu content (0.68mg/kg) in the samples. Meanwhile in gills, both Al and Cu content were lowest in Riau River (0.48 and 0.03) mg/kg. Al and Cu contents in both prawn's muscle and gill collected from Pandan River were the highest (3.95 and 7.06 mg/kg) and (14.03 and 12.28) mg/kg.

Table 3: List of permissible limit of heavy metals (mg/kg dry weight) in crustaceans set by organisations

	Al	Cu
WHO (1982) [11]	-	10
MFA (1983) [12]	-	30
US FDA (2001) [13]	-	100
JECFA (2000) [14]	-	30

In this study, the heavy metals in *M.rosenbergii* were also compared with other international organisations. Unfortunately, there was no information on permissible limit for Al in giant freshwater prawns according to international standards [11-14]. In fact, there was no description of Al toxicity in giant freshwater prawns reported in literatures especially in Kuantan area. However, this does not indicate that the presence of Al in *M.rosenbergii* may not harmful to the aquatic organism. Al is not an essential element in biological mechanism and can cause toxicity due to its peculiar chemical properties [16] and it has been recognized as a toxic element to large parts of aquatics as well as terrestrial ecosystems [15]. On the other hand, it was noticed that the content level of Cu in gills was lower than permissible limit stated by [11-14]. Even though the results obtained in present study proved that the prawns caught from the rivers were safe for human consumption however, it was demonstrated biomagnification of Al, and Cu in the prawns from Kuantan. Continuous care must be taken to monitor level of heavy metal levels in crustacean species.

The study also demonstrated that the concentration of Al was way higher than Cu in most of *M.rosenbergii* samples from all sampling sites. Results of this study showed a good agreement with report of [17-20] which various species of mollusc, crustacean and fish are able to regulate essential metals. However the bioaccumulation of non-essential metals in the organisms are depends on their concentrations in the environment. The higher the concentration of Al, the higher the bioaccumulation in species.

Al and Cu content in *M.rosenbergii* caught from Pandan River was found to be the highest in muscle (3.945 and 7.059) mg/kg and in gills (14.029 and 12.283) mg/kg. From the observation and survey carried out from the villagers, Pandan River located near to agriculture activity site, thus, the use of fertilizers could contribute to the increase level of Al in *M.rosenbergii* caught at this location.

These rivers also have the history on bauxite mining activity and until now the mining are is still exist. Al is known as one of the core metals which related to the bauxite [21] and study from [15] stated that the high values of Al in the water at the stockpile area were due to Al2O3, Fe2O3 (hematite), and SiO2 (quartz) in bauxite ores. Moreover, as the sampling sites were close to a rock quarry (Bukit Bekelah Quarry), it is understandable that this pollution was caused by the runoff from mining sites. The quarry had been running for several years and large amounts of rocks had been excavated from the ground. This may contribute to the high concentration of both Al and Cu in Pandan River.

The accumulation of Al and Cu was higher in gills of *M.rosenbergii* than in the muscle. It was confirmed that gill tend to taken up more heavy metal than edible meat as generally, gills act as water filter and had a direct contact with the polluted water. According to [22], gill is basically main sites of deposition due to their cellular configuration.

The levels of Al and Cu of those prawns varied based on the organs and location of the sampling stations. Bioconcentration of metals in an animal tissue was contributed by several factors; such as body size and mass, diet, metabolism, as well as the environment. However, food is the factor that had the most influence on the accumulation of metals in animal tissues [23]. Thus, different species may have different ability in metal accumulation into their tissue. However, information about metal metabolism in prawns are still lack, therefore it is not possible to conclude that the tiger prawn can be a suitable indicator of heavy metal pollution associated to aquatic habitat.

Heavy metals pollution in river is one of the environmental issues due to its toxicity and persistence in the environment for decades [24]. Heavy metals waste can bioaccumulate in the tissues of fish, crustaceans and shellfish [25-27] as they are sensitive to heavy metals especially for crabs and shrimp because invertebrates tend to accumulate high heavy metals than fish as a result of the differences in evolutionary phylum specific coping strategies [28-31].

IV. CONCLUSION

This present study provided data on accumulation of toxic (Al) and essential metal (Cu) in local freshwater prawn species from several rivers in Kuantan. The result showed that the concentration of heavy metal in different rivers were in the order of Al > Cu. Pandan River had the highest level of Al and Cu in *M.rosenbergii* muscle (3.95 ± 2.50 and 14.03 ± 3.55) mg/kg and gills (7.06 ± 2.53 and 12.28 ± 3.87) mg/kg compared to other sampling sites which were in order of Pandan River> Riau River> Pinang River> Kuantan River> Balok River. However, the concentrations of Al and Cu in giant freshwater prawn were still below the permissible limit and safe to be consumed. From the analysis, all crustaceans may be considered safe for consumption but they need continuous monitoring and assessment studies of heavy metals pollution in order to prevent bioconcentration and provide adequate useful data to ensure the quality of crustaceans.

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