Faculty of Resource Science and Technology

Metal Contamination in *Macrobrachium rosenbergii* from Lupar River, Sarawak and its Risk to Human Health

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Metal Contamination in *Macrobrachium rosenbergii* from Lupar River, Sarawak and its Risk to Human Health

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DECLARATION

I declare that this thesis has been written entirely by myself and has not been submitted, in whole or in part, as part of a previous degree application. All of the sources that were used for this study are fully acknowledged, and all of the quotations are properly cited.

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ABSTRACT

The abundance of *Macrobrachium rosenbergii* in the Lupar River has become a source of food and economic activity for the locals. The influence of human activity surrounding the Lupar River, has the potential to deteriorate the water quality and sediment, as well as the organic organisms inside it. The objectives of this study were to investigate the contamination level of heavy metals in the muscle tissues, hepatopancreas, exoskeletons, and gills of *M. rosenbergii* and to assess their risk to human health. The trend of heavy metal contamination in *M. rosenbergii* from the Lupar River are as follows: Cd (BDL) < Mn (3.22–24.22 mg/kg) < Cu (4.55–44.28 mg/kg < Co (33.63–126.03 mg/kg) < Al (388.42–60.67 mg/kg) < Fe (832.26–42.49 mg/kg) with their concentrations high in hepatopancreas > gills > exoskeletons > muscle tissues. There was a positive correlation of total length and total weight of *M. rosenbergii* (r = 0.941, p < 0.001), indicated an increase in total length would result in an increase of total weight. A positive correlation of Cu, Mn, and Fe in muscle tissues, gills, and hepatopancreas was detected with the increasing size. The Lupar River has a Hazard Index (HI) of 80.94 for heavy metals which is hazardous to human health. All metals were above the permissible limits. Therefore, *M. rosenbergii* from Lupar River were contaminated enough to pose a high risk to human health.

Keywords: *Macrobrachium rosenbergii,* heavy metals, human health risk, Lupar River.

ABSTRAK

Sungai Batang Lupar yang kaya dengan Macrobrachium rosenbergii telah menjadi sumber makanan dan aktiviti ekonomi penduduk setempat. Pengaruh daripada aktiviti manusia di persisir sungai Batang Lupar bepotensi untuk menyumbang kepada kemerosotan kualiti air, sedimen dan hidupan semulajadi di dalam sungai tersebut. Tujuan kajian ini adalah untuk mengkaji tahap pencermaran logam berat yang terdapat pada tisu otot, hepatopakcreas, kerangka luar dan insang serta menilai risikonya kepada kesihatan manusia. Arah aliran pencermaran logam berat pada M. rosenbergii dari sungai Batang Lupar adalah seperti berikut: Cd (BDL) > Mn (3.22 - 24.22 mg/kg) > Cu (4.55 -44.28 mg/kg) > Co (33.63 - 126.03 mg/kg) > Al (388.42 - 60.67 mg/kg) > Fe (832.26 - 42.49 mg/kg) dengan kepekatan tinggi dalam hepatopankreas > insang > kerangka luar > tisu otot. Terdapat kolerasi positif jumlah panjang dan jumlah berat M. rosenbergii (r = 0.941, p < 0.001) menunjukkan pertambahan jumlah panjang akan mengakibatkan pertambahan jumlah berat. Kolerasi positif Cu, Mn dan Fe dalam tisu otot, insang dan hepatopankreas dikesan dengan saiz yang semakin meningkat. Sungai Batang Lupar mempunyai indeks bahaya 80.94 untuk logam berat yang berbahaya kepada kesihatan manusia. Semua logam dalam M. rosenbergii adalah melebihi had yang dibenarkan. Oleh itu, M. rosenbergii dari sungai Batang Lupar telah tercemar dan mendatangkan risiko tinggi kepada kesihatan manusia.

***Kata kunci**: Macrobrachium rosenbergii, logam berat, risiko kesihatan manusia, Sungai Lupar.*

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LIST OF ABBREVIATIONS

Al	Aluminium
As	Arsenic
BAF	Bioaccumulation Factors
BC	Blue Claws
BDL	Below Detection Limit
Cd	Cadmium
Co	Cobalt
Cu	Copper
DIM	Daily Intake Metals
DW	Dry Weight
EPA	Environmental Protection Agency
FAAS	Flame Atomic Absorption Spectrophotometer
FAO	Food and Agriculture Organization
Fe	Iron
FW	Flesh Weight
HCl	Hydrochloric Acid
Hg	Mercury
HI	Hazard Index
HNO3	Nitric acid
HRI	Hazard Risk Index
ICP-OES	Inductively Coupled Plasma - Optical Emission Spectrometry
JECFA	Joint Expert Committee on Food Additives

MFA	Malaysian Food Act
Mn	Manganese
Nd	Not Detectable
Ni	Nickel
OC	Orange Claws
Pb	Lead
QC	Quality Control
Rfd	Reference Dose
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
TL	Total Length
TW	Total Weight
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
Zn	Zinc

CHAPTER 1

INTRODUCTION

The freshwater prawn (*Macrobrachium rosenbergii*) can be found through all tropical and subtropical regions of the world (Habashy *et al.*, 2012). *M. rosenbergii* is a well-known seafood product and export (Banu and Christianus, 2016). It has also gained in popularity due to its high price and delicate flavour. *M. rosenbergii* usually be sold at a price between RM50 to RM90 per kilogram depending on the grade. This species can be found in large numbers in the natural habitat in the Lupar River, Sarawak and can be caught easily by local fishermen.

Heavy metal contamination in the environment is becoming an alarming issue, and it is raising widespread concern owing to its negative consequences. Heavy metals have the potential to be toxic to the marine biota (Kahlon *et al.,* 2018). Heavy metals are not biodegradable, even low levels of heavy metals are harmful and its affecting the animals, and humans. (Brodin *et al.,* 2017; Gu *et al.,* 2018; Ferrey *et al.,* 2018; Wang *et al.,* 2020). The increase of industrialization, agricultural, urbanisation and domestic waste discharge has introduced a variety of contaminants into the aquatic environment (Sankhla *et al.,* 2016). Due to the persistence and high toxicity of heavy metals to aquatic organisms, they are considered pollutants of the freshwater ecosystem (Su *et al.,* 2017).

The rapid human activities along the Lupar River may increase the concentration of the toxic metals. Heavy metal pollution has emerged as a result of anthropogenic activity, which is the primary cause of pollution; leaching of metals from various sources such as the rubber industry, pepper, oil palm, paddy, and livestock farming are among the major activities carried out by Sri Aman's farming population (Department of Agriculture Sarawak, 2021). The discharge of industrial, municipal, and agricultural wastewaters and sewage into rivers has been identified as a major source of heavy metals into aquatic resources. They readily absorb suspended particles in water, settling in the riverbed before being discharged into the water column, where they can represent a secondary source of contamination, endangering ecosystems (Titilawo *et al*., 2018; Manoj *et al*., 2012; Yi *et al*., 2017).

Thus, the various industrial waste, domestic sewages, and agricultural run-off have all contributed to an increase in heavy metal contamination in the water bodies. The industrial and agricultural wastes were illegally dumped into rivers without proper treatment and it becomes one of the sources of heavy metal pollution in rivers (Ahmed *et al.,* 2021). Heavy metals can accumulate in prawns, which are often located at the bottom of the aquatic food chain, and they have negative health consequences on humans. Typically, prawns absorb metals from their food and water, which are then circulated and stored in specific organs (Aytekin *et al*., 2019). Due to the non-biodegradability of heavy metals, the accumulation of heavy metals in prawn has been recognized as posing significant health risks when transferred to humans via the food chain (Anani and Olomukoro, 2018). *M. rosenbergii* is one of the food sources for residents living near the Lupar River. Hence, it is crucial to assess the concentrations of heavy metals accumulated by *M. rosenbergii* to establish metal intake status around this region.

Therefore, the objectives of this study were to:

i. To assess the heavy metals content (i.e. Cd, Cu, Mn, Co, Fe, Al) in *M. rosenbergii* from Lupar River.
ii. To estimate the health risk to human nearby Lupar River.

CHAPTER 2

LITERATURE REVIEW

2.1 The Taxonomy and Morphology of *M. rosenbergii*

M. rosenbergii is a giant freshwater prawn that belong to the largest genus, *Macrobrachium* and a commercially significant species of the family Palaemonidae (New, 2002). Below shows the taxonomy classification of *M. rosenbergii*.

Kingdom	: Animalia
Phylum	: Arthropoda
Subphylum	: Crustacea
Class	: Malacostraca
Order	: Decapoda
Family	: Palaemonidae
Genus	: *Macrobrachium*
Species	: *Macrobrachium rosenbergii*

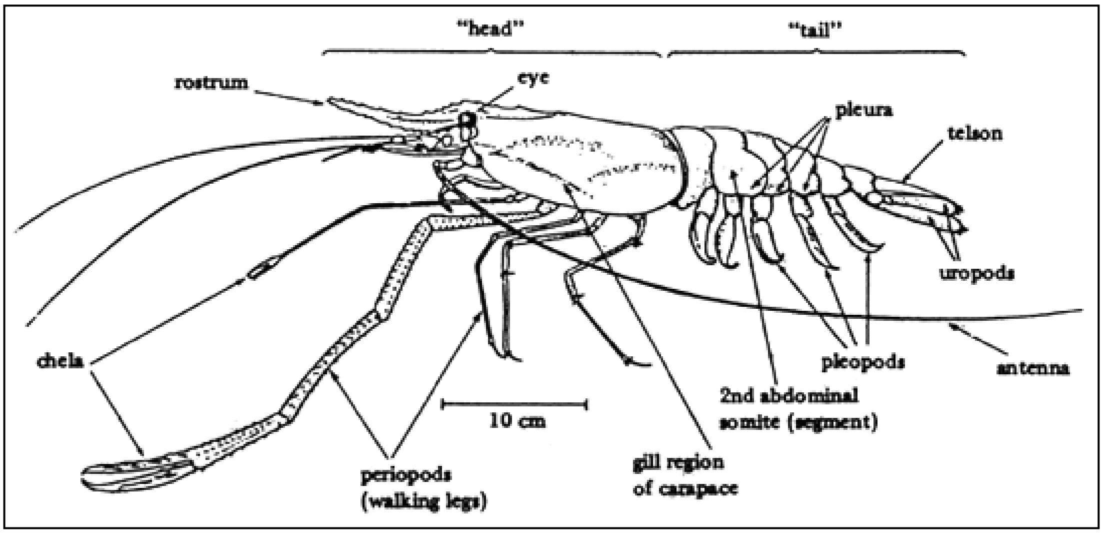

Figure 1 The external morphology of *M. rosenbergii*. Source: (New, 1988)

The morphology of *M. rosenbergii* is shown in Figure 1. *M. rosenbergii* adults are divided into two distinct parts: the cephalothorax (head) and the abdomen (tail). The cephalothorax is composed of stalked eyes, a rostrum, two pairs of antenna, and five pairs of walking legs, while its abdomen is composed of a pleura, pleopods, a telson, and a pair of uropods. The body colour of *M. rosenbergii* ranges from greenish brown to greyish blue, with darker and lighter colour streaks spreading longitudinally or irregularly (New, 2002).

Furthermore, there are differences between male and female prawns. Male prawns are significantly larger than female prawns. Males have a larger cephalothorax(head) than the abdomen while female prawns have a smaller head and a wider abdomen. The chelipeds of male prawns appeared long and massive compared to female prawns (Idrus *et al.*, 2018; Nandlal and Pickering, 2005).

2.2 Habitat and Biology of *M. rosenbergii*

This species can be found in tropical freshwater environments influenced by brackish water. It is commonly found in highly turbid environments. The life cycle of a prawn is divided into four stages: 1) egg, 2) larva, 3) postlarva, and 4) adult. The duration of each phase and the rate of growth are influenced by the environment, water temperature and food availibility. Males and females reach first maturity within four to six months with a weight of approximately 15–35 g (Nandlal and Pickering, 2005). Spawning and nursing up to the post-larval stage require brackish water, whereas juveniles are mostly found in fresh-water zones (New, 2002).

Freshwater prawns undergo a moulting process in which they shed off their exoskeleton as they grow. Females moult prior to mating and have a soft shelled, while males remain unchanged and hard-shelled. Fertilization of freshwater prawns occurs externally after the eggs are extruded. The male deposits its sperm in the form of a gelatinous mass during mating. After mating, the female will start laying their eggs after about five to six hours. Females may move from freshwater to brackish water in order to hatch the egg, as developed larvae require brackish water within one to two days or they will not survive. Alternatively, larvae hatch and migrate down the river to the coastal zone (D'Abramo *et al*., 2003; Saravanan *et al*., 2008).

Freshwater prawn metamorphoses into postlarvae after they have completed their larval life. They resemble miniature adult prawns at this point and are mostly crawling rather than swimming. Freshwater prawn postlarvae exhibit a strong tolerance for a wide range of salinities. Postlarvae begin to travel upstream into freshwater environments within one or two weeks of metamorphosis. Freshwater prawns in their postlarval stage are omnivorous. As they grow, they consume aquatic insects and larvae, algae, mollusks, worms, and other crustaceans (D'Abramo *et al.*, 2003).

The postlarva, which becomes juveniles as it matures, has five distinct horizontal lines on its carapace. Juveniles lack a crest on the rostrum, and their teeth are crowded closely together with little space between them. Adults and juveniles move slowly during the day and seek shade and cover in shallow areas of rivers, canals, and ponds, and become extremely active at night (Nandlal and Pickering, 2005).

2.3 Comparison of Heavy Metal Contents in *M. rosenbergii*

The concentration of heavy metals such as Cd, Cu, Zn, and Pb usually higher in males prawns compared to female prawns due to its size. Male prawns are larger in size and heavier in weight, which means they can accumulate a higher concentration of heavy metals. This is consistent with the findings from several studies (Idrus *et al.,* 2018,2021; Islam *et al.,* 2017; Mostafiz *et al.,* 2020). Moreover, heavy metals accumulate variably in *M. rosenbergii* depending on location. Table 1 shows the comparison of metals concentration of difference location from several previous studies.

Table 1 Metals concentration in *M. rosenbergii* of difference location from several previous studies.

Location	**Metals**	**Concentrations (mg/kg)**	**Body parts**	**References**
Sarawak River, Sarawak.	Cd, Co, Cu, Zn, Hg	Muscle tissue (range): Cd (0.46-0.50 mg/kg) Cu (6.62-10.18 mg/kg) Zn (10.87-13.76 mg/kg) Exoskeleton (range): Cd (0.57-0.79 mg/kg) Co (3.99-5.67 mg/kg) Cu (7.65-10.47 mg/kg) Zn (6.98-7.36 mg/kg) Gill (range): Cd (1.51-1.53 mg/kg) Co (13.77-15.17 mg/kg) Cu (17.09-21.58 mg/kg) Zn (52.56-59.17 mg/kg) Hg (0.11-0.13 mg/kg)	Muscle tissues, exoskeletons and gills	Idrus *et al*., 2021
Kerang River, Sarawak.	Cd, Cu and Zn	Muscle tissue: Cd (0.07±0.03 mg/kg) Cu (1.00 ± 0.18 mg/kg) Zn (1.20±0.41 mg/kg) Gill: Cu (52.25±9.37 mg/kg) Zn (14.69±3.76 mg/kg) Exoskeleton (range): Cd (0.20 – 0.28 mg/kg) Cu (3.30 – 4.00 mg/kg) Zn (1.50 – 2.30 mg/kg)	Muscle tissues, gills and exoskeletons	Idrus *et al*., 2018
Chittagong and Chandpur, Bangladesh.	Pb, Cr, No, Cd, Fe, Cu, Zn and Mn	Cephalothorax (range): Pb (5.08-5.92 mg/kg) Cr (5.37-8.3 mg/kg) Ni (0.04-0.09 mg/kg) Cd (0.4-0.28 mg/kg) Fe (121.41-190.65 mg/kg) Cu (66.8-3.94 mg/kg) Zn (97.69-211.57 mg/kg) Mn (35-38.75 mg/kg) Abdomen (range): Pb (9.55-9.66 mg/kg) Cr (4.13-5.18 mg/kg)	Cephalothorax and abdomen	Mostafiz *et al*., 2020

		Ni (0.91-3.66 mg/kg) Cd (0.11 mg/kg) Fe (45.49-67.29 mg/kg) Cu (32.98-33.49 mg/kg) Zn (45.62-49.16 mg/kg) Mn (18.09-19.75 mg/kg)		
Dhaka, Bangladesh.	Cd, Hg, Co	Muscle tissues: Cd (2.38 ± 0.69 mg/kg) Hg (6.59 ± 0.35mg/kg) Co (7.20 ± 0.12 mg/kg) Exoskeleton: Cd (2.67 ± 0.41 mg/kg) Hg (6.31 ± 0.19 mg/kg) Co (7.26 ± 0.11 mg.kg)	Muscle tissues and exoskeletons	Islam *et al*., 2017

Furthermore, wild prawns were more contaminated than farmed prawns. As wild and farmed prawns were compared, it was discovered that the majority of trace metals accumulated more commonly in wild-caught prawn samples from natural bodies of water than in farmed prawn samples in Bangladesh (Mostafiz *et al.*, 2020). Trace metal is more likely to accumulate in wild prawns than in farmed prawns due to the sources of trace metal intake in farming being limited compared to those in polluted natural waterbodies (Mostafiz *et al.*, 2020). Besides, changing of water in the prawn farms helped in reducing the metals accumulation in the water or sediment. Natural waterbodies, such as freshwater, have been rapidly contaminated with toxic metals from industrialization, agricultural, urbanisation and domestic waste discharge (Sankhla *et al*., 2016), causing wild prawns to accumulate higher concentrations of metal than farmed prawns.

2.4 Permissible Limit of Metals and Potential Human Health Risks

Contaminants from the environment can make their way into food. Permissible limits for levels of heavy metals in food for human consumption was established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2012). The permitted levels of heavy metals for human consumption are 2 mg/kg for Cd, 30.00 mg/kg for Cu, 1 mg/kg for Mn, 100 mg/kg for Fe and 50-70 mg/kg for Al. Exceeding these limits could pose a potential negative health impact to human.

Some heavy metals have functional roles that are required for a variety of physiological and biochemical activities in the body. However, high doses of heavy metals pose a number of hazards to human health. According to Tong *et al.* (2013), cobalt (Co) contamination in the human body can be harmful to the developing foetus in pregnant women. Frequent vomiting and diarrhea, changes in blood pressure, or headaches are some of the other symptoms. Moreover, high Co concentrations can wreak havoc on the immune system (Bezerra *et al.,* 2014; Valera *et al.,* 2014). Cadmium (Cd) is efficiently kept in the human body once absorbed, where it accumulates throughout life. However, Cd is most toxic to the kidney, especially the proximal tubular cells, which accumulate the most. The impact of Cd is bone demineralization, which occurs direct or indirect through renal failure (Bernard, 2008). Other than that, the excessive of copper (Cu) causes acute effects such as nausea or abdominal pain (Taylor *et al.,* 2020). Moreover, neurotoxicity, including cognitive and learning deficits and hyperactivity in children, is one of the major adverse effects reported in several studies of occupational and environmental manganese (Mn) exposure (Hermes *et al.*, 2013; Carvalho *et al.*, 2018; Bouchard *et al.,* 2018). Meanwhile, aluminium (Al) in high concentrations can have serious health consequences, including damage to the central nervous system, dementia, memory loss, and listlessness accompanied by severe trembling as reported by Rostern (2017) and a higher iron (Fe) concentration in the human body can cause hemochromatosis, which can lead to end organ damage. Thus, it is concerning that a high intake of contaminated prawns with these metals may threaten human health.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Location

The study location was at the Lupar River in Sri Aman, Sarawak. The sampling was carried out in three stations. A total of 41 samples of *M. rosenbergii* were collected during this study. Figure 2 and Table 2 illustrate the coordinates of each sampling stations and the map of the sampling location. The sample collections were done from 6th December 2021 to 30th January 2022.

Figure 2 A map showing three sampling stations for sample collection in the Lupar River. (Source: Google Earth)

Table 2 The coordinates of 3 sampling stations in Lupar River, Sri Aman.

Stations	Coordinates	Station description
1	1°13'29.64"N 111°29'24.47"E	• Cement factory • Oil palm plantation
2	1°14'9.77"N 111°28'9.06"E	• Agriculture farm • Human settlement
3	1°14'59.64"N 111°27'38.04"E	• Agriculture farm • Construction site • Human settlement

3.2 Collection of Samples

The samples were collected using cast nets and fishing rods. The prawn samples were then identified following New (2002) reference, and their total length and weight were measured. At low tide, about 5 cm from surface sediment was collected from the river's bank. The sex of each prawn sample was also determined before being kept in a clean plastic zipper bag with a label in the ice box and was kept frozen before being transported back to the laboratory for further analysis.

3.3 Acid Wash

An acid wash procedure was performed to prevent contamination of the apparatus used for sample digestion, sample dilution and standard calibration. All the apparatus were rinsed with water, soaked for 24 hours in 10% HCl, rinsed three times with distilled water, and finally rinsed with deionized water. The apparatus were then air-dried on the acid wash racks.

3.4 Sample Digestion

Muscle, hepatopancreas, exoskeleton, and gills were dissected and weighed before being dried in an oven (at 60°C) until a uniform weight was obtained to assure full drying. Prior to the digestion procedure, the dried prawn samples were ground into powder using a mortar and pestle. The sediment samples were also dried in a 60°C oven overnight and ground before being digested. For prawn samples, the exoskeletons, muscle tissues and gills were weighed at $\pm$ 0.30 g while hepatopancreas was weighed at $\pm$ 0.10 g in 6 ml of HNO_3. As for sediment samples, $\pm$ 0.10 g of the sample were weighed in 10 ml of HNO_3. Then, the mixture were then placed in the inert polymeric microwave vessels and were digested using the Multiwave Go Plus microwave digestion system (Anton Paar). The microwave digestor system sealed the vessel and heated it according to the method chosen for digesting the sample. For prawn sample, Organic B method on the Multiwave Go Plus microwave (Anton Paar) was used, and for sediment sample, EPA Method 3051a (USEPA, 2007) was used. After the contents of the vessel had cooled, they were filtered using 0.45µm fibre filter paper, diluted in a volume of 100 ml, and placed in the bottles.

3.5 Heavy Metals Analysis

The heavy metal concentrations of Cd, Cu, Mn, Co, and Fe in all samples, including standard solutions, were determined by using a Flame Atomic Absorption Spectrophotometer (FAAS; Thermo Scientific iCE 3500), while Al was determined using Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES; Perkin Elmer Optima 800). The blank that consists of the digestion acids was also analysed following the same method.

3.6 Blank and Standard Calibration of Metals

During the preparation of sample digestion, sample blanks were prepared using the same method as samples, with acid diluted in deionized water. Each metal's calibration standard was prepared by diluting master solution (Cd, Cu, Mn, Co, Fe and Al) with 100 ml with deionized water, according to the required concentrations as shown in Table 2. The table shows the standard solution concentrations and the calibration fit for each metal. Cd, Cu and Mn were analysed using (FAAS; Thermo Scientific iCE 3500) while Co and Fe were analysed using (FAAS; Analytik Jena).

Table 3 The standard concentration of each metal, Cd, Cu, Mn, Co, and Fe was determined using FAAS. The calibration fit value of metals must exceed the acceptable fit limit of 0.995.

Element	Quality Control (ppm)	Blank	Standard 1 (ppm)	Standard 2 (ppm)	Standard 3 (ppm)	R^2
Cd	1.0	0.00	0.5	1.0	1.5	0.9971
Cu	1.0	0.00	1.0	3.0	5.0	0.9998
Mn	1.0	0.00	1.0	2.0	3.0	0.9994
Co	1.0	0.00	1.0	3.0	5.0	0.9998
Fe	1.0	0.00	1.0	3.0	5.0	0.9977

Calibration curves that are linear ($R^2 > 0.995$) are preferred because they produce the highest accuracy and precision. The result of the calibration process was shown in the Table 4.

Table 4 The calibration process result.

Element	R^2	RSD (%)	Blank (mg/L)	Detection limit (mg/L)
Cd	0.9971	0.2	-0.001	0.0028
Cu	0.9998	0.3	-0.001	0.0045
Mn	0.9994	0.5	0.002	0.0016
Co	0.9998	3.2	0.000	0.0100
Fe	0.9977	3.0	0.000	0.0043

Al concentration was analysed using ICP-OES because the absent of appropriate lamp for Al in FAAS. Table 5 shown a standard concentration and the calibration fit value ($R^2 > 0.999$).

Table 5 The standard concentration of Al was determined using ICP-OES. The calibration fit value of metal must exceed the acceptable fit limit of 0.99.

Metal	Blank	QC (ppm)	Standard 1(ppm)	Standard 2(ppm)	Standard 3(ppm)	Standard 4(ppm)	Standard 5(ppm)	Calibration fit
Al		1.0	0.5	1.0	2.0	5.0	7.5	0.999960

The result of the calibration process was shown in the Table 6.

Table 6 Result of calibration process of Al.

Element	R^2	RSD (%)	Blank (mg/L)	Detection limit (mg/L)
Al	0.999960	1.85%	0.000	Not specified

3.7 True Metal Concentration Values Calculation in Samples

3.7.1 *M. rosenbergii* samples

The results were expressed in mg/L from the FAAS and ICP-OES. The true metals concentrations were then calculated (Equation 1) and expressed in mg/kg flesh weight (FW) for prawn samples. The formula was used to convert the concentration of metals in aqueous to metals concentration in the form of flesh weight.

$$Metal\ (mg/kg\ FW) = \frac{A\ (mg\ /L)\ x\ B\ (L)}{Weight\ (kg)} \qquad \ldots(1)$$