Toxicity Level of Heavy Metal Mercury (Hg) to Post Larvae of Giant Freshwater Prawn, *Macrobrachium rosenbergii*

By

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the report of this final year project entitled “Toxicity Level of Heavy Metal Mercury to Post Larvae of Giant Freshwater Prawn, *Macrobrachium rosenbergii*” by Nur Nazatul Najwa bt Muda matric number F14A0252 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honors, Faculty of Agro Based Industry, Universiti Malaysia Kelantan.

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Toxicity Level of Heavy Metal Mercury to Post Larvae of Giant Freshwater Prawn, *Macrobrachium rosenbergii*

ABSTRACT

Nowadays, farming activities of *Macrobrachium rosenbergii* is one of importance sector around the world. The growth of human population will increase the production from industries and human activities. However, according to increasing the human activities a lot of heavy metals are generated. Heavy metals are harmful to most organisms especially for aquatic species even in low concentration. The mercury level in this species will be accumulated when this species consumed by their predators. This study was conducted to observe the survival rate of *Macrobrachium rosenbergii* against the effect of heavy metal of Mercury (Hg). The post larvae of *Macrobrachium rosenbergii* were exposed for four days in different concentration of mercury which is 0.01 ppb, 0.03 ppb and 0.05 ppb. The effect of heavy metal of mercury on mortality, resistance and bioaccumulation were studied. Survival of post larvae that exposed to 0.05 ppb were significantly lower than of those exposed to lower doses which are 0.03 ppb and 0.01 ppb. From this study, the result showed that when the concentrations of heavy metal of mercury increased, the percentages of mortality rates of giant freshwater prawns also increased which was 14.44% on 0.01 ppb, 28.89% on 0.03 ppb and 51.11% on 0.05 ppb. Hence, this research was conducted to investigate the effect of mercury toxicity on mortalities and resistance in giant freshwater prawn, *M. rosenbergii*. For bioaccumulation analysis, the result cannot be determined due to technical error. The finding of this research will provide a platform for future research and study on giant freshwater prawn against heavy metal.

Keywords: *Macrobrachium rosenbergii,* heavy metals, mercury, bioaccumulation and toxicity.

Tahap Ketoksikan Logam Berat Merkuri untuk Larva Udang Air Tawar Giant, *Macrobrachium rosenbergii*

ABSTRAK

Pada masa kini, aktiviti pertanian *Macrobrachium rosenbergii* adalah salah satu sektor penting di seluruh dunia. Pertumbuhan populasi manusia akan meningkatkan pengeluaran dari industri dan kegiatan manusia. Bagaimanapun, menurut peningkatan aktiviti manusia banyak logam berat dihasilkan. Logam berat bahaya kepada kebanyakan organisma terutamanya bagi spesies akuatik walaupun dalam kepekatan rendah. Tahap merkuri dalam spesies ini akan terkumpul apabila spesis ini dimakan oleh pemangsa mereka. Kajian ini dijalankan untuk memerhatikan kadar ketahanan *Macrobrachium rosenbergii* terhadap kesan logam berat Mercury (Hg). Larva *Macrobrachium rosenbergii* didedahkan selama empat hari dalam kepekatan merkuri yang berbeza iaitu 0.01 ppb, 0.03 ppb and 0.05 ppb. Kesan logam berat merkuri pada mortaliti, rintangan dan bioakumulasi dikaji. Ketahanan larva yang terdedah kepada 0,05 ppb jauh lebih rendah daripada yang didedahkan kepada kepekatan yang rendah iaitu 0,03 ppb dan 0,01 ppb. Dari kajian ini, hasilnya menunjukkan bahawa apabila kepekatan logam berat merkuri meningkat, kadar peratusan kematian larva untuk udang air tawar juga meningkat iautu sebanyak 14.44% pada 0.01 ppb, 28.89% pada 0.03 ppb and 51.11% pada 0.05 ppb. Oleh itu, kajian ini dijalankan untuk mengkaji kesan ketoksikan merkuri terhadap kematian dan ketahanan ke atas udang air tawar. Analisis bioakumulasi, keputusan tidak dapat ditentukan disebabkan oleh kesilapan teknikal. Penemuan penyelidikan ini akan menyediakan platform untuk penyelidikan dan kajian masa depan mengenai udang air tawar terhadap logam berat.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF ABBREVIATION AND SYMBOLS

°C	Degree Celsius
°F	Degrees Fahrenheit
%	Percent
g/cm^{-3}	Gram per cubic centimeter
ppb	Part per billion
ml	Milliliter
L/min	Liter per minute
Mg/L^{-1}	Mili gram per liter
kW	Kilo wat
nm	Nanometer
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
Hg	Mercury
ICP-OES	Inductively – Coupled Plasma Optical Emission Spectrometry
PL	Post larvae

CHAPTER 1

INTRODUCTION

1.1 Research Background

Giant freshwater prawn or scientifically known as *Macrobrachium rosenbergii.* In Bangladesh, their common name is Mota Chingris and Mocha Chingri in India. Locally in Malaysia and Thailand, *M. rosenbergii* is known as “udang galah”. *M. rosenbergii* is one of the most economically important. This prawn lives in the tropical and subtropical zones around worlds. This species also can be found in canal and ponds, rivers, lakes, swamps, irrigation ditches and usually around estuarine areas but it depends on natural for seed (New, 2002).

Asian country were dominated the production of aquaculture (Sri Widada *et a*l., 2003). The production of aquaculture sectors comes from many different sources such as fish pond, fish cages and pens and agriculture. Todays, tilapia, seaweeds, prawn and milkfish are the majors products compared with others. The *M. rosenbergii* are one of economic aquaculture because they contain higher meat quality, while omnivorous feeding habit. In environment issue, pollution of water totally can give bad impression to the aquatic species such as bivalve, crustacean and fish also. Nowadays, urbanization and agriculture activities are major sources of heavy metal such as phosphorus and nitrogen which is give impact to aquatic ecosystem. This current situation are harmful to aquatic ecosystem such as toxic algal blooms, losses sources of oxygen, biodiversity

loss and fish kill (Carpenter *et al.*, 2010). The pollution of water will be happen when availability of heavy metals to aquaculture species (Zodape, 2014).

Examples of heavy metals are chromium, lead, cadmium, mercury, copper and argentum (Golinska & Bany, 2000). Heavy metals are essential for living organisms but it will be toxic in higher concentration. Toxic heavy metal can caused bad impact such as dermatological disease, skin cancer, and internal cancers. Heavy metal poisoning in human was analyses from the toxic accumulation of heavy metal in soft tissue. Heavy metals emissions in our environment occur by a varied range of process and pathways such as combustion, extraction and processing. Besides, in surface water such as through direct deposition. Lastly, in soil it comes from crops process. However, majority of heavy metal are harmful to aquatic animals especially.

Mature female eastern king prawn could be some times substantial than a mature male (Montgomery, 2010). *M. rosenbergii* usually lives in freshwater habitat, however during its larval phase *M. rosenbergii* grow in brackish water, water that has more salinity than freshwater, once it has grown out from it planktonic stage.

The present of this study were undertaken to investigate the effect of mercury toxicity on mortalities and resistance in giant freshwater prawn (*M. rosenbergii*) and also to investigate the bioaccumulation of mercury residue in their tissue.

1.2 Problem Statement

Heavy metal is common to be found at industrial area. Water pollution will occurs if waste from industrial is not controlled properly treated and monitor by government and those responsible. Water pollution is known to have adverse effect on the aquaculture industry. Pollution that effected aquatic species such as shrimps, fishes, prawn and crustacean has become extreme issues nowadays and requires universal attention in order to control this problem. This situation may reduce the diversity, quantity and quality of the aquatic species in aquatic environment. The aim of this research was to identify effect of pollution caused by heavy metal from mercury. The present study investigated if the toxicity level of mercury in the water effects the survivability and growth rates of *M. rosenbergii.*

1.3 Hypothesis

H0 : The toxicity level not affected by mercury.

H1 : The toxicity level affected by mercury.

1.4 Objectives

The objectives of this study were:

- To identified the toxicity level of heavy metal of mercury on post larvae of giant freshwater prawn, *M. rosenbergii.*
- To investigate the effect of mercury metal on the bioaccumulation.

1.5 Significant of Study

This research is important to discover the effect of heavy metal on the *M. rosenbergii,* to survive with the presence of heavy metal of mercury. Outcome from this study will determine the effect of mercury on water and growth of post larvae (PL) of giant freshwater prawn, *M. rosenbergii.* The different concentrations of mercury determined to identify the toxicity level in PL of *M. rosenbergii*. The present study is also important to prevent extinction *M. rosenbergii* around the world cause by pollution of heavy metal mercury.

CHAPTER 2

LITERATURE REVIEW

2.1 General background of *M. rosenbergii.*

M. rosenbergii are the largest freshwater prawn. During breeding season they will migrates from a region of lower to higher salinity (Madlen & Montaser, 2011). From the previous study, by (Chowdhury, Bhattacherjee, & Angell, 1993), they state that around 150 species of *M. rosenbergii* (de Man,1879) around the world which only 49 species where selected to be commercial. Besides, from the study of (Alam & Alam, 2014) which *M. rosenbergii* was called as galda in Bangladesh. In United State of America (USA) this species was called as freshwater shrimps compared to Australia which is called as freshwater prawn. The different names of this species occur because of different geographic structure of the country. This species commonly cultured at tropical and subtropical zones around the world (Thanh et al., 2009). From the researcher the habitats of giant freshwater prawn *M. rosenbergii* are around lake, rivers, irrigation ditch, swamp, canal and pond. *M. rosenbergii* are adopted both under monoculture and polyculture. Besides, prawns were considered to be sensitive indicator of marine and estuarine pollution (Das & Sahu, 2002).

Mostly, the *M. rosenbergii* like to spent their daily life in brackish water area during initial stage for survival (Chowdhury et al., 1993). Brackish water area is a place where their salinity is higher than freshwater. Brackish water is connector of sea weather

directly or indirectly. *M. rosenbergii* is a species which have a good nutritional source of protein, low amount of fat. Broodstock referred to female M. rosenbergii which is kept in hatcheries until hatching their eggs (New, 2002). Furthermore, berried female is terms for female of *M. rosenbergii* carrying eggs of *M. rosenbergii* (Louis & Martin, 1996). Based on (New, 2002) berried female from natural waters are higher qualities than pond reared ones. The size of female affecting the quantity of egg produced (Louis & Martin, 1996). *M. rosenbergii*, carried out their eggs below the tail of female which is in oviduct (New, 2002). The oviduct made by three layers which is connective tissue, basal lamina, and layer of columnar epithelial cells without a muscular layer. Female prawn will migrate across saline gradients to estuaries where eggs are hatching and development of larvae (Ismael & New, 2000; Madlen & Montaser, 2011). They also state that to improve the hatching rates of their eggs, berried female will transferred from freshwater to brackish water.

Male of *M. rosenbergii,* could be divided into three major types which are blue-claw males (BC), small males (SM) and orange claw males (OC). In mating process, BC is more successful compared to other. They can maintain territory associated with a group of female. The size of SM of *M. rosenbergii* is equal to the size of juveniles (New, 2002). On other hand, *M. rosenbergii* could be categories into three different reproductive statues which mature ovary, berried and spawned females (Dinh & Nguyen, 2014).

Figure 2.1: The differentiation of male (upper) and female (lower) of *M. rosenbergii.*

(Sources: National Research Council of Thailand, 2016)

Before hatching, the newly spawned eggs from bright-yellow to orange color will change to orange, then brown and finally greyish-brown. First phase, the eggs of *M. rosenbergii* is orange color in first seven days. Second phase, the egg turn to yellowish or brown. Lastly, the eggs will turn to greyish-brown with the formation of larvae that will inherit the generation of giant prawns. (New, 2002), he stated that the exchange of color occurs because embryos utilize their food reserve. After that, they change to larvae phase and metamorphosis. After metamorphosis, they change to post larvae state. Post larvae can survive in wide range of salinities. Post larvae also known as juvenile which is, basically the term of juvenile refer for *M. rosenbergii* between post larvae and adult. In adult stages, normally they come in form blue-green color and sometimes on brownish hue. The color of *M. rosenbergii* show off their quality and type of diet (Louis & Martin, 1996). When selecting a healthy berried female for nursery purpose, few parameters must be considered such as the berried female should be healthy and absolutely actives,

well pigmented, complete appendages and lastly carrying large egg masses (New, 2002).

In nature, the diet of larvae is zooplankton such as small worms compared to post larvae; they can utilize the larger pieces of organic materials on this stage even for origin from animals or vegetables. When they grows, the natural diet of post larvae is aquatic insects and their larvae, algae, nuts, grains, fruits and small molluscs (New, 2002).

2.2 Morphology of *M. rosenbergii.*

The morphology of *M. rosenbergii* can be divided into two segments. First is cephalothorax (head) and secondly is abdomen (tail). Each parts of their body consist of segments that are covered with exoskeleton. The bodies of *M. rosenbergii* were divided to 12 segments which are also known as somites. In head, they consist of 14 segments which all segments fused together and invisible under a large dorsal and lateral shield. This segment knows as carapace. Cephalon is the front portion of the cephalothorax, which have six segments and it include eyes and five pairs of appendages (New, 2002).

Their head or cephalothorax is covered by carapace and their tail or abdomen clearly segmented. From their structure, their periopods and pleopods are functioning as walking legs. Besides legs, they have an antenna. An antenna is important to sense the surrounding environment. First antenna have three segments of peduncles from three tactile plagella emerge. Second antenna having five segments of peduncles and single long flagellum. Their legs have pincer which is also called as chelipeds. In thorax, they

consist of 8 fused segments which is consists pairs of appendages that contain 3 sets of maxillipeds and 5 pairs of pereiopods. Maxillae and maxillipeds which both of that are play an important role which is gripped and chopped their food for survival (New, 2002). The tail which is abdomen part, it divided into 6 segments. Each part consist a pair of appendages knows as pleopods.

Their eggs are extruded from oval gonophores in the base of the third pereiopods of female and it is covered by membrane. For males, their sperm were extruded from gonophore which is covered by flaps. Adult males having been reported with a total body length up to 33 cm and adult females up to 29 cm. *M. rosenbergii* is the largest macrobrachium species.

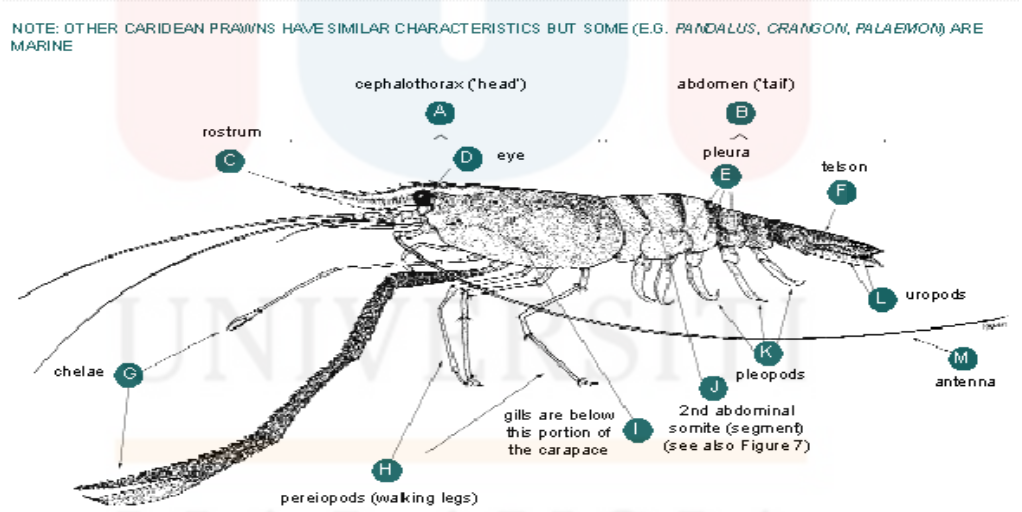


Figure 2.2: The external features of *M. rosenbergii.*

(Sources: New, 2002)

For *M. rosenbergii* they have four different stages to complete their life cycle which is eggs, larvae, PL and adults (New, 2002). Eggs were fertilized by non-motile sperm. The quantity of the eggs depends on the size of female. The long axis of prawn eggs is between 0.6-0.7 mm (New, 2002). Their eggs were incubated around 21 days before hatching, while hatching process will take around 20 days at temperature below 28˚C (Chowdhury et al., 1993). Time for larvae hatching is during night. At this stage larvae need brackish water for survival. Larvae are photo tactic which is light sensitive. In hatching, larvae will take time around 26 days to metamorphose into post larvae. After metamorphosis, they change to adult. When adult, usually their color becomes blue-green or brownish hue. The size of cephalothorax of adult male and berried female is around 10 cm.

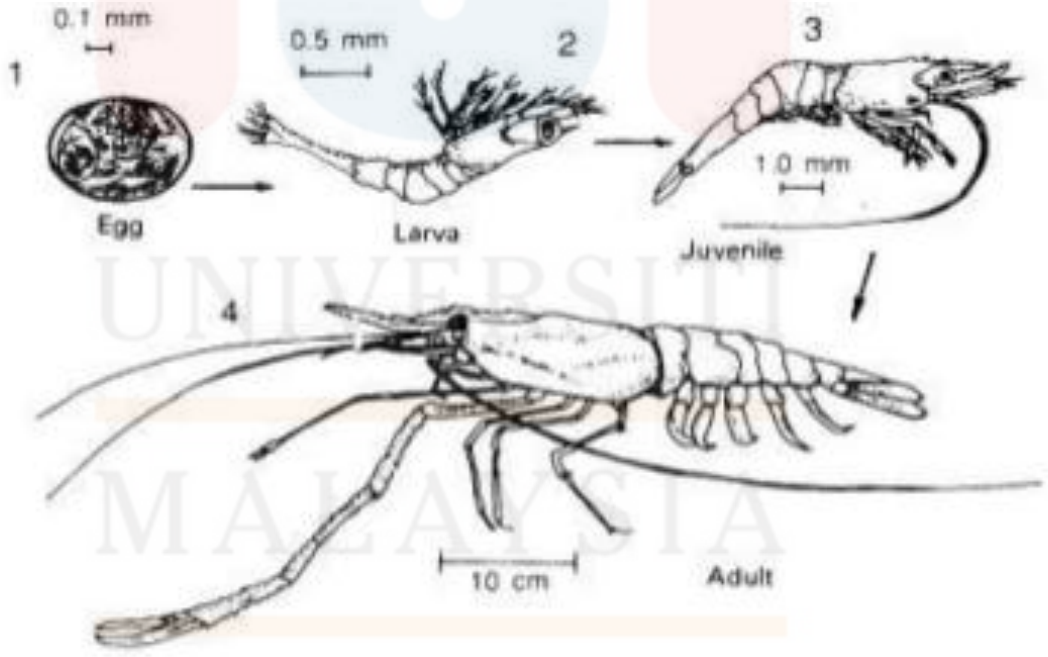


Figure 2.3: The life cycle of *M. rosenbergii.*

(Sources: New, 2002)

M. rosenbergii are categories of omnivore's species because they feeding on plants, mollusks, algae, fish and worms. Basically they eat feed such as aquatic worm, small, molluscs and insect larvae. Besides, they also eat nut, fruit and algae. They search food using antennae and antennules (Ling, 1969).

Besides, the haemocyte in crustacean are important in non-specific cellular immunity on pathogens and parasites which act as phagocytosis, encapsulation, nodule formation and cytotoxic mediation (Anderson, 1992; Chen & Sung, 2005). To avoid parasite and pathogen or non-pathogenic animal from entering the hemocoel, the blood cell and seric factors in arthropods are important as defence mechanisms (Ravindranath 1980, Ratchiffe et al., 1984; Lorena Vazquez, 1997). Besides, in decapod, circulating haemocytes appear to be critical to defense (Johansson & Soderhall, 1989; Martin & Hose, 1992,; Lorena Vazquez, 1997). Decapod contain three types of circulating haemocytes which is hyaline cells, semi-granular cells and large granular cells (Sung *et al*., 2011).

The taxonomy of *M. rosenbergii* based on the domain, kingdom, phylum, subphylum, class, subclass, order, suborder, genus and species of giant freshwater prawn as followed:

Table 2.1 show the taxonomies class of *M. rosenbergii*

Domain	Eukayota
Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Crustacean
Class	Malacostraca
Subclass	Eumalacostraca
Order	Decapoda
Suborder	Natantia
Family	Palaemonoidea
Genus	Macrobrachium
Species	*rosenbergii*

2.3 Optimum Water Quality of Prawn

To keep shrimp growth in optimum condition, water quality parameter need to monitor daily. The management of water quality is important to avoid environmental stress to shrimp. Based on the research from Southern Regional Aquaculture Centre (2016), *M. rosenbergii* are more tolerant to high salinity, high water temperature, low dissolved oxygen, and low ammonia concentrations. Besides, to maintain the water

quality, 50% to 60% water needs to change every 3 days from 10th day to the end of nursing period. This is to avoid deterioration of water quality.

2.3.1 pH

The measure of the acidity or alkalinity in water is the term of pH or the concentration of hydrogen ions (H+). The scale of pH is from 0 to 14. Being of pH 0 is most acidic and pH 14 is most alkaline. For neutrality condition, the pH is 7. In aquaculture the suitable range is 7.0 to 9.0. The optimum range is between 7.5 to 8.5. The pH for culturing prawn is range from 7.5 to 9.0. This ranging is suitable for production of shrimp. However, if the pH below than 5.0, the growth of shrimp will be fall down. In contrast, if the pH higher than 9.0, the health of prawn will detrimental (Tharavathy, 2010).

2.3.2 Temperature (˚C)

The optimum water temperature for *M. rosenbergii* growth is about 25˚C to 30˚C. The metabolic process in the body of shrimp will be inhibited if the water temperature is low. In fact, the shrimp will stop from feeding. The high temperature will lead to mortality of giant freshwater prawn (Tharavathy, 2010).

2.3.3 Alkalinity

Alkalinity can be defined as an amount of carbonate, bicarbonate and hydroxide that contain in water. Alkalinity is important factor in water quality because it able to

sustain pH level in water. The standard value for alkalinity in pond is around 80ppm or above.

2.3.4 Salinity

Based on previous study by Tharavathy, (2010) stated to ensure the metabolic process for shrimp in proper condition, the salinity of water must be in optimum condition. The optimum salinity for growth and culture of prawn is ranging from 15 to 30 ppt. The water in the shrimp body will come out if the salinity of environmental condition is higher than the salinity in the body fluid of shrimp.

2.3.5 Dissolve oxygen

The amount of DO can be measured by using unit of mg per litre (mgl^{-}). Process of diffusion can generate oxygen frim air to water. Based on Ryan, 2007, dissolve oxygen level between 5 to 7 mg/L is suitable to nursing prawn. Lack of dissolved oxygen in water and above water level can cause mortality of PL because PL jump at times and stranded on tank wall.

2.3.6 Ammonia

From overfeeding, high level of ammonia can be generating. Decay of feed can liberate toxic ammonia gas which is harmful to aquatic species. Based on Jain et al. (2008) stated that high level of ammonia can cause osmo-regulatory imbalances.

2.4 General Background of Heavy Metals

The farming industries of giant freshwater prawn are growing rapidly nowadays. However with increased level of toxicity, the production of prawn will decline and more prone to get disease infection. Chen & Sung (2005) stated that, the poor management of pond will encourage the disease outbreak. It also can decrease the production of giant freshwater prawn. Heavy metals are one of the factors of pollution in our environment. High level of heavy metal may cause industrial pollution (New, 2002). Heavy metal can be defined as refers to any metallic element which has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004; Duruibe, 2007). Trace metal are synonyms with heavy metal which is essential and non-essential trace metals (Rainbow, 1995). Even in low concentration, heavy metal have a potential to be harmful to most organism (Zodape, 2014). Went our environment are affected with heavy metal, the cost for reduce the pollution will be expensive (Tangahu et al., 2011). Heavy metals are divided to three types which is toxic metal, precious metal and radionuclides (Bishop, 2002).

Examples of heavy metal are mercury, cadmium, copper, magnesium, chromium, lead and arsenic. According to Bishop, (2002) stated these metals were be classified into 3 categories which is toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru, etc.) and radionuclides (such as U, Th, Ra, Am, etc.)

Biological system will be polluted if exposed to the metal pollution. This is because heavy metal pollutant cannot be degradation (Rajeswari & Sailaja, 2014). For aquatic environment, this situation which is toxicants will damage their environmental. The big issues is too human, when they depending on aquatic products as sources of food. The accumulations of hazardous environmental pollutants along the aquatic food chain will severe high risk for animal and human health (Cleary, Battaglene, & Pankhurst, 2002; Desi, Nagymajtenyi, & Schulz, 1998; Zodape, 2014). Heavy metal cannot be destroyed and degraded, so, they are classified as natural constituent for earth and persistent to environmental contaminants (Duruibe et al., 2007).

Previous study by Mahmud, (2005), chromium is a soluble in non-oxide acids such as hydrochloric acid (HCl) but it passive in nitric acid (HNO_3). Even at high temperature, chromium is a hard steel-gray metal that is highly resistant to oxidation. In earth, Cr is 17th most abundant element (Oliveira, 2012). From her research, she states that chromium formed through natural processes as chromite ($FeCr_2O_4$) in ultramafic and serpentine rocks or other metals like crocoite ($PbCrO_4$), bentorite $Ca_6(Cr,Al)_2(SO_4)_3$ and tarapacaite (K_2CrO_4), vauquelinite ($CuPb_2CrO_4PO_4OH$), among others (Helena, 2012). Based on Prakash Chandra & Kamla Kulshreshtha, 2004, chromium is element which is form naturally from combination of compound (chromium + oxygen + iron) knew as chromite.

According to the Toxics Release Inventory, in 1997, estimated releases of chromium was 111,384 pounds to water from 3,391 large processing facilities which accounted for about 0.3% of total environmental releases.

The particle of chromium from industry comes through effluents discharged from tanneries, textiles, electroplating, mining, dyeing and printing industries (Zodape, 2014) where will be transferred to aquatic medium. She also state, reduction, oxidation, sorption, desorption, dissolution and precipitation suffered to aquatic environment.

2.4.1 Mercury

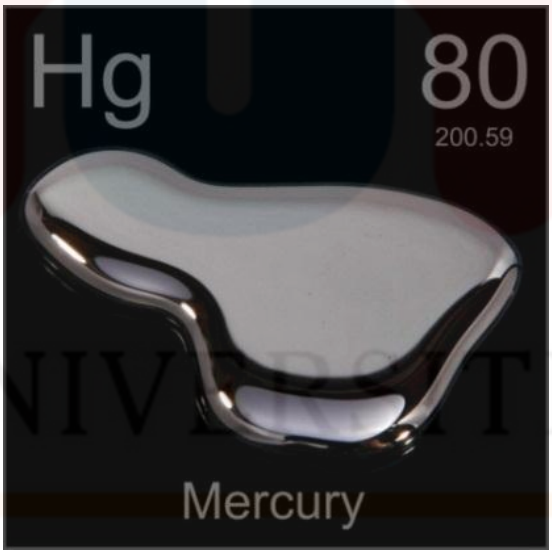


Figure 2.4 show the Mercury poster sample.

(Source, http://www.periodictable.com/Items/080.14/index.html, accessed on 11/12/2017)

One of most harmful heavy metal is mercury. Mercury is an element of heavy metal that comes from group 12 in Periodic Table. This chemical element represented by the symbols of “Hg”. This element also known as quicksilver or hydrargyrum which is liquid silver (Hare, D, 2007). Usually mercury where used in production of scientific apparatus such as thermometer, barometers, manometers and sphygmomanometers (Hare, 2007). Besides, the particle of mercury from industry comes from cosmetic sector which is usually in mascara products (CIDPUSA Foundation, 2008).

In aquatic fauna, concentration of mercury in some species such as shark, swordfish, king mackerel and tilefish is higher than other species. The mercury level in this species will be accumulated when this species consumed by their predators (Hare, 2007).

The physicals properties of mercury are this element is the only liquid metal. However, Hg can change to the solid went put in frozen condition. Hg also can turn to gas when we boiled it. This element is good conductor for electricity. Besides, the chemical property of Hg is moderately active. In air, Hg not reacts with oxygen. However, in hot condition, they can react with some acids.

Table 2.2: Physical and chemical properties of mercury

Name	Mercury
Symbols	Hg
Atomic Number	80
Atomic Mass	200.59 amu
Melting Point	-38.83 °C (234.32 K, -37.89 °F)
Boiling Point	356.73 °C (629.88 K, 674.11 °F)
Number of Electron	80
Number of Neutrons	121
Classification	Transition element
Crystal structure	Rhombohedral
Density @ 293 K	13.456 g/cm^{-3}
Color	Silver

2.5 Effect of Heavy Metal on Prawn

Nowadays, the fast growing population, urbanization and industrialization have significantly contributed to the higher amount of waste water that contains heavy metal (Golinska & Bany, 2000). Random heavy metal ion discharges from a wide range of anthropogenic and industrial process lead to serious environmental problems. Unlike organic contaminations, heavy metals ions are non–biodegradable and tend to

accumulate in living organisms. Previous research by Kadirvelu et al., (2001), the major sources of aquatic pollution is occurs from industrial that used water. The contamination of heavy metal exists in aqueous waste of many industries such as metal plating, mining operations and tanneries. They state a few treatments are used for metal removal from waste water such as precipitation and membrane filtration. Several heavy metals are essential to prawn tissue metabolism.

2.6 Bioaccumulation in Aquatic species

Bioaccumulation is a process when toxins enter to our ecosystem and generally observed more concentrated per unit of biomass at higher level trophic (John *et al.,* 2010). The toxic substances can be taking up to organism through their lung, gill, skin or direct point when transferred to the environment. Aquatic species can accumulate heavy metal such as mercury contaminated in the water. Bioaccumulation could occur when animals accumulate the heavy metal if exposed to the contaminant. Bioaccumulation and biomagnification can be occurring when the element cannot be excreted easily (Bishop, 2002).

De Smet & Blust (2001) stated that heavy metal cadmium can enter the tissue of *Cyprinus carpio* using different routes. In this species, cadmium can be accumulating more in gills compared in their gills. Based on Zahir et al. (2005) stated that human that consumed their dietary intake based on fresh or marine environment have high exposure to mercury. In United States, their people were exposed to mercury through consumption of fish or shellfish that containing methylmercury (John et al, 2010). Poisoning of

methylmercury will increase when people routinely consume fish or particular species of fish (Zahir et al., 2005)

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The materials that were used are same size of post larvae of *M. rosenbergii,* nitric acid, hydrogen peroxide, anti-chlorine, mercury and giant freshwater prawn feed.

3.2 Apparatus and Equipment

The apparatus that have been used are aerator, fiber tank, green net, tank (2' x 1'x 1'), conical flask, beaker, hot plate, volumetric flask, blender, drying oven, falcon tube, mask, syringe filter, filter funnel, Whatman filter paper, dropper and glove.

3.3 Method

3.3.1 Preparation of Working Place

Procedures for cleaning the tanks were followed according to the standard protocol. The aquariums were cleaned using tap water and left for one night. It is because; any detergent such as soap will make surface tension of the water will be low. The aquariums were washed with chlorox in left over night. After that, the aquariums were rinsed thoroughly and soaked in tap water for one night. The water was supplied with dissolve oxygen for three days prior for the experiment. On the next day, the aquarium was filled up with de-chlorinated water and the aerators were plugged in the aquarium for about 24 hours. To neutralize the water, anti-chlorine were added into the

water, to destroy harmful bacteria that exist in the water. Water quality was checked to ensure the optimum level of water is same with their natural habitat.

3.3.2 Sample Collection and Maintenance of Prawn

A total of *M. rosenbergii* PL were purchased from Pusat Penetasan Udang Galah PPUG), Setiawan, Perak. During collected, 1/3 of the plastic cover was filled up water from the original pond. After that, during packaging, the plastic cover was filled with oxygen. During transportation, the temperature was maintained between 27-31 (℃), the pH between 7.0-8.5 and salinity less than 10. The collected PL were brought to the Fish Propagation House (FPH), Politeknik Jeli in a plastic bag. The PL were transferred into fiber tank with sufficient aeration. The water was changed weekly and they were feeding daily with commercial pelletized.

Figure 3.1: Location of sampling site in Setiawan, Perak

(Source: http://maps.google.com on 7th September, 2017)

3.3.3 Experimental Design

Post larvae of *M. rosenbergii* were used for experimental studies. A total 360 of PL were used in this study and divided into 4 groups. Each group contains 30 of post larvae with three replicates. PL were exposed to three different levels of mercury which were 0.01 ppb, 0.03 ppb and 0.05 ppb. Aquarium tanks without heavy metal were used as a control group (0.00 ppb).

Table 3.1 show the experiment design for

C1 A	C1 B	C1 C
T1 A	T1 B	T1 C
T2 A	T2 B	T3 C
T3 A	T3 B	T3 C

C = Control T = Treatment A, B, C = Replication

3.3.4 Stock Solution

Stock solution for heavy metal of mercury solutions were purchased from Environmental Laboratory at Universiti Malaysia Kelantan. The stock solution was diluted to 0.01 ppb, 0.03 ppb and 0.05 ppb. The stock solution was diluted by using the standard formula which is:

$m_1v_1 = m_2v_2$

When,

m_1	Stock concentration
v_1	Stock volume required
m_2	Concentration needed
v_2	Volume for final dilution

Firstly, 1000 ppb stock was diluted to 50 ppb. The dilution was measured by using volumetric flask for accuracy and convenience. The calculation was re-run by using the dilution formula. The serial dilution technique was used to reduce the concentration of heavy metal.

3.3.5 Mercury Exposed

Post larvae were randomly assigned and divided into 4 identical tanks and remained as unexposed (control group) and exposed to a sub-lethal dosage of mercury.

The concentration of mercury was used is 0.01 ppb, 0.03 ppb and 0.05 ppb. The different concentrations were used to identify the survivability of *M. rosenbergii* on different toxicity level of mercury.

Table 3.2: Level of Mercury used in the present study

Tank	Level of Mercury (Hg) (ppb)	Concentration of Mercury (ppb)
A	Minimum	0.01
B	Optimum	0.03
C	Maximum	0.05
D	Control	0.00

3.3.6 Sample preparation and preservation

The length and weight of the post larvae sample were measured and noted after exposed on heavy metal. The samples were stored in clean glass bottle separately at -20 °C for 24 hours. All samples were dried separately at 120 °C for 24 hours in dry oven. Then, each sample was blended homogenously until the sample turned into powdered form and then packed in zipped begs and sealed separately before acid digestion process.

3.3.7 Sample Digestion and Extraction

Total of 0.5 gram of dried sample were weighed and put on 100 ml beaker. About 6 ml of (65%) concentrated nitric acid (HNO_3) was added into the beaker then, followed by adding 2 ml of (30%) hydrogen peroxide (H_2O_2) into the beaker. All the beakers were placed on the hot plate separately at 70 °C for 3 hours until becomes dryness and this to ensure complete digestion of all organic matters. 3% of diluted nitric acid (HNO_3) was dropped into the beakers placed on the hot plate after 20 minutes. The digested solutions were left to cool down in ambient temperature.

After cooling, the digested solution were filtered through 0.45 µm of Whatman filter paper into falcon tubes and doubled rinsed with deionized water to ensure that the entire digest were transferred into the tube. The filtrated samples were made up to 30 ml by adding distilled water for the dilution. The laboratory works were done in Materials Science Laboratory at Universiti Malaysia Kelantan, Jeli.

The determination of heavy metal mercury was carried out using inductively coupled plasma-optical emission spectrometry (ICP-OES).

3.3.8 Instrument of ICP-OES

Heavy metal of mercury was analysed by Inductively – Coupled Plasma Optical Emission Spectrometry (ICP–OES) model (Varian 725-ES, Australia) at Veterinary Medicine Faculty, UMK Kampus Kota for metal speciation. ICP–OES is an analytic technique used for element determinations. The element at ppb level can be identified.

3.3.9 ICP-OES Condition

The wavelengths of mercury used were 184.887 nm, 194.164 nm and 253.652 nm. ICP-OES conditions were stated below.

Table 3.3: The condition for ICP-OES

Condition	1	2	3
RF power (kW)	1.2	1.2	1.2
Viewing mode	Axial	Axial	Radial
Nebulizer flow (L/min)	0.7	0.5	0.5

3.3.10 Statistical Analysis

All the data were collected and statistically analysed by using One-Way Analysis of Variance (ANOVA) by using Tukey test available from SPSS package for the social science software version 22.0.

CHAPTER 4

RESULT

4.1 Collected Data

This experiment was conducted at Fish Propagation House (FPH) located at Politeknik Jeli. From this study, different concentration of heavy metal were used which was 0.01ppb (tank T1 A, T1 B and T1 C), 0.03ppb (tank T2 A, T2 B, T2 C), 0.05 ppb (tank T3 A, T3 B, T3 C) and control (tank C1 A, C1 B and C1 C).

Table 4.1 shows the percentage of mortality of *M. rosenbergii* in 4 days during exposed to different heavy metal mercury.

Concentration of heavy metal mercury (ppb)	Percentage of mortality (%)
Control	0
0.01	14.44 ± 1.1645
0.03	30.00 ± 2.5346
0.05	51.11 ± 2.3677

4.2 The LC_{50} of Mercury on *M. rosenbergii*

Toxicity occurs when the rate of metal uptake into the body exceed the limit (Suhaimi-Othman *et al*., 2006). Since, mercury was non-essential element for organism. So, in low concentration of heavy metal it is not necessary and give harmful effect to the prawn. From this experiment, LC_{50} cannot be recorded because on the maximum concentration of mercury which is 0.05 ppb they are not 100% of mortality of PL was recorded.

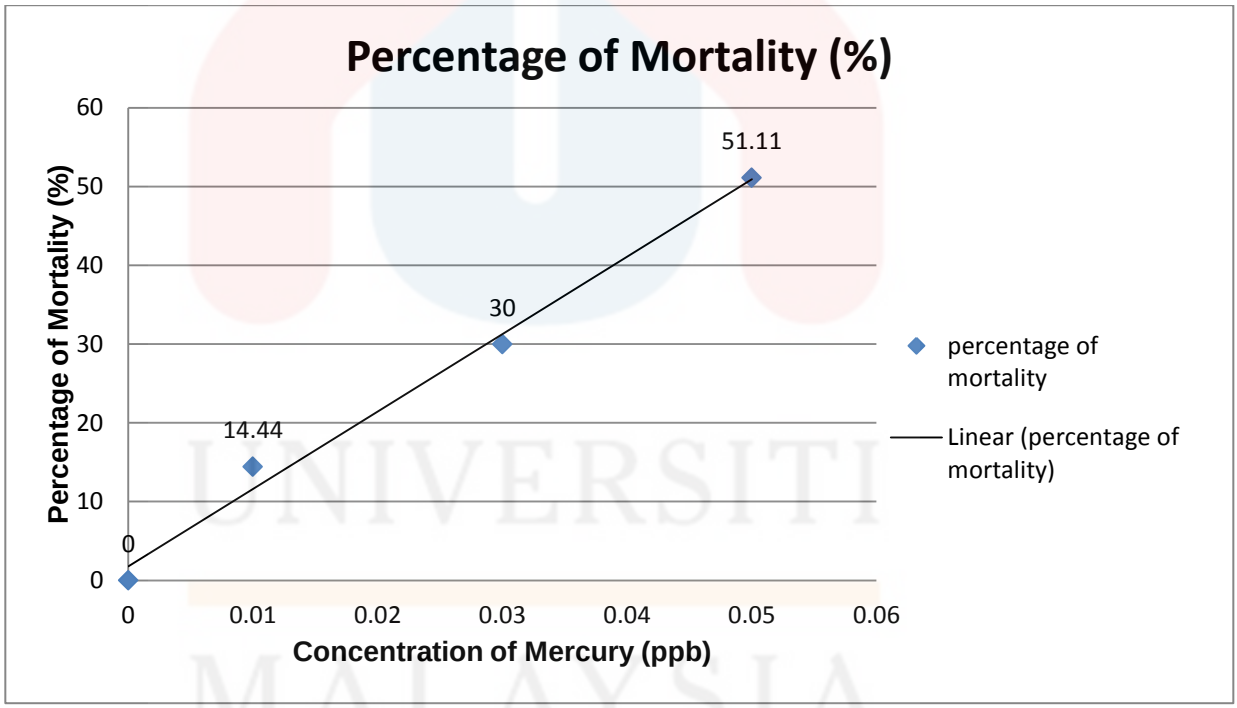


Figure 4.1: The graph of percentage of mortality that shows the concentration of mercury that causes 50% mortality of *M. rosenbergii.*

This graph showed a good linear relationship among concentration of heavy metal mercury and percentage value for mortality.

4.3 Data Analysis

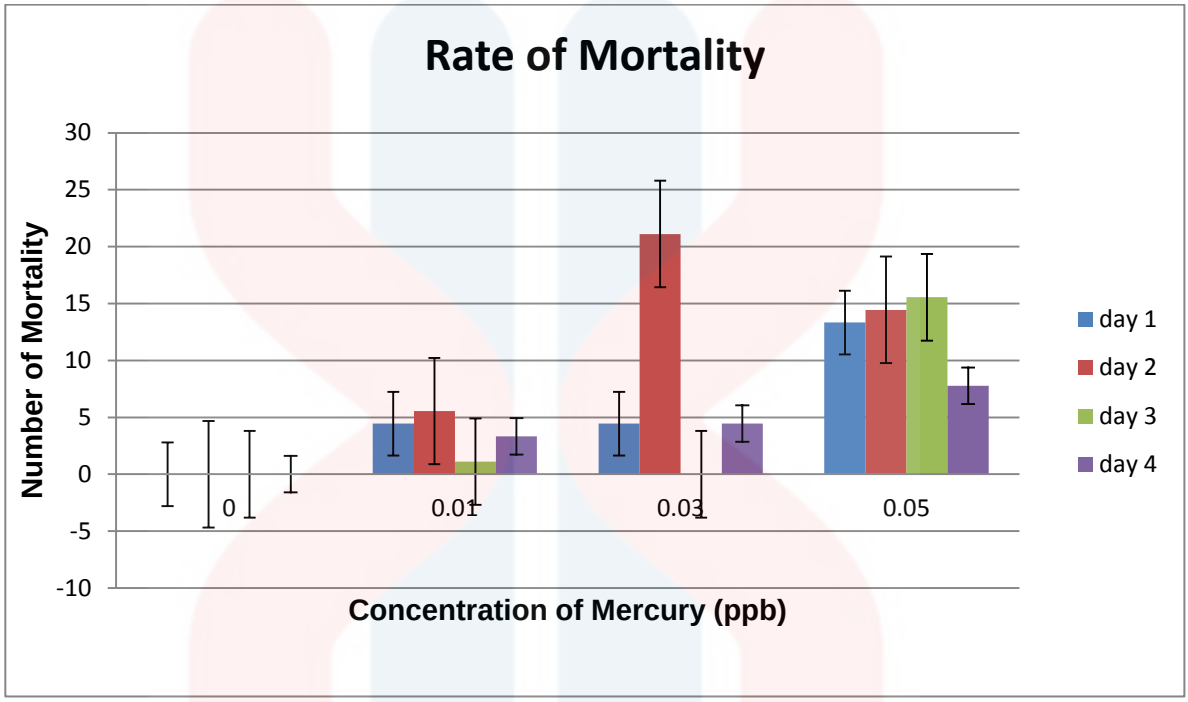


Figure 4.2: Rate of mortality of *M. rosenbergii* in different concentration of Mercury

Based on figure 4.2, the bar graph included all the treatment that showed the mortality of PL within concentration of mercury 0.01 ppb, 0.03 ppb and 0.05 ppb. During first day of experiment, the highest mortality of PL was on the concentration of 0.05 ppb. Then, for second days, exposed to heavy metal of mercury, the highest mortality was on concentration 0.03 ppb. On the second day, the rate of mortality of PL was most higher compared to other days. During third days, on the concentration 0.03 ppb no mortality of PL was recorded. On the last day, the amount of mortality was higher when exposed to 0.05 ppb.

Based on table A.4, rate of mortality of *M. rosenbergii* during experimental period ranged at 0 to 21. The rate of mortality at the beginning of experiment were homogenous and were not significantly different ($p>0.05$). The mean of rate of mortality of giant freshwater prawn on first day for treatment 1 (0.01 ppb), 2 (0.03 ppb) and 3 (0.05 ppb) were 4.4433, 4.4433 and 13.3333. During second days of exposed to heavy metal the control group (0.00 ppb) and treatment 2 which is 0.03 ppb are significant. The mean of rate of mortality of giant freshwater prawn on second day for treatment 1, 2 and 3 were 5.5533, 21.1100 and 14.4433. On the third days, control group (0.00 ppb) and treatment 3 (0.05 ppb) are significant. The mean of rate of mortality of giant freshwater prawn on third day for treatment 1 and 3 were 1.1100 and 15.5533. In contrast, on the last day exposed to heavy metal of mercury no significant value between the treatments. The mean of rate of mortality of giant freshwater prawn on last day for treatment 1, 2 and 3 were 3.3333, 4.4433 and 7.7800.

4.4 Environmental Condition

During this experiment, the water quality was controlled checked daily. Table 4.2 showed the reading of water quality during the period of experiment.

Table 4.2: Water quality parameter through experimental period for 4 days.

Water quality parameter	Experimental Data
Temperature (°C)	26.7 – 27.5
pH	6.8 – 8.0
Dissolved Oxygen (mg/L^{-1})	6.5 – 8.1

The temperature was in the range 26.7 – 27.5 °C in the period of experimental. While the pH was between 6.8 to 8.0 and the DO was maintained in the range of 6.5 to 8.1 mg/L^{-1}.

4.5 Bioaccumulation Analysis on *M. rosenbergii*

4.5.1 ICP-OES Condition

There are four conditions to run the ICP-OES. In condition 1, the RF power used was 1.2 kW, plasma flow was 12L/min, Auxillary Flow was kept at 1 L/min, Nebulizer Flow was kept at 0.7 L/min, heavy metal mercury was monitored at wavelength of 184.887 nm, 194.164 nm and 253.652 nm and plasma view was in axial mode. While, in condition 2, the RF power was used is 1.2 kW, plasma flow was used 12L/min, Auxillary Flow was kept at 1 L/min, Nebulizer Flow was kept at 0.5 L/min, heavy metal mercury was monitored at wavelength of 184.887 nm, 194.164 nm and 253.652 nm and plasma view was in axial mode. Lastly is condition3, the RF power was used is 1.2 kW, plasma flow was used 12L/min, Auxillary Flow was kept at 1 L/min, Nebulizer Flow was kept at 0.5 L/min, heavy metal mercury was monitored at wavelength of 184.887 nm, 194.164 nm and 253.652 nm and plasma view was in radial mode. The heavy metal mercury was monitored at wavelength of 184.887 nm, 194.154 nm and 253.652 nm.

4.5.2 Wavelength of Calibration Curves

Calibration curves for absorption of the cadmium metal were performed with the reference metal standards, at 184.887 nm, 1.94.164 nm and 253.652 nm wavelengths for mercury. Subsequent to the calibration, samples were aspirated to get the absorption at the specific wavelengths and concentration of the metal. The wavelengths of

calibration curve were observed. The results of wavelength were presented in Figures 4.3, 4.4 and 4.5.

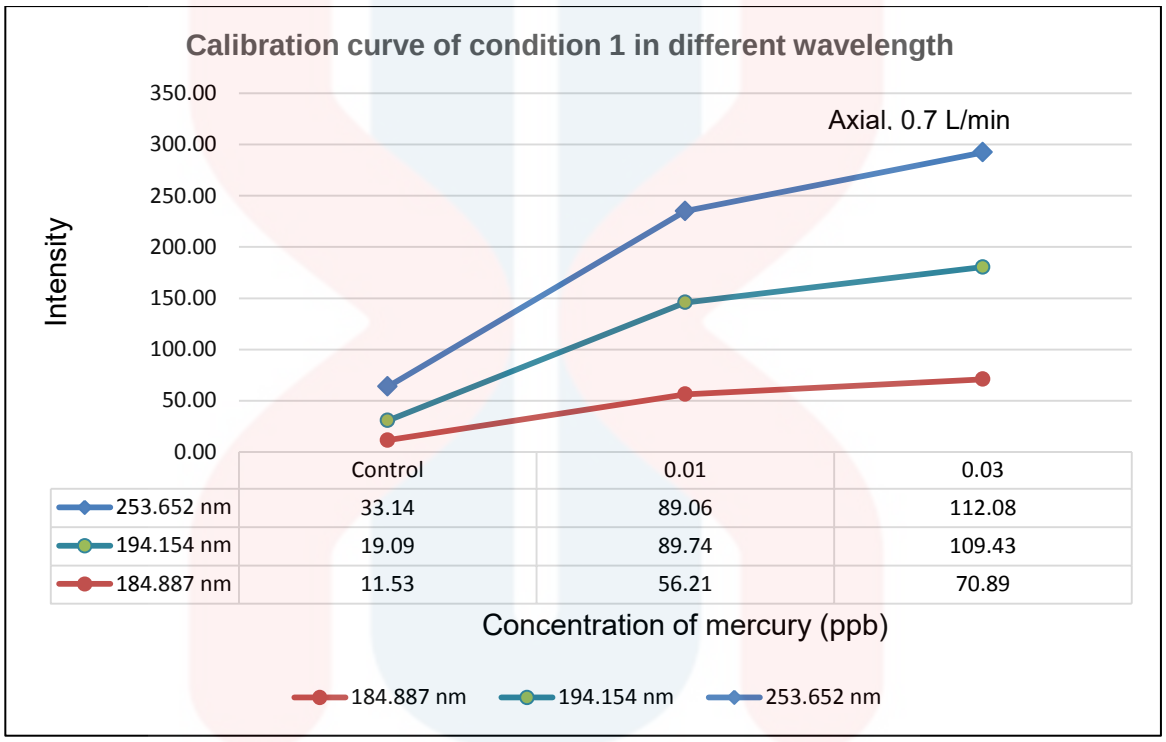
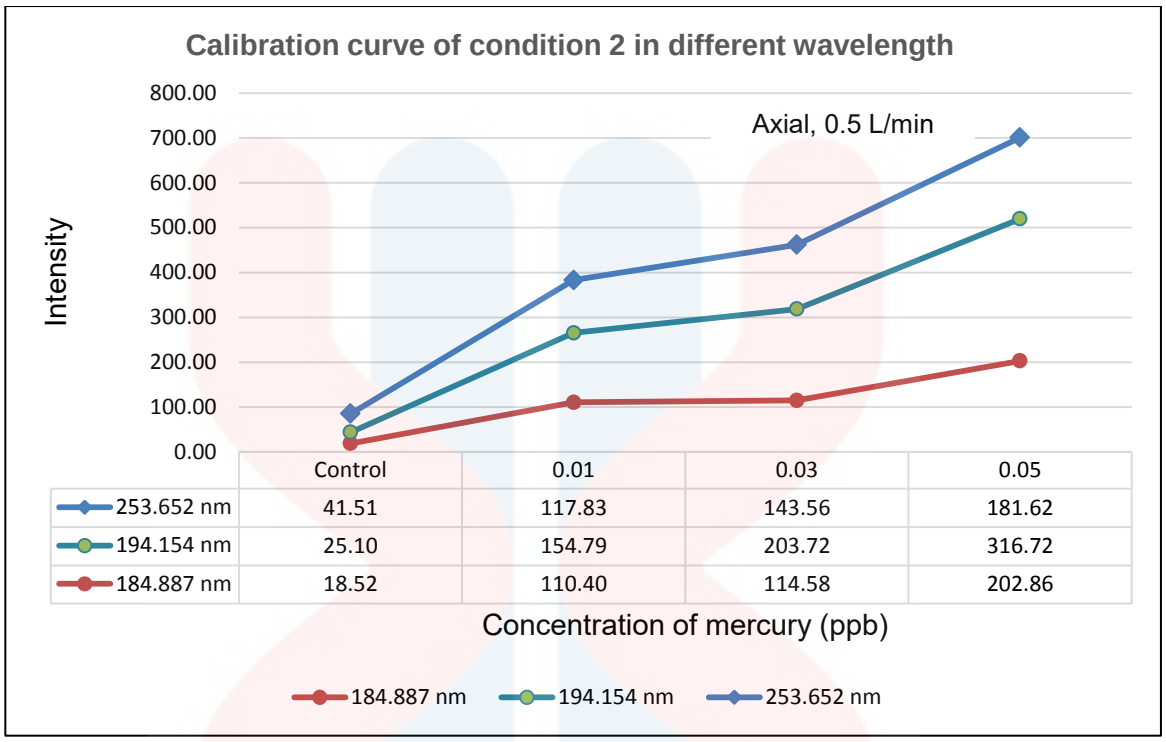


Figure 4.3 shows the standard calibration curve of condition 1 in different wavelength using viewing mode of axial and nebulizer flow of 0.7 L/min.



	Control	0.01	0.03	0.05
253.652 nm	41.51	117.83	143.56	181.62
194.154 nm	25.10	154.79	203.72	316.72
184.887 nm	18.52	110.40	114.58	202.86

Figure 4.4 shows the standard calibration curve of condition 2 in different wavelength using viewing mode of axial and nebulizer flow of 0.5 L/min.

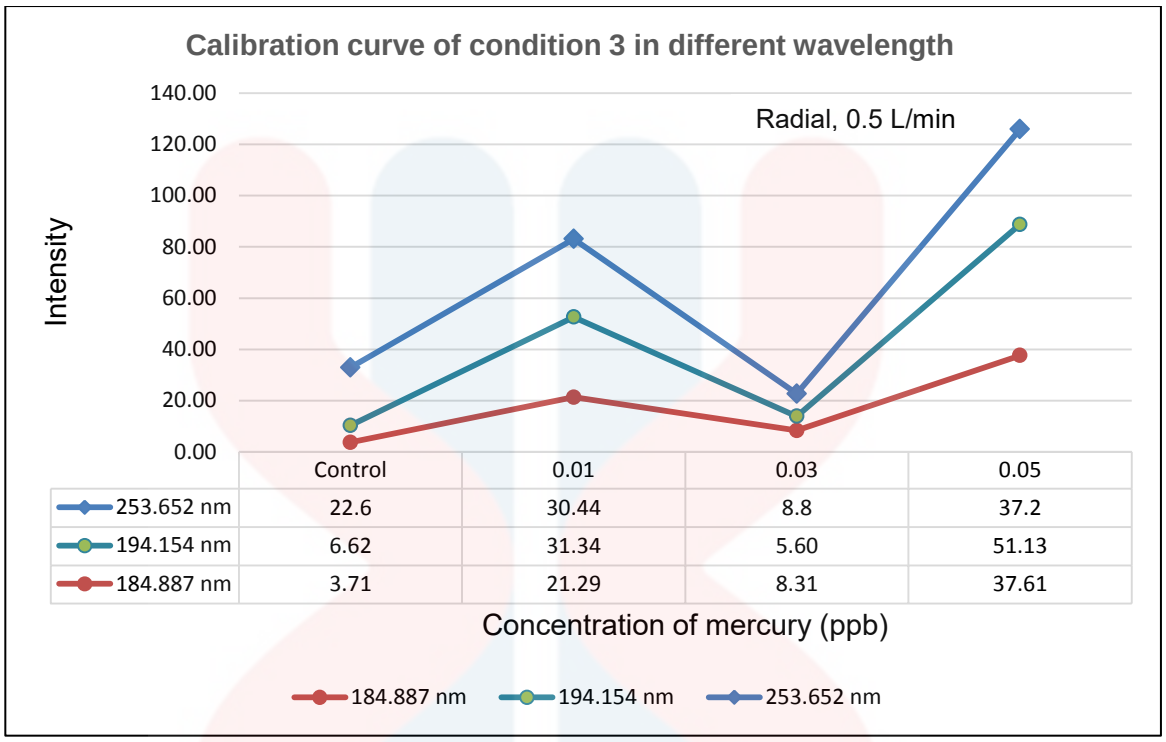


Figure 4.5 shows the standard calibration curve of condition 3 in different wavelength using viewing mode of radial and nebulizer flow of 0.5 L/min.

CHAPTER 5

DISCUSSION

5.1 Toxicity

Based on Table 4.1, it shows the different concentration of Mercury (Hg) can affect the rate of mortality of post larvae of giant freshwater prawn *M. rosenbergii*. When the concentration of heavy metal of Mercury (Hg) that exposed to post larvae of giant freshwater prawn, *M. rosenbergii* was increased, the rate of mortality of *M. rosenbergii* rate was increased. From the previous study it show the same result for study on heavy metal of cadmium effect in *M. rosenbergii* (Kaoud & Eldahshan, 2010).

This situation can be seen based on Table A.3 which is at concentration of 0.05 ppb is the highest mortality rate on post larvae of *M. rosenbergii* was represented which is 51.11%. It can be seen that during the 24 hours period of exposed the PL of giant freshwater prawn to heavy metal of Mercury, the amount of mortality in 0.01 ppb was lower compared to 0.05 which is 4.44% and 13.33%. During 48 hours exposed to heavy metal mercury, the amount of mortality in 0.03 ppb is higher compared to 0.01 ppb which is 21.11% and 5.56%. While, during 72 hours exposed to heavy metal mercury, the amount of mortality on 0.05 ppb is higher compared to 0.01 ppb which is 15.56% compared to 1.11%. For the last days exposed to heavy metals mercury which is during 92 hours, the amount of mortality in concentration of 0.05 ppb is higher compared to 0.01 ppb and 0.03 ppb which is 7.78% compared to 3.33% and 4.44%. On second days exposed to heavy metal mercury, there is significance to other groups.

Lethal concentration 50 (LC_{50}) cannot be measured because during 4 days experiment was conducted, no 100% mortality was recoded in all concentration. It is because the concentration of mercury is in small amount. To observe the LC_{50}, the percentage of the sample under observation must die 100%. During this experiment, no giant freshwater prawn in the control group died. The 96 hours percentage for mercury exposure in *M. rosenbergii* of the concentration 0.01ppb, 0.03ppb and 0.03 ppb were calculated which are: 14.44%, 28.89% and 51.11%. The effects that cause the acute lethal effect on *M. rosenbergii* by three different concentration of heavy metal mercury were shown in figure 4.1. The percentage for mortality rate of 0.05 ppb is 51.11% was higher compared to other concentration which is 0.01 ppb and 0.03 ppb.

From the previous study, lethal effect of heavy metal from mercury, copper, cadmium and lead causes coagulation of mucus which is the insoluble metal of proteins compound was precipitate on their gills and cause failure on respiration (Dandroff & Katz, 1953). Bhamre et al. (2010) stated that the increasing the concentration of heavy metal will affect the rate of mortality of *Lamellidens consobrinus.* Increase the duration time exposure to heavy metal lead to the death. From this research, the toxicity level of heavy metal on *L. consobrinus* affects respiratory system and nervous system of this species and caused death. They also stated that high sensitivity on mollusc to mercury is higher compared to other heavy metal which is cadmium and zinc

The water quality parameter such as temperature was maintained at optimum range throughout the experiment. Based on table 4.3 water condition during this experimental period was suitable for rearing giant freshwater prawn *M. rosenbergii.* The average of water temperature for this experimental ranged from 26.7 °C to 27.5°C.

These temperatures were in preferred range of temperature recorded by Yogi Himawan and Ikhsan Hasani (2010). The study that conducted by Yogi Himawan and Ikhsan Hasani stated that the optimum temperature for growth of giant freshwater prawn is range between 28.0 °C- 32.0°C.

During this experimental period, pH value was at minimum 6.8 and maximum is 8.0. The range was in the optimum values of pH stated by Yogi Himawan and Ikhsan Hasani (2010) at range level 7.2 to 8.5 in order to obtain optimum development and survival rate.

Besides that, dissolve oxygen (DO) was at minimum 6.5 mg/L and maximum is 8.1mg/L. Based on Yogi Himawan and Ikhsan Hasani (2010), they stated that the DO for rearing of giant freshwater Yogi Himawan and Ikhsan Hasani stated that the suitable condition for rearing giant freshwater prawn is between 28 prawn is more than 3mg/L.

5.2 Bioaccumulation Analysis

Aquatic species is one of human consumption nowadays which is as a source of food. Bioaccumulation can be defined as the net uptake by all possible sources of pollution by organism from their surrounding environment.

From this experiment, prawns get their mercury exposure due the three different concentration of treatment which is 0.01 ppb, 0.03 ppb and 0.05 ppb. Bioaccumulation of heavy metal Mercury cannot be defined. It was due to the some factor like there is other elements in the sample. So, ICP-OES cannot read the specific value for the mercury.

According to graph on Figure 4.3, it shows that viewing mode of axial and nebulizer flow of 0.7 L/min when using wavelength of 253.652 nm have a potention to create a calibration curve. Calibration curve must be create in straight line. The straight line is important to show the consistence result of heavy metal mercury that accumulates in PL. From this experiment, the calibration curve cannot be determined. This is due to some parallax error during preparation of stock solution. During preparing the stock, the stock solution was transferred to volumetric flask by using measuring cylinder. The accurate method is the dilution need to be transferred by using pipette. The calibration curves can be created based on linear response between instrument signals an analytic concentration.

Based on previous studies, the level of bioaccumulation is based on the role of age, species and trophic transfer. The concentration of metal may vary with the age and bodyweight within the same species (Islam et al., 2014). Islam et al. (2014) also stated

another factor that influences the concentration of metals is feeding habits of the species.

According to the research from Shuhaimi-Othman (2006), he stated that the bioaccumulation of heavy metal in prawn increases with the increasing concentration exposure. Experiment was same like previous study but the mercury bioaccumulation in *M. rosenbergii* cannot be determined. Based on Shuhaimi-Othman et al., 2006, accumulation pattern of heavy metal were depend on metal concentration and exposure time. From this experiment, the concentration of heavy metal mercury only in small amount and the duration of exposed to heavy metal is short time. In the study that conducted by Green et al. (1976), they stated that by using post larvae *Penaeus setiferus* there is no effect on growth, respiratory rate or moulting rate when using 1.0 ppb.

Based on previous study, the levels of heavy metal that accumulate in *P. corruscans* were greater in their liver, spleen and muscle (Arantes *et al*., 2015). From the study that conducted by Dang & Wang (2012) stated that, high level of mercury in fish cause significant risk in the aquatic ecosystem and can affect the human health.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Present study revealed that the accumulation of heavy metal of mercury can affect the life span of PL of giant freshwater prawn, *M. rosenbergii.* Based on this study on toxicity level, on the concentration of 0.05 ppb of mercury it is harmful to PL. even in small amount of heavy metal, it can affect the mortality to PL. Based on this study on bioaccumulation, the result cannot be determining in the PL. This problem was due to technical some error when preparing the standard solution and not used suitable apparatus to prepare the standard solution. In fact, the results of standard curves were not being identified in this research.

6.2 RECOMMENDATION

For future study, the exposed of another concentration of heavy metal mercury should be run to other aquatic species such as fish. It is because fish is common source of food to human. The heavy metal exposed should be in different concentration and the histopathology and bioaccumulation of the fish should be observed. Besides that, more research on evaluation of ammonia and nitrite toxic effect in aquatic species should be conducted. The study and research on the river around Malaysia to detect the amount of

mercury level should be conducted continually to assess the level of pollution. This research is important to generate maximal growth and optimum survival rate of this species. Besides, the most important things avoid the error in lab work and need to have basic lab skill to improve the result of research in the future.

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APPENDICES

Table A.1: Post Hoc Analysis using Tukey Test for Mortality Rate.

Mortality

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Control 1st	3	.0000		
Control 2nd	3	.0000		
Control 3rd	3	.0000		
Control 4th	3	.0000		
Treatment2 3rd	3	.0000		
Treatment1 3rd	3	1.1100	1.1100	
Treatment1 4th	3	3.3333	3.3333	
Treatment1 1st	3	4.4433	4.4433	
Treatment2 1st	3	4.4433	4.4433	
Treatment2 4th	3	4.4433	4.4433	
Treatment1 2nd	3	5.5533	5.5533	
Treatment3 4th	3	7.7800	7.7800	7.7800
Treatment3 1st	3	13.3333	13.3333	13.3333
Treatment3 2nd	3	14.4433	14.4433	14.4433
Treatment3 3rd	3		15.5533	15.5533
Treatment2 2nd	3			21.1100
Sig.		.091	.262	.159

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.2: One-Way ANOVA

Mortality

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control 1st	3	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
Control 2nd	3	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
Control 3rd	3	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
Control 4th	3	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
Treatment1 1st	3	4.4433	1.92835	1.11333	-.3470	9.2336	3.33	6.67
Treatment1 2nd	3	5.5533	6.93755	4.00540	-11.6805	22.7872	0.00	13.33
Treatment1 3rd	3	1.1100	1.92258	1.11000	-3.6659	5.8859	0.00	3.33
Treatment1 4th	3	3.3333	3.33500	1.92546	-4.9513	11.6179	0.00	6.67
Treatment2 1st	3	4.4433	1.92835	1.11333	-.3470	9.2336	3.33	6.67
Treatment2 2nd	3	21.1100	1.92258	1.11000	16.3341	25.8859	20.00	23.33
Treatment2 3rd	3	0.0000	0.00000	0.00000	0.0000	0.0000	0.00	0.00
Treatment2 4th	3	4.4433	1.92835	1.11333	-.3470	9.2336	3.33	6.67
Treatment3 1st	3	13.3333	8.81602	5.08993	-8.5669	35.2335	6.67	23.33
Treatment3 2nd	3	14.4433	12.61862	7.28537	-16.9031	45.7897	.00	23.33
Treatment3 3rd	3	15.5533	6.93755	4.00540	-1.6805	32.7872	10.00	23.33
Treatment3 4th	3	7.7800	1.92258	1.11000	3.0041	12.5559	6.67	10.00
Total	48	5.9717	7.62420	1.10046	3.7578	8.1855	0.00	23.33

Table A.3: The rate of mortality of *M. rosenbergii* per hours.

Concentration (ppb)	Tanks / Hours	24 hours	48 hours	72 hours	96 hours	Total numbers of mortality (Out of 90)	Percentage death (%)
0	C1 A	0	0	0	0	0	0
	C2 B	0	0	0	0		
	C3 C	0	0	0	0		
0.01	T1 A	1	1	1	1	13	14.44
	T1 B	1	0	0	2		
	T1 C	2	4	0	0		
0.03	T2 A	1	6	0	1	27	30.00
	T2 B	2	7	0	2		
	T3 C	1	6	0	1		
0.05	T3 A	2	0	3	2	46	51.11
	T3 B	7	7	4	3		
	T3 C	3	6	7	2		

Table A.4: Rate of mortality (%) of *M. rosenbergii* in different concentration of Mercury

Concentration of Mercury (ppb) / Days	Mean ± SD			
	Days 1	Days 2	Days 3	Days 4
Control	0.0000[a]	0.0000[a]	0.0000[a]	0.0000[a]
0.01	4.4433 ± 1.9284[ab]	5.5533 ± 6.9376[ab]	1.1100 ± 1.9226[ab]	3.3333 ± 3.3350[ab]
0.03	4.4433 ± 1.9284[ab]	21.1100 ± 1.9226[c]	0.0000[a]	4.4433 ± 1.9284[ab]
0.05	13.3333 ± 8.8160[abc]	14.4433 ± 12.6186[abc]	15.5533 ± 6.9376[bc]	7.7800 ± 1.9226[abc]

Values are means ± SD of three replicates. Values in the same column with different superscripts are significantly different ($p<0.05$).
*Control: Without heavy metals of mercury; Treatment 1: 0.01ppb concentration of heavy metal of mercury; Treatment 2: 0.03ppb concentration of heavy metal of mercury; Treatment 3: 0.05ppb concentration of heavy metal of mercury.

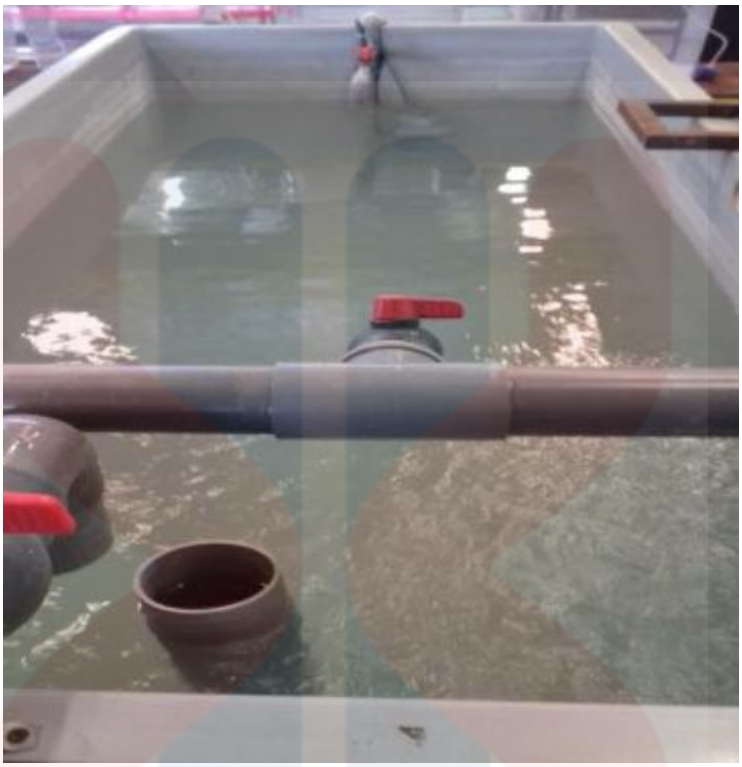

Figure A.1: Preparation of lime water to stabilize the pH of water

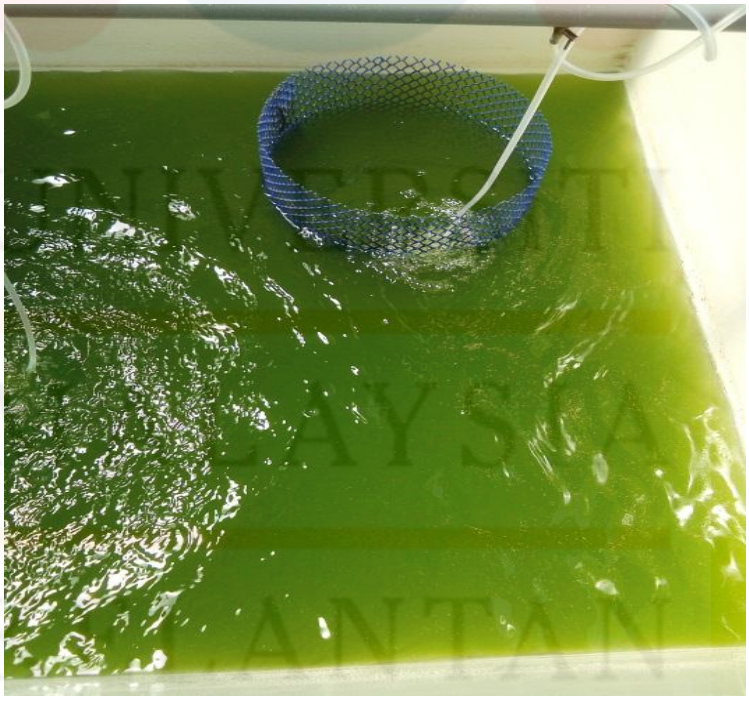

Figure A.2: Preparation of green water.

Figure A.3: Weighing post larvae of *M. rosenbergii.*

Figure A.4: Drying process of sample in drying oven.

Figure A.5: Set the temperature and hours for drying the post larvae

Figure A.6: Weighing the post larvae after drying.

Figure A.7: Blended of sample.

Figure A.8: The sample after blended.

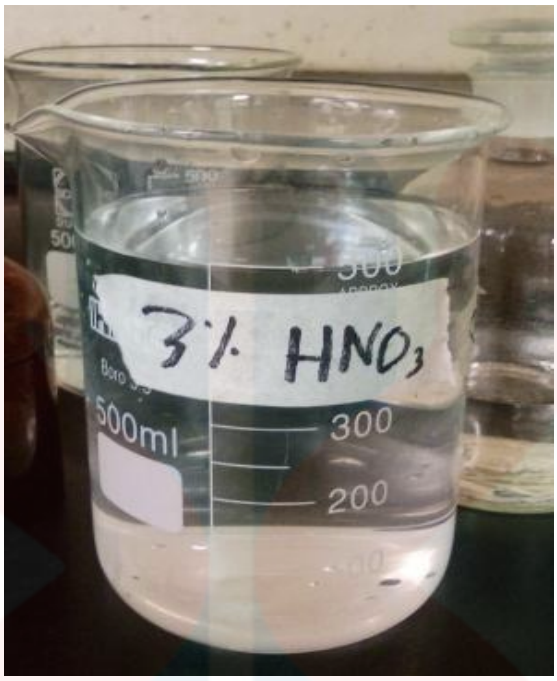

Figure A.9: Preparation of 3% of HNO_3

Figure A.10: Weighing the powder of post larvae.

Figure A.11: Acid digestion process

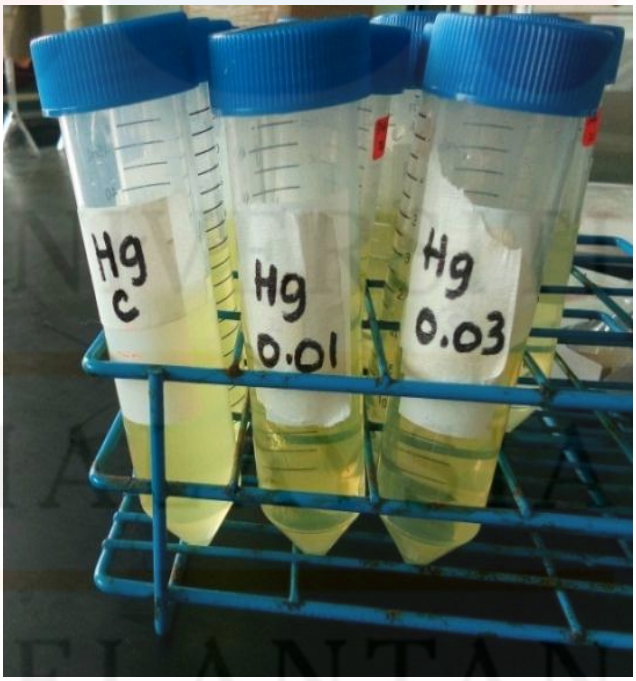

Figure A.12: filtering process.

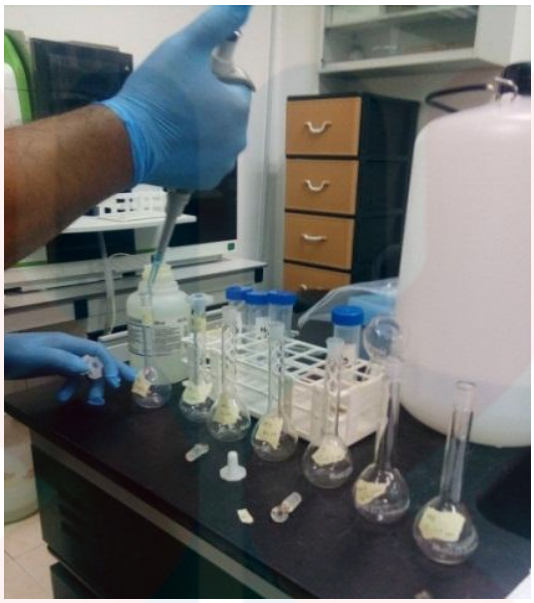

Figure A.13: The sample after filtering

Figure A.14: Preparation of stock solution for ICP-OES

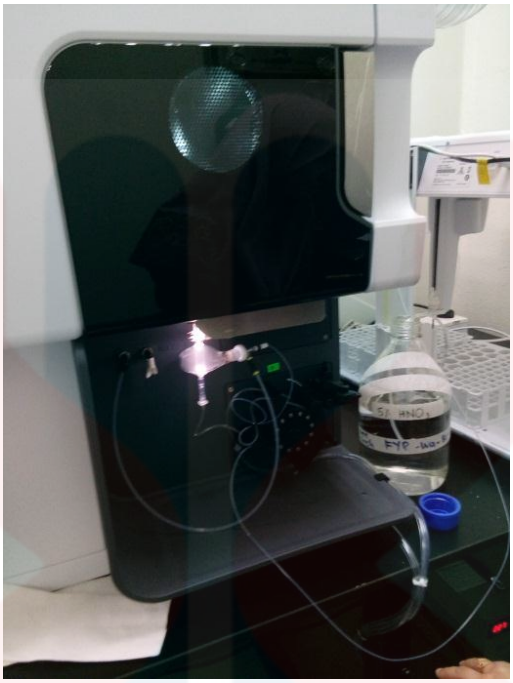

Figure A.15: Run the process of bioaccumulation.

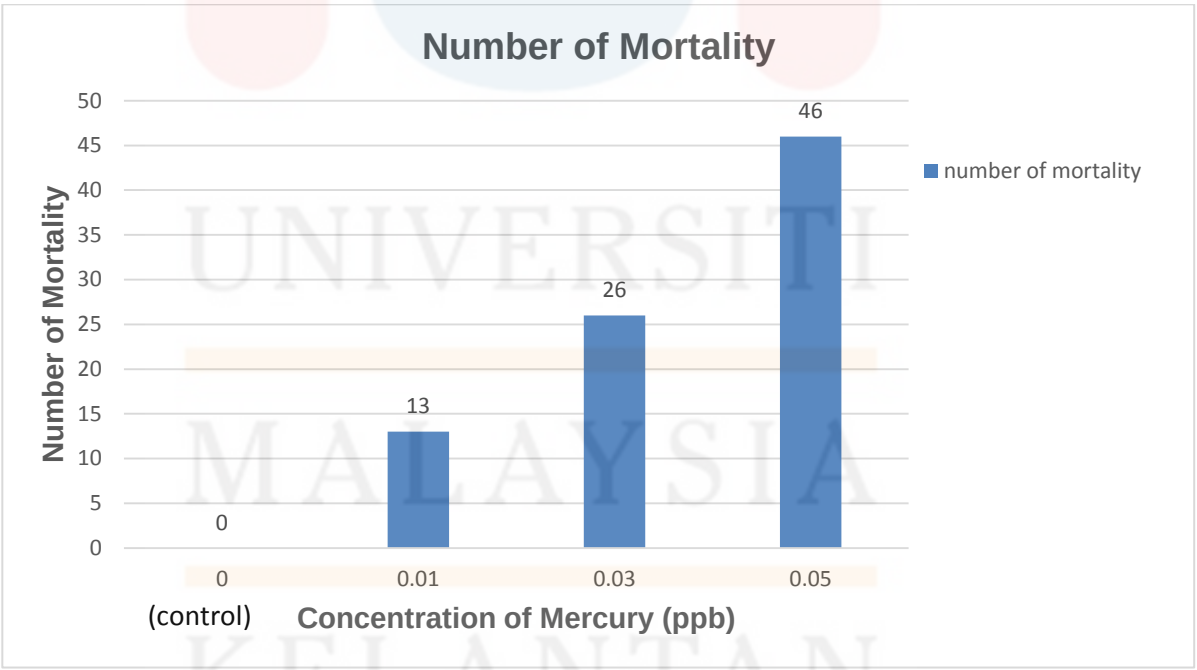

Figure A.16: The number of mortality of *M. rosenbergii.*