The Toxicity of Cadmium (Cd) on The Giant Freshwater Prawn (*Macrobrachium rosenbergii)*, Post-Larvae.

By

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A report submitted in fulfillment of the requirement for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours.

Faculty of Agro Based Industry

UNIVERSITI MALAYSIA KELANTAN

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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 fir higher degree to any universities or institutions.

_____.

Student

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Date:

I certify that the report of this final year project entitled "_____'' by _____, matric numbers _____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 Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

Approved by:

_____

Supervisor

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Date

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The Toxicity of Cadmium (Cd) on The Giant Freshwater Prawn (*Macrobrachium rosenbergii)*, Post-Larvae.

ABSTRACT

Heavy metal has gravely increased worldwide attention and under certain environmental condition. The aquatic organisms such as freshwater prawn may concentrate large amount of metal from water in their tissue. Cadmium is a heavy metal that considerably toxicity with destructive impact on most organ systems. Cd was former used in the many industrial activities for a long period of time. In this review, the post larvae *M. rosenbergii,* were exposed to different concentration of Cd in soft water. The concentrations of Cd that has been used were 20 ppb, 40 ppb, 60 ppb, 80 ppb and 100 ppb and control without Cd concentration. The post larvae were used because they consistently more sensitive. A 4 days exposure period 24, 48, 72 and 96 hours appears to be appropriate for determining post larval sensitivity to Cd. The post larvae(PL) exposed at 28 °C, salinity is 28, pH 7.76 and the dissolved oxygen is 4.8 mg/L. The concentrations were triplicate and 30 post larvae per tank were used. The objective of this study was to determine the acute level of cadmium on the freshwater prawn, *M. rosenbergii*, PL.

After 96 hours exposed to the different concentration of Cd, the result showed higher significant effect between the between the studied concentrations of Cd. Result also demonstrated the highly toxic even for short term exposure to 20-100 ppb of Cd in PL. In conclusion, the 100 ppb was the maximum concentration that showed the 100% mortality on post larvae.

Keywords: M*. rosenbergii*, post larvae, heavy metal, Cadmium, toxicity.

Ketoksidan Kadmium (Cd) kepada Udang Air Tawar (*Macrobrachium rosenbergii*),larva-pasca

ABSTRAK

Logam berat telah mendapat perhatian yang meluas dari seluruh dunia dan berada di bawah keadaan persikitaran yang tertentu.Organisma akuatik seperti udang air tawar menyerap jumlah yang besar logam dari air kedalam tisu organisma tersebut. Kadmium adalah logam yang berat yang sangat bertoksid dengan memberi kesan kerosakan pada kebanyakan sistem organ.Kadmium telah digunakan di dalam industri untuk jangka masa lama.Di dalam kajian ini, larva pasca *M. rosenbergii* didedahkan kepada kepekatan Kadmium yang berlainan di dalam air suling. Kepekatan Kadmium yang digunakan adalah di antara 20 ppb, 40 ppb,60 ppb and 100ppb. Kawalan digunakan tanpa sebarang kepekatan Kadmium. Larva pasca digunakan kerana mereka secara konsisten lebih sensetif .Tempoh pededahan selama 4hari dalam tempoh 24, 48, 72 dan 96 jam agak sesuai untuk menentukan kepekaaan larva pasca kepada Kadmium. Larva pasca didedahkan pada suhu 28 ° C, saliniti 28 , pH 7.76 dan oksigen terlarut adalah 4.8 mg/L. Setiap kepekatan digandakan kepada tiga dengan jumlah pasca larva sebanyak 30 ekor di dalam setiap tangki. Objektif untuk eksperimen ini adalah menentukan ketoksidan kadmium terhdap udang air tawar ,*M. rosenbergii*, pasca larva.

Selepas 96 jam didedahkan kepada kepekatan larutan Kadmium yang berbeza,keadaan menunjukan terdapat kesan yang besar di antara kepekatan rendah dengan kepekatan yang tinggi ($p < 0.05$). keputusan yang ditunjukkan ketoksidan tinggi pada 20-100 ppb kepekatan Kadmium dalam larva pasca. Bagi rawatan kawalan yang tidak didedahkan kepada kepekatan Kadmium menunjukan tiada perubahan keputusan dalam masa 24 jam, 48 jam,72 jam dan 96 jam. Konklusinya adalah 100 ppb adalah kepekatan maksimum dengan menunjukan kematian pasca larva 100%.

Kata kunci :*M. rosenbergii*, larva pasca, logam berat, Kadmium, Ketoksidan.

TABLE OF CONTENT

LIST OF TABLE CONTENT

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis Of Variance
Ppb	Parts per billions
Ppm	Parts per millions
Ppt	Parts per trillions
Cd	Cadmium
g	Gram
mg	Milligram
µg	Microgram
M	Meter
Mm	Millimeter
Cm	Centimeter
%	Percentage
PPUG	Pusat Penetasan Udang Galah
mg/L	Milligram per liter
L	Liter
d	Days
PL	Post larvae

Chapter 1

Introduction

1.1 Research Background

Crustaceans, below the order of Decapoda like shrimp, crab, prawn and lobster which are ecologically and economically imperative. *Macrobrachium rosenbergii* as giant freshwater prawn is locally known as udang galah. Moreover, it is important crustacean species produced inland aquaculture in many tropical countries worldwide. The species of the freshwater prawn genus Macrobrachium are distributed throughout the tropical and subtropical zones of the world provided useful information on the distribution, local name, habitats and maximum size of commercial *M. rosenbergii* (Holthuis, 1980).

Natural habitat for *M. rosenbergii* is in freshwater and this prawns required the brackish water during larval period (New, 2005). The average weight for the prawn in the male populations can reached up to 473g within a period of 150 d. Females the size can be reached up to 248 g and 260 g respectively (Hartnoll, 1982).The female prawn will extrude eggs to be fertilize with the male prawn spermotophores. So, when the eggs are fertilized the female will carry the eggs till they hatch into zoeae. For the freshwater prawn eggs, they are slightly elliptical, with long axis 0.6-0.7 mm, before they hatching the eggs turn to bright orange colour within 2-3 d and they become grey-black (Michael, 2002).The zoeae undergoes the metamorphose turn to PL. They will be around 8 mm with adult characteristics at this stage (Michael, 2002).

Heavy metal contamination of the environment which has been predictable as a serious pollution problem is capable of exerting considerable biological effect even at low because of their pervasiveness persistence natural (Singh and Chandleb, 2006).There are numerous human activities that can generate heavy metal in high quantity such as industrial, agriculture and also domestic pursuits into the water that contain the aquatic resources (IEA, 2015).

Heavy metals such as Cu, Zn, Mn, Fe, Co, Cr, Ni, Pb, Cd and Hg are potentially harmful to most organisms even in very low concentration and have been reported as hazardous environmental pollutant able to accumulate along the aquatic food chain with the serves risk for animals and also human health (Desi et al.,1998).

1.2 Problem Statement

Metal pollution has harmful effect on the biological system and does not undergo biodegradation. Many industrials activity emitted the heavy metal and they likely exposed to the environment. For example, toxic heavy metal such as Cd are potential harmful to many organism even though in very low concentration. Cd can enter into the aquatic body in many ways. The post larva, *M. rosenbergii* were very sensitive to the heavy metal and give the several effects into their body tissue. The toxicity of Cd in different concentrations was exposed on the post larvae stage of *M. rosenbergii* and could cause mortality due to exposed. This study aimed to determine the acute toxicity of Cd on the fresh water prawn *M. rosenbergii*, PL.

1.3 Objective

Objective of the present study was to determine the acute toxicity levels of Cd on fresh water prawn *M. rosenbergii*, PL.

1.4 Hypothesis

The toxicity levels of Cd in different concentrations have significant effect to the PL.

1.5 Scope of Study

This study manipulated different concentrations of heavy metal Cd to investigate the effect on freshwater prawn *M. rosenbergii,* PL. The *M. rosenbergii* samples used in this study obtained from the Pusat Penetasan Udang Galah (PPUG), Perak.

The PL was subjected for 96 h exposure to the different concentration of Cd within 4 d. The mortality was observed and recorded and aquarium conditions were checked maintained at optimum.

1.6 Significance of Study

In this study, the different concentrations of Cd were determined. The different concentrations of Cd are determined to know the ability survival and observed the mortality in post larvae stage *M. rosenbergii*. It is important to know the maximum and minimum concentration of Cd should contain in the water. It is important to prevent and minimize the extinction of the freshwater prawn if the water exposed with the heavy metal such as Cd that can caused toxic effect to the aquatic organisms.

Chapter 2

Literature review

2.1 General Background of *M. rosenbergii.*

M. rosenbergii was one of the species that become scientifically known and the early recognizable illustration appearing in 1705. This species can be easily found in throughout the tropical and subtropical area of the Indo-pacific region which is from India to southeast Asia and Northern Australia (Mutoh and Kuronuma, 1980).

Asian country was dominated the production of aquaculture. Nowadays, *M. rosenbergii* become economic aquaculture because they have higher meat quality, while they also have omnivores feeding habit .The male size for *M. rosenbergii* can reach total length 320mm while the female *M. rosenbergii* size 250mm (de Man, 1879). On this time, Department of Fisheries in Malaysia have been given priorities for the farmers on giant freshwater prawn, *M. rosenbergii* among commercial aquaculture (Rubia et al., 2016).

The morphology of *M. rosenbergii* is the body segment are divided into two parts which cephalothoraxes head and chest and another one is abdomen that called as the body and tail. Then, the carapace acts as cover for body and head of *M. rosenbergii*. They eat both plant material and small animals and known as omnivores. During juvenile stage, they eat plankton and also seaweed. Each part of their body consists of segment that is covered by exoskeleton (Houng et al., 2010).

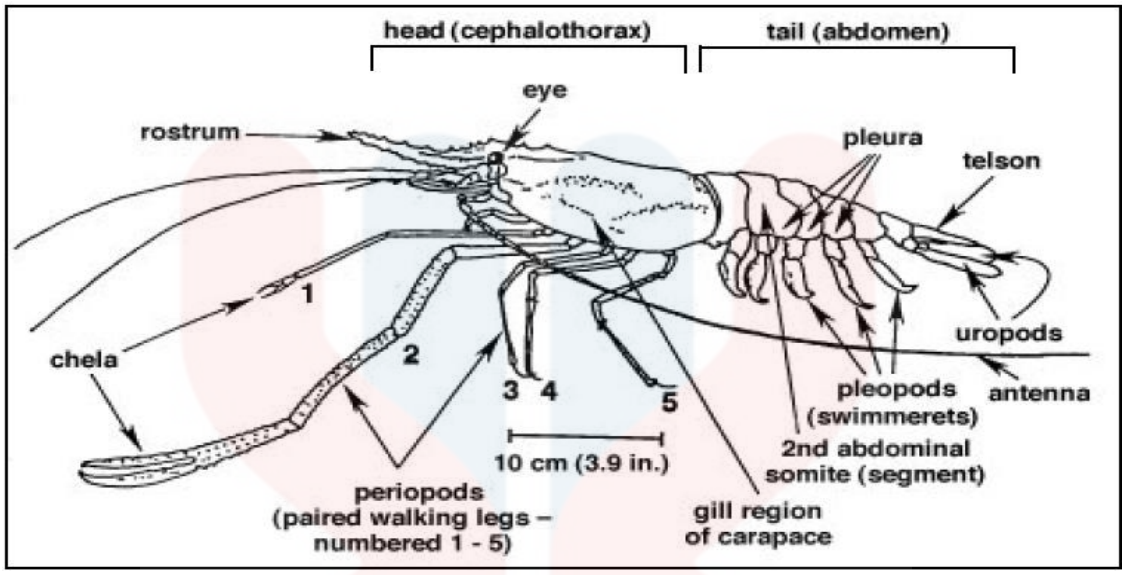

Figure 2.1: The External Anatomy of Freshwater Prawn (Louis et al., 1996)

The sex of *M. rosenbergii* can be recognized by observe their physicals. As male, their size more larger and also faster growth compared to the female which is fatter because there is space for storage of eggs. For life cycle, they have different stage to become complete and adult. The stage is eggs, larvae, PL and juvenile. The egg will fertilize with the non-motile sperm and the quantities of the eggs are also depending on the size of the female (Houng et al., 2010).

The eggs that fertilize were incubated around 21 d before them hatching. The hatching process will take around 20 d at temperature below 28ºC (Rafiqul et al., 1993).For the larvae stage, the time for they hatch is during night and they need the brackish water for survival. Larvae are photo-tactic which they sensitive to the light. The next stage is, the larvae are become the PL. During this stage, they feed on the insect and also worm. The PL are able to adapt with the salinity of freshwater. After

metamorphosis, the PL become juvenile. When the PL become an adult, their colour become blue-green or brownish hue and the sizing for the cephalothoraxes of adult for the male and berried female are around 10cm (Rafiqul et al., 1993).

M. rosenbergii can tolerate a wide range of temperature about 14-35 °C and a wide range of salinity level 0-25 ppt .For growth, the optimal temperature is 29-31 °C, the optimal pH is 7-8.5 and the optimal salinity 0-10 ppt (New, 1995).The effect of pH ,temperature and salinity on the oxygen consumption and nitrogen excretion of *M. rosenbergii* have been studied by Nelson et al., 1997 and also by Chen and Kou, 1996.

2.2 Heavy Metal Contaminant

Heavy metal referred as a metal that toxic at very low quantity and that was relatively high density. Some trace elements which can classified as heavy metal could be toxic at higher concentration although it is essential to maintain the body condition and also body metabolism. Moreover, these toxic affects the organism through metabolic interference and also could be via mutagenesis (Duruibe et al., 2007).

The example of heavy metals is like copper, magnesium, chromium, cadmium, lead and arsenic. Even in low concentration, heavy metals have potential to be harmful to most organisms (Zodape, 2014). If the environment is affected with heavy metal which is essential and non-essentials trace metals, they can risk the animals and also human health (Philips, 1995).

Previous studied showed that, the biological system will affect and polluted if metal pollution are exposed because they do not undergo biological degradation, so they have the capability to exposed and spread to the environment (Na Li et al., 2007 ; Cleary et al., 2002 ; Farombi et al., 2007).For aquatic environment, this problem and situation will also harm the environment.

When the environments are effected to the heavy metal, the expensive costs are needed to reduce the pollution (Bieby et al., 2011). The challenge issue to human is when they are depending on aquatic product as sources of food that affected to the pollution from the heavy metal. The accumulation of hazardous environmental pollutant along the aquatic food chain will serve high risk for animal and also human health (Zodape, 2014).

Heavy metal are very toxic metals having density time greater than water and their toxic are effect to all living organism and human. In human they enter into the body through various ways like ingestion, absorption, etc. They become harmful when their accumulation rate is more than their discharge (Kamran et al., 2013).

Heavy metal cannot be destroyed and also degraded because of that they are classified as natural constituent for earth and persistent to the environmental contaminants (Duruibe, 2007). All heavy metal can be toxic when they exceed the threshold concentration. The contamination of natural waters by heavy metal affects

aquatic biota and poses considerable risk and concerns to the environment and human health (Otchere, 2003 ; Amisah et al., 1999).

2.3 Toxicology of Heavy Metal

To estimate or evaluate the effect of a suspected residual on an organism, the toxicity study has been used. Based on (Hassall,1969) there are two types of distinct toxic effect which firstly the acute toxic effect could happen when the abruptly after application of only poisons of a single dose, and the substances can be traced to a specific biochemical effect oftentimes. In generally, 50 percent mortality is observed in the term of dose is acute toxicity and commonly seen as LD_{50} with the present of (unit toxicant weight /body weight). A directly applied dose and when the toxicant not be able to apply topically is refer to LD_{50}, then it should times be placed in the organisms immediate environment at known concentration.LD_{50} in X hours, or the time at which 50 percent mortality detected at a known concentration (LT_{50}) at X concentration expressed as the concentration at which 50 percent mortality occurs in a known time increment.

The quantity depending on the parameter observed and result from repeated small, non-lethal doses of toxic material show that chronic toxic effects are more complicated (Hassall, 1969). The toxicant must reach a physiologic site and be able to interfere with some function that is important to the organisms to act (Karl, 1976). The part of the central nervous system was may be the physiological site, or they may be critical enzyme systems that responsible for respiration, membrane transport, etc. The

ability of the substances to pass the layer found on insects and crustacean may be dependent on its temperature, lip -solubility, stability and etc (Winteringham, 1969).

Brown and Ahsanullah (1971), Collier et al. (1973) and Thurburg (1973) reported about the toxicity of the Cd on the marine organism. A decrease in gill tissue oxygen uptake in the mud crab *Eurypanopeus depressus* caused by the Cd. LC_{50} for the 72 h was 4.9 ppm, 1 ppm for the LC_0 , and 11.0 ppm for the LC_{100} (Collier et al., 1973). The 20-25 percent was reduced in the gill tissue consumption rates of the green crab *Carcinus maenas*_and the rock crab *Cancer irroratus*, (Brown and Hasnullah, 1971) performed the test only Cd toxicity on *Artemia salina*. Based on reported ,the value LT_{50} at 100 ppm being in 7 d, 16 d was 50 ppb.

Table 2.1: The Maximum Concentration Levels for Heavy Metal Concentration in Air, Soil and Water (Duruibe et al.,2007)

Heavy metal	Max conc. in air (mg/m^3)	Max. conc. In sludge (soil) (mg/kg or ppm)	Max. conc. In drinking water (mg/l)	Max conc. In H_2O supporting aquatic life(mg/l or ppm)
Cd	0.1-0.2	85	0.005	0.008^{δ}
Pb	- -	420	0.01^{π} (0.0)	0.0058^{δ}
Zn^2	1,5*	7500	5.00	0.00766^{δ}
Hg	- -	< 1	0.002	0.05
Ca	5	Tolerable	50	Tolerable > 50
Ag	0.01	- -	0.0	0.1
As	- -	- -	0.001	- -

2.4 Effect of Cadmium Pollution to The Environment

Metals are considered major sources of environmental pollutant. Cd which is the one of these pollutants has received considerable attention for its toxic effect on the living individuals. Metals contamination is typically derived from different sources such as mining, industrial waste discharged, etc. Cd is very unique because it's toxicity at a very low dosage, long half-life and its low excretion from the body (Jones and Charlen, 1990).

Cd is a soft, silvery white, fusible metallic element. Cd symbolize with symbol Cd, is a heavy metal with high toxic. Among the heavy metal, it is considered most serious metal contaminant since the occurrences of Itai-Itai disease in Japan (Yukimasa, 1975). It is heavy metal having half-life of ten to thirty years and it is also a non-essential highly toxic (Jan et al., 1999). Toxic of Cd is at very low exposure levels since it is non degradable in nature and hence once released to the environment and it has chronic and also acute effect on health and environment. Besides, compounds of Cd are relatively water soluble compared to others that being more mobile, they easily bioavailable and bioaccumulate (Pinto et al., 2004).

Cd is a naturally occurring metal situated in the Periodic Table of the Element between Zinc (Zn) and mercury (Hg), with chemical behavior similar to Zn (Robin, 2013). Commercially, Cd is used in television screens, lasers, batteries , paint pigments, cosmetics and in galvanizing steel, as a barrier in nuclear fission and was used with Zinc to weld seals in lead water pipes prior to the 1960s (Frigberg, 1983). In the electroplating industry, Cd is used intensively and it is also used as a corrosion protectant for many metals. Pigment and plastics stabilizers are other uses of Cd (National Academy of Science, 1974). Based on American Public Health Association (1971), Cd being toxic elements in some cases of food poisoning and has a relative and high potential poisoning has been reported.

2.5 Effect of Cadmium on *M. rosenbergii*

Cd can enter into the aquatic bodies through sewage sludge and with the runoff from agriculture lands as its one of the major components of phosphate fertilizers. Also, the major sources of contamination include electroplating, paper, PVC plastics, pigments and ceramic industries and many other modern industries (Gupta et al., 2003)

Metals such as Cd are known to accumulate in marine organisms and cause rapid genetics change (Nimmo *et al.,* 1978). It is also possible that environment toxicant may increase the susceptibility of the aquatic animals to various of disease by interfering with the normal functioning of their immune, reproductive and development processes (Couch 1978, Brock 1997).

(Kaoud and Rezk, 2010) stated that the survival of prawn exposed to Cd dose over 60 $_{\mu}$ gl^{-1}-$_1$ of Cd were significantly lower than of those exposed to lower dose. However in similar study, after the prawn was exposed to > 40$_{\mu}$ gl^{-1}-$_1$ of Cd had a greater reduction and phagocytic activity was observed than those exposed to lower concentration.

2.6 Effects of Cadmium on Human

Cd exposure from ingestion of contaminant food or water from old Zn or Cd sealed water pipes on industrial pollution and can produce long term health effect. Contamination of drug and dietary supplement may also be sources of contamination (Arbernethy et al., 2010).

Toxic heavy metal can caused bad impact to human such as dermatological disease, skin cancer and internal cancers, cardiovascular disease, diabetes and anemia as well as reproductive developmental, immunological and neurological effect in human body (Rose et al.,1992; Lukawaki et al., 2005)

Cd-contaminated will affect the respiratory system by the inhalation and will caused shortness of breath, lung edema and destruction of mucous membrane part of cadmium-induced pneumonitis (Seidal et al., 1993). Acute gastrointestinal effect likes vomiting and diarrhea caused by the intake of Cd contamination food (Nordberg, 2004).

Exposed to Cd caused kidney damage has long since been described to be the main problem for patient chronically (Barbier et al., 2005). Cadmium-metallothionein (Cd-MT). Cd-MT is filtered in the glomerulus and subsequently reabsorbed in the proximal tubulus is the form of cadmium reached in the kidney. It remains in the kidney tubulus cells, then makes up for the major part of the cadmium body burden (Svatergren

et al., 1986). A higher calcium excretion caused by an increasing of cadmium content in kidney is also discussed to cause in leading to a higher risk of kidney stones

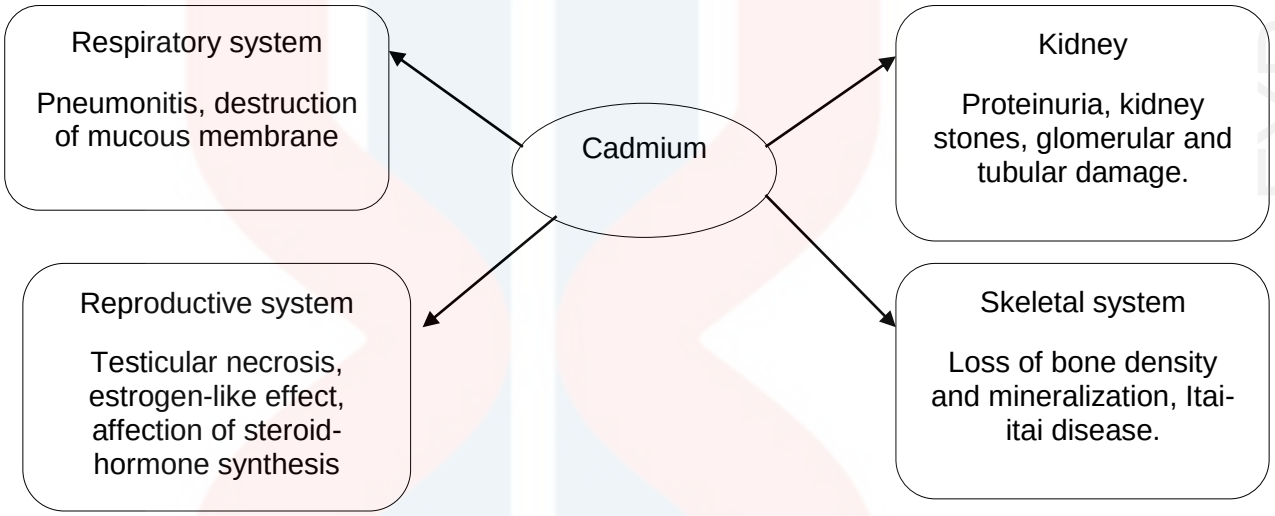

Figure 2.2: Effect of Cd on Several Organ System.(Johannes et al., 2006).

Chapter 3

Methodology

3.1 Methodology

3.1.1 Sample collection

Sampling PL *M. rosenbergii* for this research was obtained from “Pusat Penetasan Udang Galah”(PPUG), located in Setiawan, Perak. The sampling was bring out and then transferred to the “Fish Propagation House” (FPH) located in Politeknik Jeli. The sample was acclimatized for 4 d in a tank before use for 96 h acute toxicity trials.

The sample of PL that obtained were stored in the fiber tank before the experiment started. The PL were exposed about 4 days in tank (12 cm x 18 cm x 12 cm). The concentrations were diluted with serial dilution method by using the pipette to avoid parallax error. The initial weight of the PL was 0.53 g and with length 1.5 cm. The average of weight PL used was 0.50-0.55 g with length between 1.5 and 1.8 cm.

Figure 3.1: Location of The Sampling site.

Source: https://www.google.com.my/maps?source=tldsi&hl=en. Accessed on December 2017.

3.1.2 Experimental Design

Four days experiment was designed. Cd was dissolved in distilled water and stock solution was made. The PL were exposed to the different concentration of 100, 80, 60, 40 and 20 ppb or µg/l of Cd for 4 d**.** The concentrations were introduced into each tank in triplicate treatment and 0 ppb or µg/l as control. Total 30 individuals of PL were exposed to the different concentration in each tank.

Cleaning of aquarium tank was followed the standards procedure. The tank should clean with water without using any detergent. Then, the tanks were filled with the water and let them for one night. Later, the tanks were cleaned again and they were ready for used to transfer the PL. The anti-chlorine was used to neutralize the harmful chlorine from the tap water. Then, the PL were acclimatized about 4 d before experiment were started. The tanks also were prepared with filtration to make sure the water keep clean and they also prepared with the aeration that function to give the oxygen and circulates water. 100 W bulbs were placed on the top of the tank to keep the temperature at optimum (27-30ºC) especially during night and rainy days. Then, post larvae were feed daily with the commercially available pellet throughout the experiment.

The initial weight and length of PL were recorded. Each of the PL weighed was between 0.53 g and 1.5 cm. For each tank, data of the mortality and the survival of the PL were recorded within 24 h, 48 h, 72 h and 96 h. Observation for mortality were made daily at regular intervals. Dead PL were removed at each observation.

3.1.3 Preparation of The Standard Cadmium Solution

Before the experiment was started, the standard solution concentration supposedly determined first. The concentration of the standard solution might be based on the possible content of heavy metal from journal reading based on the toxicity level. The ranges of the concentrations used are between 0ppb to 100ppb.

Stock of Cd 1000mg/L of Cd metal was used. About 100ml of Cd solution were pipette into flacon tube and then, the stock was diluted with distilled water depend on the volume of tank or aquarium. The procedure is repeated by prepared the 20 ppb, 40 ppb, 60 ppb, 80 ppb and 100 ppb using formulae:

$$M_1V_1=M_2V_2$$

Where M_1: Molarities concentration 1

V_1: volume concentration 1

M_2: Molarities concentration 2

V_2: volume concentration 2

3.1.4 Statistical Analysis

The mortality rate was analyzed using the One-way Analysis of Variance (ANOVA) and where appropriated, IBM SSPSS statistical were applied. Significant level was set at $P \leq 5\%$. All the data were presented as mean ± SD.

Duncan's multiple range tests was used to compare the significance of the mortality in different concentration of Cd 100 ppb, 80 ppb, 60 ppb, 40 ppb 20 ppb within 24 h, 48 h, 72 h and 96 h.

Chapter 4

Result

4.1 Environmental condition of aquarium tank during toxicity test:

Table 4 .1: The environmental condition maintained during the 4 days toxicity test.

Temperature	28°C
Salinity	28 PSU
Ph	7.78
Dissolved oxygen	4.5 mg/L

Table 4.1 shows the environmental condition maintained during the 4 d toxicity test. The environmental condition was checked daily using multi parameter. The average of temperature, salinity, pH and dissolved oxygen were recorded. The conditions in the table above show that it is suitable value because there show no mortality in the control treatment**.**

4.2 Data collection

Table 4.2: Total mortality percentage of PL in different concentration of Cd

Concentration (ppb)	Mortality%
Control	0
20	5.56
40	44.44
60	76.67
80	98.9
100	100

The table 4.2 showed that the total percentage of mortality in different concentration of Cd. The percentages were calculated to determine the median lethal concentration. Mean mortality was 0% ,5.56 %, 44.44 %, 76.67 %, 98.9 % and 100% in the control treatment, 20, 40, 60, 80 and 100 ppb or μg/L of concentration Cd respectively.

The value of LC_{50} was determined as the Figure 4.1 with using equation of straight line:

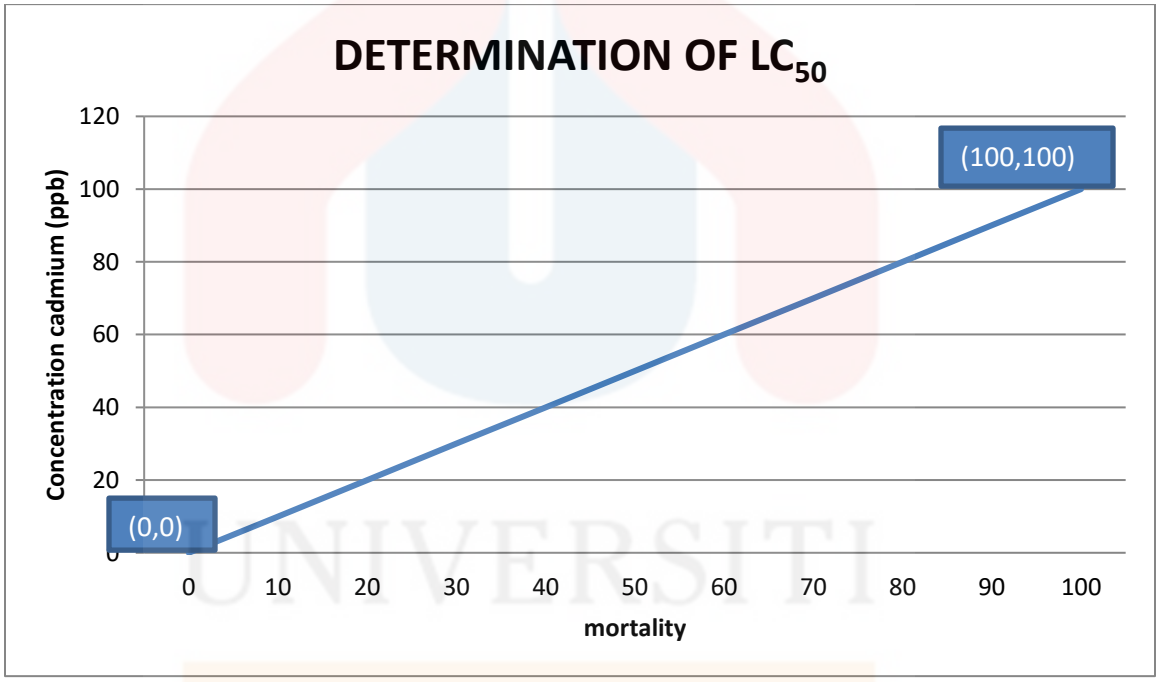

Figure 4.1 : Determination of the 50 % of mortality.

The LC_{50} was determined with plotting the graph of mean mortality 0%, 2.22% ,5.56 %, 44.44 %, 76.67 %, 99 % and 100% in the concentration 0, 20, 40, 60, 80 and 100 ppb or μg/L respectively.

The 50% of mortality were calculated by using equation:

$y = mx + c$

So, the m was obtained from the straight line.

$$m = \frac{y_2 - y_1}{x_2 - x_1}$$

$$= \frac{100-0}{100-0}$$

$$=1$$

So, the x were obtained from x-axis = 50

$y = 1 (50) + 0$

= 50 : the concentration at 50% mortality.

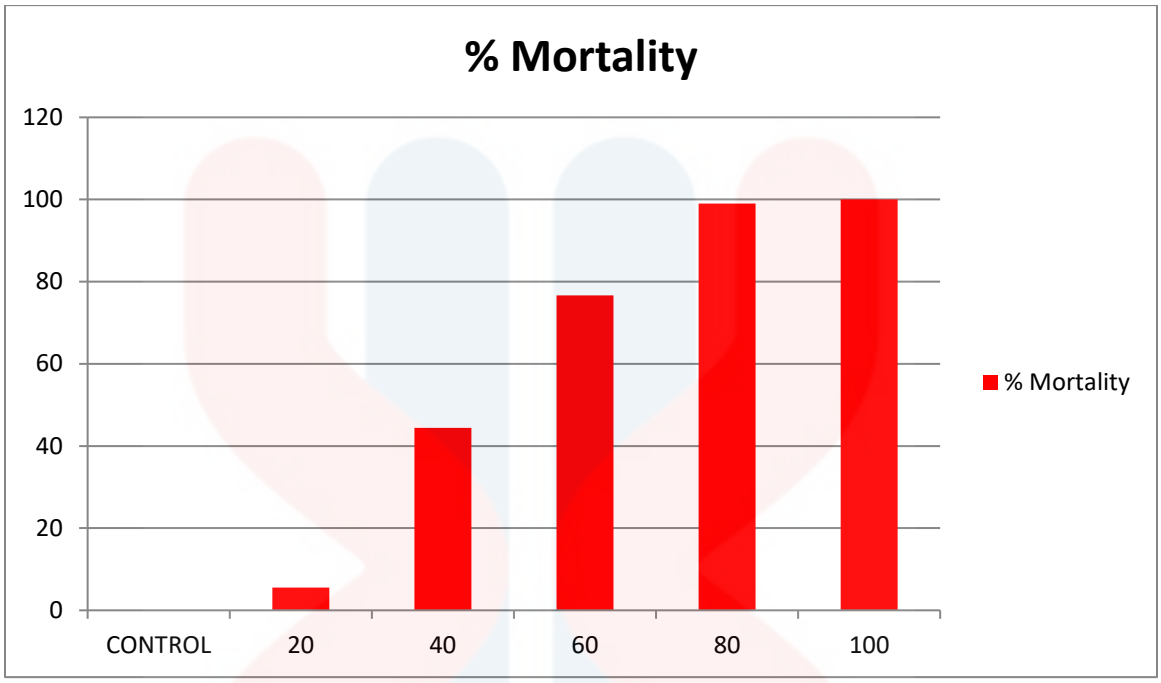

Figure 4 .2: The total mortality percentage of PL in different concentration.

The bar graph (Figure 4.2) included all treatment mortality percentage. As compare to all the all treatment together, this study indicated that the PL raised mortality percentage 100% in concentration 100 ppb. The second highest mortality in concentration 80 ppb showed that the 76.67%. Then the control show that no mortality percentage.

4.3 Data analysis

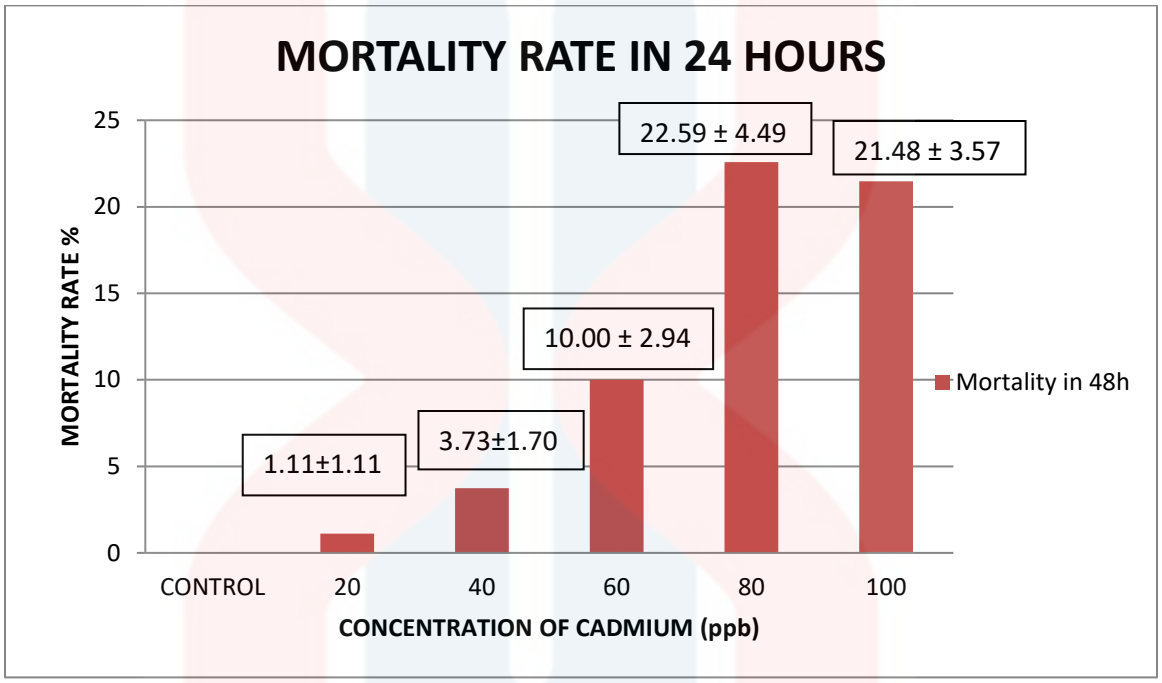

Figure 4.3 : The bar graph for the mortality rate within 24 h in different concentration of Cd.

The bar charts (Figure 4.3) include the entire mortality rate in 24 h in different concentration of Cd solution. As to compare the mortality in 24 h experiment time, it showed that in concentration 80 ppb show the highest mortality compare with the other treatment. The second highest mortality within 24 h was in concentration 100 ppb followed up by the 60 ppb, 40ppb and then 20 ppb concentration.

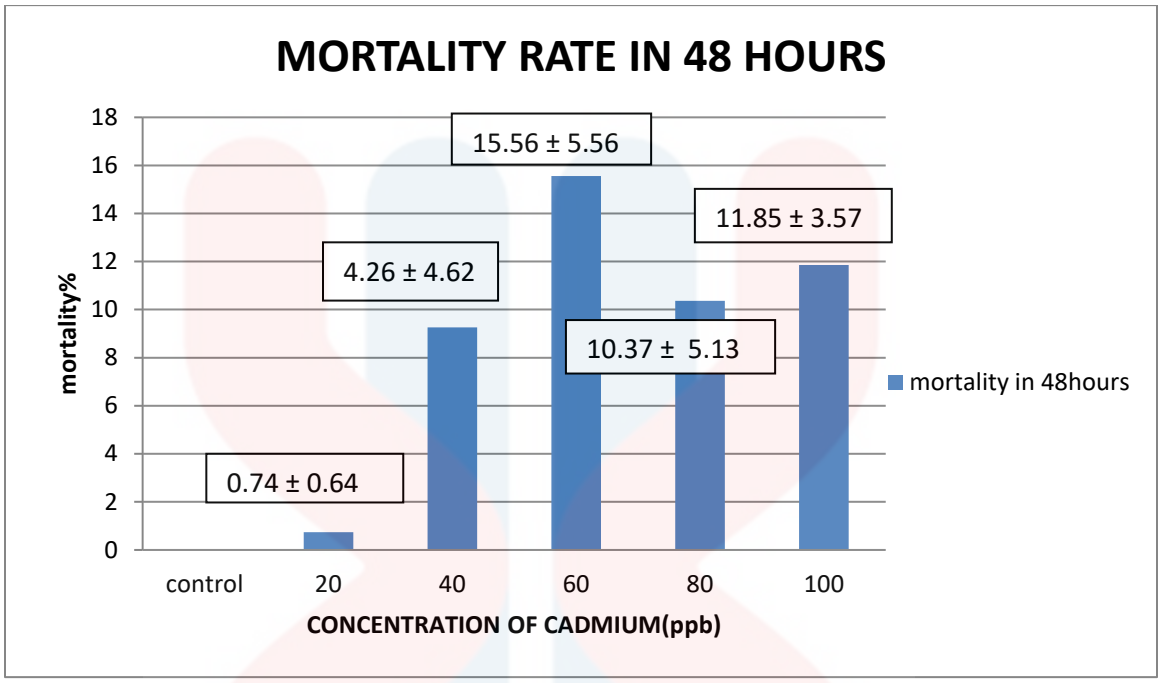

Figure 4.4 : The bar graph for the rate mortality in 48h in different concentration of Cd.

After exposed 48 h, the highest mortality showed in concentration 60 ppb and the second highest is in concentration 100 ppb followed up by the 80 ppb, 40 ppb and lastly 20 ppb.

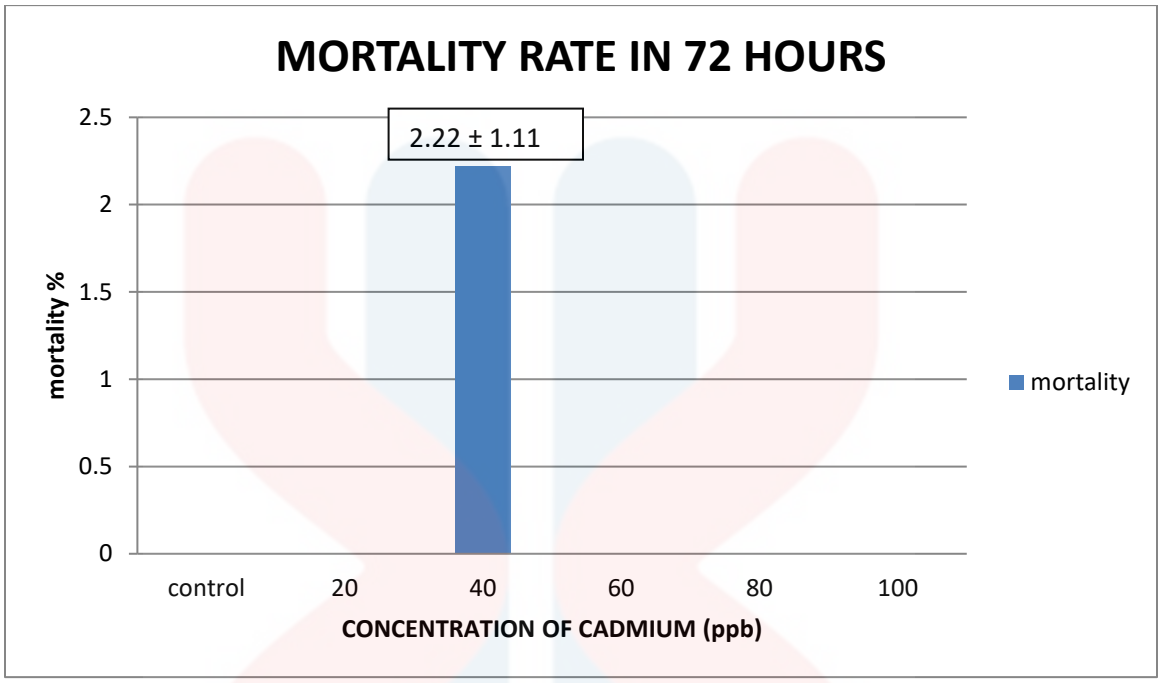

Figure 4.5: The bar chart for the mortality rate within 72 h in different concentration of Cd.

The bar chart above shows the mortality rate in 72 h in difference concentration of Cd. There was only mortality in 40 ppb concentrations. After 72 h, there were no mortality recorded in control, 20, 60, 80 and 100. For the mortality rate in 96 h, there was no mortality rates recorded in all concentrations.

4.4 Conclusions of The Result:

The bar graph (Figure 4.2, 4.3, 4.4 and 4.5,) include all the experiment hours within 24 h, 48 h, 72 h and 96 h. As to compare the mortality in 24 h experiment days, it shows that in concentration 80 ppb show the highest mortality compare with the other treatment. As to compare the mortality in 48 h, it showed the highest mortality in concentration 60 ppb. After exposed in 72 h, only the concentration 40 ppb has the mean

mortality of PL. Only in the concentration 40 ppb present the mortality after 72 h exposed to Cd. Others concentrations show that mortality occur within 24 h to 48 h only. No mortality recorded in the 96 h exposed.

Chapter 5

Discussions

Based on the result show in the Table 4.1, the environmental condition was checked daily to make sure the test condition and apparatus appeared suitable for maintaining and exposing the PL *M. rosenbergii*. The air-lift system provided constant circular flow and maintained saturated levels of dissolved oxygen in the tank. The used of serial dilution method to deliver exposed solutions minimized variation in exposed concentration. During the toxicity test the temperature was maintained at 28° C. Then, for the salinity is 28 PSU. The pH should maintain in 7.78 and the dissolved oxygen 4.8 mg/L.

The mortality in control treatment shows no significant effect ($p < 0.05$) with the percentage of mortality, 0% for up to 4 days demonstrating that the holding facilities, water, control environment condition and handling technique were acceptable. The mortalities checked regularly in the control treatment within 24 h, 48 h, 72 h and 96 h. Thus we conclude that our test apparatus provided the condition necessary for the survival of the post larvae in control treatment without any concentration Cd solution. The same exposed apparatus also provided suitable for toxicity test with post larvae *M. rosenbergii* which the environmental condition were set as table 4.1.

In unpolluted freshwater, the level of Cd range from 0 to 4µg/L respectively. Contaminated water often contains much more 40- 120µg/L of Cd.

Based on the result show in Table 4.2 and Figure 4.2, the total mortality percentage of post larva, *M. rosenbergii* present that the highest mortality show in the concentration 100 ppb. From the experiment, it shows that dramatic increasing of mortality percentage when exposed to the higher concentration as long as the experiment is executed. The second highest mortality show in concentration 80 ppb with the percentage 98.9% compare with the mortality percentage of PL in concentration 40 ppb and 20 ppb with mortality 5.56 %. In the concentration 100 ppb, all the PL were died within 96 hours exposed to the Cd. This was because the PL cannot adapt with the concentration 100 ppb and reached the maximum level because PL were very sensitive to the heavy metal exposed compared to the adult and others species. Based on the previous study (Dave and Xiu, 1991), fish embryo and larvae are more sensitive to the environmental impacts, including toxic substances compared to juvenile and adult.

Exposure of fertilized of egg of *Oryziaz javanicus* to low level of Cd at concentration (10-50 µg/l) caused developmental disorder, while in concentration of 100µg/l completely inhibited development and resulted in death of all embryo (Ismail and Yusof, 2011). In the present of study, the result show all the PL died in same concentration, 100 ppb or 100µg/l that stated by the author of the previous study.

Based on the result in Figure 4.1, the graph was plotted to obtain the lethal median concentration at 50% of mortality. The LC_{50} PL was at concentration 50 ppb. In the concentration 20 ppb and it shows only 10% of mortality occurs. That value can be stated as minimum concentration of Cd can exposed to the PL.

Justyna and Jacek (2006) reported that throughout Cd exposure, only in the in the control group did fish losses not occur. At the end of the D1, larval survival rate was calculated based on the number of dead specimen found were then 100, 98.5, 95.0 and 90.3 % respectively for the consecutively increasing of Cd solution.

Based on their report, the *S. erythrophthalmus* larvae to be highly sensitive to the Cd intoxication at a concentration 0.1 mg dm^{-3} when it show the final survival rate ranged from 86.7 % to 62.7%.Based on reported by the (Finalayson and Verrue, 1982; Hansen *et al.,* 2002),the higher percent of mortality occurred with increase in concentration and exposure period, hence confirms the observation was made in case of salmonids, *Oncoryhnchus mykiss*, *Salvelinus confluents* and *Oncorhynchus tshawytscha*.

So, for the *M. rosenbergii* PL show that, they highly sensitive to the 0.1 mg/L concentration of Cd show that 100% mortality but not for *S. Erythrophthalmus.*

The concentration 20 ppb show that the percentage of mortality not more than 10%. The minimum level concentration of Cd is 20 ppb and the maximum concentration of Cadmium is 100 ppb. *M. rosenbergii* PL were acutely sensitive to concentration in order 100> 80 > 60 >40> 20. Medial lethal concentration LC_{50} of Cd in this test was 50 ppb.

The 96 h LC_{50} was Cd in *M. rosenbergii* was calculated to be 74 ppb by the (Kaoud and Rezk, 2011) is higher compare with the result from present of study is 50 ppb. Trusscott and White, 1990 reported that there a significant reduction in phagocytosis of *Bacillus cereus* was observed in the shore crab *Carcinus maenas* following 14 d exposure to 500 μgl^{-1} Cd..

Regarding the toxicity of Cd on the marine organism, there are a few studies that have been reported by (Brown and Ahsanullah, 1971: Collier et al., 1973; Thurburg, 1973). A decrease in gill tissue oxygen uptake in the mud crab *Eurypanopeus depressus* was caused by Cd. The 72 h LC_{50} was at concentration 4.9 ppm, the LC_0 was 1 ppm, and the LC_{100} was 11.0 ppm (Collier et al., 1973). The gill tissue consumption rates of the green crab *Carcinus maenas* and the rock crab *Cancer irroratus* were reduced by 20-25 percent. Only Cd toxicity test on *Artemia salina* that performed by Brown and Hasnullah (1971). They reported that being 7 d , LT_{50} value at 100 ppm, being 16 d in 50 ppm..

The LC_{50} of *M. rosenbergii* also show that in 72 h exposed to the 50 ppb of Cd concentration, same with the *Eurypanopeus depressus* in 72 h but not in concentration, 4.9 mg/L.

Based on the report (Edison, 2008), after 96 h exposed the medium lethal concentration of Cd for *F. paulenis* show at 0.83 mg/L. Exposure shrimp to zinc and Cd caused an exhibition in oxygen consumption of 25 % and 32.4 % respectively relative to

the control. But on *M. rosenbergii* show that at 0.1 mg /l of Cd concentration are 100% were died.

St-Amand et al. (1999), the respiration of decapods crustacean demonstrated the decrease in oxygen consumptions rate was related to concentration, exposure time and larval stage is affected by the heavy metals.

Based on the Figure 4.3, 4.4 and 4.5 showed the relation between Cd concentration and the mortality rate of *M. rosenbergii* according to the Duncan's prohibit analysis. There were no significant differences in the control treatment ($p<0.005$) with 0.00 ± 0.00 in triplicate. This due to the test condition and the apparatus provided is necessary for the PL to maintain survival. (Bat et al., 2014) reported that the toxicity of Cd for the larvae of *P. adspersus* increase with increasing concentration and exposure time. The 4 d value of LC_{50} of Cd for aquatic organism differ from the other species and according to the type of heavy metal.

The result can be proved with the other authors (Chadurvelan et al., 2017), found that acute 96 h lethal concentration of 0.45 mg/L was derived for *Paratya curvirostris*, placing it among the most tolerant of fresh water shrimp.

For the concentration 20 ppb, the mortality mean was 1.11 ± 1.11 in 24 h and after 48 h is 0.74 ± 0.64. There shows high significant ($p< 0.05$).after 72 h and 96 h

there are no significant different between this hours. Next, in the concentration 40 ppb, the table 4.1 showed that total percentage of mortality was less than 50%. Within 24 h, there were 3.73 ± 1.70 shows that significant higher $p<0.05$ compare with the 0.74 ± 0.64 in concentration 20 ppb. All the results were significance.

So the conclusion is, the *M. rosenbergii* are very high sensitive to the heavy metal even in very low concentration. So the mortality rates are increasing in the term of increasing the concentration. The minimum value of concentration of Cd that can show only 10 % the PL were died at concentration 20 ppb or 0.02 mg/L and maximum concentration at 100 ppb or 0.1 mg/L.

Chapter 6

Conclusions and Recommendations

6 .1: Conclusion

The study was carried out to investigate the toxicity of Cd is has significant effect to the *M. rosenbergii*, PL. The rising levels of concentration Cd leading to increase the mortality of PL *M. rosenbergii* due to its toxic effect. For the PL, the minimum of concentration that can be exposed is 20 ppb and maximum 100 ppb that show the entire PL were died. For the present of the heavy metal such as cadmium are decreased the time period to post larvae maintenance to survival. The high mortality rate shows in concentration 80 ppb and 100 ppb.

For the 24 h exposed to the Cd solution, there showed high mortality in concentration 80 ppb followed by the 100 ppb. For the 48 hour, in concentration 60 ppb followed by 100 ppb was higher and in 72 h there was only in 40 ppb showed the mortality. The entire PL in 100 ppb died within 48 h and there were only the PL died within 72 h in 40 ppb and 48 h in 20 ppb, 60 ppb and 80 ppb.

All the result obtained in the present of study has significant effect ($p < 0.05$). So, the hypothesis from this study were accepted where the toxicity of Cd at 20 ppb, 40 ppb , 60 ppb , 80 ppb and 100 ppb has significant effect to the PL which show the mean mortality percentage 5.565, 44.44%, 76.67 %,98.9% and 100%.

6 .2: Recommendations

As the *M. rosenbergii* become the more important target species, the studies must be shared more and acts as references to others to increase the its production. The water quality must be checked every day and should in optimum condition to avoid the PL are exposed to the trace metal. The next study should focus on the technique in dilution of heavy metal to avoid the parallax error. The result should be prove with the Atomic spectrometer reading to make sure that concentration in correct value to use to exposed to the PL. Next the future work also should focus on the effect in growth performance of *M. rosenbergii* when exposed to the heavy metal, effect of Cd on metabolism, moulting effect, effect of heavy metal in different temperature, salinity level because the growth rate are also important in the prawn culture industry.

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APPENDICES A

Table 4.3: The percentages of mortality post larvae, in 24 hr, 48 hr, 72 hr and 96 hr in different concentrations of Cadmium.

Concentration/ Hours	Mean ± SD			
	24	48	72	96
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20	1.11 ± 1.11	0.74 ±0.64	0.00 ± 0.00	0.00 ± 0.00
40	3.73 ± 1.70	9.26 ± 4.62	2.22 ± 1.11	0.00 ± 0.00
60	10.00 ± 2.94	15.56 ± 5.56	0.00 ± 0.00	0.00 ± 0.00
80	22.59 ± 4.49	10.37 ± 5.13	0.00 ± 0.00	0.00 ± 0.00
100	21.48 ± 3.57	11.85± 3.57	0.00± 0.00	0.00 ± 0.00

Values are means ± SD of three replicate. (n=3). Value in the same column with different superscripts are significantly different (p< 0.05). Control: without concentration. Treatment 1= 100 ppb concentration, treatment 2= 80 ppb concentration, treatment 3= concentration 60ppb, treatment 4= 40 ppb and treatment 5= concentration 20 ppb concentration of heavy metal of Cadmium.

Table 4. 1. 1: The total mortality post larvae in different concentrations of cadmium.

Concentration of cadmium (ppb)	Total mortality
Control	0
20	5
40	40
60	69
80	89
100	90

APPENDECES B

Descriptives

Percentage

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control 1st	3	.0000	.00000	.00000	.0000	.0000	.00	.00
control 2nd	3	.0000	.00000	.00000	.0000	.0000	.00	.00
control 3rd	3	.0000	.00000	.00000	.0000	.0000	.00	.00
control 4th	3	.0000	.00000	.00000	.0000	.0000	.00	.00
conc.20 1st	3	1.1100	1.11000	.64086	-1.6474	3.8674	.00	2.22
conc.20 2st	3	.7400	.64086	.37000	-.8520	2.3320	.00	1.11
conc.20 3rd	3	.0000	.00000	.00000	.0000	.0000	.00	.00
conc.20 4th	3	.0000	.00000	.00000	.0000	.0000	.00	.00
conc.40 1st	3	3.7033	1.70101	.98208	-.5222	7.9289	2.22	5.56
conc.40 2nd	3	9.2600	4.62130	2.66811	-2.2199	20.7399	5.56	14.44
conc.40 3rd	3	2.2200	1.11000	.64086	-.5374	4.9774	1.11	3.33
conc.40 4th	3	.0000	.00000	.00000	.0000	.0000	.00	.00
conc.60 1st	3	10.0000	2.93678	1.69555	2.7046	17.2954	6.67	12.22
conc.60 2nd	3	15.5567	5.55500	3.20718	1.7573	29.3561	10.00	21.11
conc. 60 3rd	3	.0000	.00000	.00000	.0000	.0000	.00	.00
conc. 60 4th	3	.0000	.00000	.00000	.0000	.0000	.00	.00
conc.80 1st	3	22.5933	4.49179	2.59333	11.4351	33.7515	20.00	27.78
conc.80 2nd	3	10.3667	5.13264	2.96333	-2.3835	23.1169	4.44	13.33
conc.80 3rd	3	.0000	.00000	.00000	.0000	.0000	.00	.00
conc.80 4th	3	.0000	.00000	.00000	.0000	.0000	.00	.00
conc. 100 1st	3	21.4833	3.57385	2.06337	12.6054	30.3613	18.89	25.56
conc. 100 2nd	3	11.8500	3.56815	2.06007	2.9862	20.7138	7.78	14.44
conc.100 3rd	3	.0000	.00000	.00000	.0000	.0000	.00	.00
conc.100 4th	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	72	4.5368	7.31534	.86212	2.8178	6.2558	.00	27.78

ANOVA

Percentage

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3522.241	23	153.141	26.512	.000
Within Groups	277.264	48	5.776		
Total	3799.505	71			

Percentage

Duncan[a]

		Subset for alpha = 0.05			
TREATMENT	N	1	2	3	4
control 1st	3	.0000			
control 2nd	3	.0000			
control 3rd	3	.0000			
control 4th	3	.0000			
conc.20 3rd	3	.0000			
conc.20 4th	3	.0000			
conc.40 4th	3	.0000			
conc. 60 3rd	3	.0000			
conc. 60 4th	3	.0000			
conc.80 3rd	3	.0000			
conc.80 4th	3	.0000			
conc.100 3rd	3	.0000			
conc.100 4th	3	.0000			
conc.20 2st	3	.7400			
conc.20 1st	3	1.1100			
conc.40 3rd	3	2.2200			
conc.40 1st	3	3.7033			
conc.40 2nd	3		9.2600		
conc.60 1st	3		10.0000		
conc.80 2nd	3		10.3667		
conc. 100 2nd	3		11.8500	11.8500	
conc.60 2nd	3			15.5567	
conc. 100 1st	3				21.4833
conc.80 1st	3				22.5933
Sig.		.127	.237	.065	.574

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

APPENDICES C

Preparation of the standard solution :

Solution 1: Preparation of 100 ppb of cadmium solution

$M_1V_1 = M_2V_2$

V1 = 0.1 (15 L)/ (2)

= 750 ml of cadmium solution

Solution 2 : Preparation of 80 ppb of cadmium solution

$M_1V_1 = M_2V_2$

V1= 0.08 (15 L)/1

= 1200 ml of cadmium

Solution 3 ; Preparation of 60 of cadmium solution

V1 = 0.06 (15)/ 1

=900 ml of cadmium

Solution 4 ; preparation of the 40 cadmium solution

V1 = o.o4 (15)/0.08

=7500 ml of cadmium

Solution 5 ; the preparation of 20 of cadmium solution

V1 = 0.02 (15)/ 0.08

= 3750ml of cadmium