CADMIUM (Cd) AND LEAD (Pb) DETERMINATION IN ASIAN SWAMP EEL, *Monopterus albus*, COLLECTED FROM PADDY FIELD IN PASIR MAS AND BACHOK, KELANTAN

by

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DECLARATION

I declare that this thesis entitled Cadmium (Cd) and Lead (Pb) Determination in Asian Swamp Eel (*Monopterus Albus*) Collected from Paddy Field in Pasir Mas and Bachok, Kelantan is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in the candidature of any degree.

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Date : ______________________

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Cadmium (Cd) and Lead (Pb) Determination in Asian Swamp Eel, (*Monopterus Albus*) Collected from Paddy Field in Pasir Mas and Bachok, Kelantan

ABSTRACT

Monopterus albus is a common fish that was found in paddy field and therefore, it is fit to be used as bio-monitor for heavy metals pollution in paddy field. The aim of this study is to determine the concentration of lead (Pb) and cadmium (Cd) in the organs of *Monepterus albus* collected from paddy field in Pasir Mas and Bachok, Kelantan. The heavy metals concentration in the organs of the *Monopterus albus* was determined by using Atomic Absorption Spectrophotometer (AAS). Among the selected organs, bone had the highest levels of Cd and Pb (0.1584 and 0.0462 µg/g) for paddy field in Pasir Mas while for Bachok was (0.0358 and 0.1302 µg/g) respectively. Meanwhile, muscle showed the lowest total metal accumulation for both metal where the concentration of Cd (0.0188 and 0.016 µg/g) and for Pb (0.0314 and 0.0264 µg/g) at Pasir Mas and Bachok respectively. The levels of Pb and Cd in the muscle of swamp eel was lower than permissible limit set by WHO/FAO and Malaysian Food Regulations (MFR) but for liver and gills, it exceeds the limits set by both organizations. The permissible limit is 2 for WHO/FAO for both metals while MFR, the limit set is 2 for Pb and 1 for Cd. The result for the level of Cd in water sample from both site showed that it is lower than permissible limit, which is 0.003mg/L. While for Pb, (0.2611 and 0.0682 mg/L) exceed the limits set by WHO which is 0.01mg/L. For the target hazard quotient (THQ), the concentration values of Pb and Cd were below one in the muscle. Thus, humans can still consume the muscle of swamp eel. This study suggests that *Monopterus albus* collected from paddy fields in Pasir Mas and Bachok are safe for human consumption.

Penentuan Kadmium (Cd) dan Plumbum (Pb) di dalam Organ Belut Sawah, (*Monopterus albus*) Diambil dari Sawah Padi di Pasir Mas dan Bachok, Kelantan

ABSTRAK

Monopterus albus adalah salah satu ikan yang biasa terdapat di sawah padi dan oleh itu, ia sesuai untuk dijadikan sebagai bio-pemantau untuk pencemaran logam berat di kawasan sawah padi. Tujuan kajian in adalah untuk menetukan kandungan logam berat, plumbum (Pb) dan kadmium (Cd) di dalam organ *Monopterus albus* yang diambil dari sawah padi yang terletak di Pasir Mas dan Bachok, Kelantan. Kepekatan logam berat di dalam organ-organ *Monopterus albus* ditentukan dengan menggunakan mesin Atomic Absorption Spectrophotometer (AAS). Antara organ terpilih, tulang mempunyai kepekatan Pb dan Cd yang tertinggi (0.1584 dan 0.0462 µg/g) untuk kawasan padi di Pasir Mas manakala bagi Bachok (0.0358 dan 0.1302 µg/g). Sementara itu, otot menunjukkan jumlah terkumpul yang terendah untuk kedua-dua logam di mana kepekatan Cd (0.0188 dan 0.016 µg/g) dan untuk Pb (0.0314 dan 0.0264 µg/g) masing-masing di Pasir Mas dan Bachok. Tahap Pb dan Cd di dalam otot belut sawah adalah lebih rendah daripada had yang telah dibenarkan oleh WHO/FAO dan Peraturan Makanan Malaysia tetapi bagi hati dan insang, tahap logam berat melebihi had yang telah ditetapkan oleh kedua-dua organisasi tersebut. Had yang dibenarkan oleh WHO/FAO ialah 2 untuk kedua-dua logam manakala MFR menetapkan 2 untuk Pb dan 1 untuk Cd. Hasil untuk kepekatan logam Cd di dalam sampel air (0.0015 dan 0.0016 mg/L) dari kedua-dua tempat menunjukkan nilai yang diperoleh tidak melebihi had yang ditetapkan oleh WHO iaitu, 0.003 mg/L. Namun, untuk kepekatan Pb, nilai yang diperoleh (0.2611 dan 0.0682 mg/L) telah melebihi had Pb yang telah ditetapkan. Untuk *target hazard quotient* (THQ), nilai kepekatan Pb dan Cd yang berada dalam otot adalah bawah nilai satu. Oleh itu, otot belut sawah masih selamat untuk dimakan oleh manusia. Kajian ini menunjukkan *Monopterus albus* yang diambil dari sawah padi di Pasir Mas dan Bachok adalah selamat untuk dimakan oleh manusia.

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LIST OF ABBREVIATIONS

Zn	Zinc
Cd	Cadmium
Pb	Lead
Cu	Copper
Mg	Magnesium
Ni	Nickel
HRA	Health Risk Assessment
AAS	Atomic Absorption Spectroscopy
WHO	World Health Organization
HCl	Hydrochloric Acid
HNO_3	Nitric Acid
SD	Standard Deviation
THQ	Target hazard quotient
MFR	Malaysian Food Regulation
WHO	World Health Organizations

LIST OF EQUATIONS

$$THQ = \frac{EFr \times ED \times FIR \times C}{RfD \times WAB \times TA} \times 10^{-3}$$

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Heavy metals are intrinsic and are natural constituents of the environment. It has a high density, which five times greater than the density of water because of the metallic chemical element. It is also lethal and fatal even it present at low concentration. Heavy metals are known to cause harm to the human's organ even the exposure is at low concentration and for that, heavy metal elements are considered as systemic toxicants (Tchounwou et al., 2012). However, heavy metals also can act as vital nutrients that needed for numerous physiological and biochemical functions (WHO, 1996). Some of the examples of heavy metals are lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), and magnesium (Mg) and nickel (Ni).

Even though heavy metal is an element that occurs naturally and can be found in the earth's crust, but most of the environmental pollution and human exposure occurred because of anthropogenic activities such as mining, industrial production, and use, and domestic and agriculture use of heavy metals (Tchounwou et al., 2012). According to Bradl (2005), there has been an increase in ecological and international public health concern related to environmental contamination by heavy metals. From the contaminated environment, organic and inorganic pollutants that bound to the aquatic systems may contaminate human by the consumption of the contaminated fish and other aquatic foods (Baharom & Ishak, 2015).

In Malaysia, agriculture is one of the main sectors in the economy. Thus, the usage of pesticides and fertilizers to increase the productivity and yield of crops to satisfy the demands are one of the causes that can elevate the heavy metal levels in paddy soils and water source at paddy field area. According to Parveen & Nakagoshi (2001), while only two applications of hazardous insecticides already sufficient, farmers tend to spray it up to five times in one cropping season in order to remove pests. Uncontrolled application of fertilizers and pesticides to the paddy field area might increase the heavy metal absorption in aquatic organisms in the area thus resulting in high bioaccumulation of heavy metals.

Aquatic organisms, such as fish and mollusk can absorb directly pollutants that presence in water and indirectly from food chains. According to Semenovich (2002), heavy metals enter the fish by mechanical capture of suspended particles of hydroxides in gills, while chemical absorption occurs on the mucous membrane.

As a main source of protein for human, heavy metals contamination in fish considered as a very serious issue because it is being consumed by human and for people that live close to the river, fish is one of their main food source (Hashim et al., 2014). Humans are one of the last receivers in the food chain, thus they tend to accumulate more pollutants in their body (Yin, Ismail, & Zulkifli, 2012). According to Lee et al. (2006), human exposure to high concentrations of heavy metals will lead to their accumulation in the human body.

Asian swamp eel, *Monopterus albus* can be found in various aquatic habitats including rivers, lakes and paddy fields (Ai Yin et. al, 2018). It is a long-lived organism that are known to survive months without food and water (Bob, 2009). Thus, in paddy ecosystem, Asian Swamp Eel has higher chances to accumulate heavy metals because their habitat is in the paddy fields.

According to Al-Mahaqeri (2015), it is very important in two major aspects to assess heavy metals concentration in fish from the contaminated area. The first aspect is from the point of view of public health. Ingestion of fish from the contaminated area can cause harm to human thus, it is important to assess the potential health risk that related to the consumption of the fish.

The next aspect is from the point of view of aquatic environment where, by assessing the heavy metals in fish, our knowledge on the biological status of the aquatic ecosystems can be improved. It also can help us to understand the changes and adaptation to aquatic ecosystem according to the changes in surrounding environmental conditions.

1.2 Problem Statement

Uncontrolled use of fertilizers and pesticides to increase the production of crops has affected aquatic organisms that live in paddy field area. This is because heavy metals from the pesticides can enter the food chain and food web and cause adverse effects on biotic elements of earth and accumulate in the ecosystem. Contamination of heavy metals in aquatic organisms is dangerous because heavy metals cannot be degraded and removed from the environment unlike, other pollutants. Fish can take up the heavy metals that exist in their surroundings through the food chain and water, and ultimately will end up in their body, where the heavy metals accumulate in various organs and tissues.

The use of pesticides to control the presence of pest organism at paddy field such as Apple snail may be effective, however, long exposure of these pollutants might increase the accumulation of heavy metals in living aquatic organisms particularly the swamp eel. As this eel is a long-lived organism, the longer exposure of this eel, the higher the bioaccumulation in the organs of the eel. Moreover, eel is considered as one of the protein source to human beings. There is high tendency for the human to get contaminated by toxic metals because human being is located at the top of food chain as end user. However, people that consume the eel may not know about the contents of heavy metals in the eel. Long-term consumption of heavy metals may result in heavy metals accumulation in the human body where they can pose chronic toxicity when their presence exceeds the concentration level required by the body.

Therefore, the objective of this research was to determine the Cd and Pb concentrations in the organs of *Monopterus albus* that were collected from the paddy field in Pasir Mas and Bachok, Kelantan. Health Risk Assessment was conducted to get the data on the potential risks to the human health from the consumption of eel.

1.3 Objectives

1) To analyzed cadmium (Cd) and lead (Pb) concentrations in selected organs of *Monopterus albus* collected from the paddy field located in Pasir Mas and Bachok, Kelantan.
2) To evaluate the potential health risks from the consumption of *Monopterus albus* by using the Health Risk Assessment (HRA) method.

1.4 Scope of Study

This study focuses on the determination of Cd and Pb concentration in the organs of *Monopterus albus* that were collected from the paddy field around Pasir Mas and Bachok in Kelantan. Pasir Mas and Bachok had been choose as the study area because it has an abundant area of paddy fields. This study involves field and laboratory works. Fieldworks involve collecting eels from paddy fields at 10 different points using tools called *tukil*. Water sample also will be collected from the paddy field. Then, in the lab, selected organs of the eel will be dissected which are heart, liver, esophagus and stomach contents, gonad, kidney, bone, muscle, skin, and gill.

The heavy metals concentration, which are Cd and Pb in final solutions, will be determined by using Atomic Absorption Spectroscopy (AAS). AAS machine is use to determine heavy metal or chemical elements by using the absorption of optical radiation by free atoms in a gaseous state. From the AAS result, HRA will be conducted in order to evaluate the possible health risk from the heavy metal exposure.

1.5 Significance of Study

Many researchers had drawn attention to the increase of heavy metal contamination of aquatic ecosystems that rise above the natural levels. This is because when the contamination content and the exposure to human health are significant, it can accumulate in aquatic species, enter the food chain, and cause harm to human health. In paddy field, the inappropriate use of pesticides and fertilizers could lead to the destruction of the ecosystem. A greater problem lies in the bioaccumulation of pesticides in organisms like fish. Since fish are at the top for the aquatic food chain, it is possible for the fish to accumulate a large amount of metals from the surrounding environment and for that, the organisms had been largely used as the valuation for the quality of aquatic systems.

By monitoring the contamination of the Asian swamp eel's tissues, it allows consumers to detect any toxic pollutants in fish that may be hazardous to them. Then, a right action can be taken to safeguard the environment and public health. For Asian swamp eel, there were only a few researches in determining the bioaccumulation of the heavy metals in the organs of *Monopterus albus*.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metal

Heavy metal is a metallic element that has a relatively high density compared to water and the heaviness and toxicity of the elements are inter-related (Duffus, 2002). The stability of heavy metals makes them a persistent toxic substances in the environment because being metals ions, heavy metal cannot be destroyed or degraded (Ayangbenro & Babalola, 2017). According to Huggett (1995), there are some heavy metals that essential for human health such as iron (Fe) and zinc (Zn) but there are also metals that can be toxic in certain forms or larger amounts. WHO (1996) also stated that Fe, Zn, and selenium (Se) are some of the essential nutrients that are needed for numerous biochemical and physiological functions where, if the supply of these nutrients are inadequate, it can results in deficiency diseases or syndromes.

Heavy metals capable in entering the food chain, where they will accumulate and cause damage to living organisms because even at low concentrations, majorities of the metals are toxic. At higher concentrations, all metals have the potential to show harmful effects and the toxicity of each metal depends on the amount available to organisms, the absorbed dose, the route and the duration of exposure (Mani & Kumar, 2014). Heavy metal as the environmental contaminants can be found in the air, soil and water, which can pose a health hazard to the public. Over a period of time, accumulation of heavy metals can occur and the concentrations will become obvious and measurable.

2.1.1 Lead (Pb)

Lead possesses the general physical properties of a metal. It conducts electricity and heat even though it is not as good as some other metals, such as copper. Lead also has high density, has a metallic lustre and albeit a dull one. Furthermore, compared to other metals, it has a very low melting point, which is 327ºC (Thompson & Clearinghouse, 1995).

Lead is an element that is found in the earth's crust and it occurred naturally. Even though lead has its beneficial uses, it also can give effect to health and can be poisonous to people and animal (EPA, 2012). Even though lead is naturally occurred, fossil fuels burning and mining are some of the anthropogenic activities that cause the release of lead into the environment to increase rapidly.

According to Atsdr (2007), inhaling dust particles that contaminate with lead, consuming food that are contaminate with lead, and paints are the main cause for the exposure of lead for the human. The symptoms of lead exposure on the central nervous system are headache and dullness because the most defenseless target of lead poisoning is the nervous system (Gerberding et al., 2005). Brains and nervous systems of children are vulnerable to the effects of lead so, if they are exposed to lead, their bodies can absorb more lead compare to the adults (EPA, 2012).

2.1.2 Cadmium (Cd)

Cadmium is commonly found as a mineral combined with other elements such as oxygen, chlorine or sulfur and pure cadmium is a soft, silver-white mineral (Godt et al., 2006). In industrial processes, cadmium is widely used in fabrication of nickel-cadmium batteries, and as an anticorrosive agent. A big amount of cadmium also presence in phosphate fertilizers and because of that, plants gradually take up these metals and get accumulated and concentrate along the food chain, ultimately will reaching the human body (Jaishankar et al., 2014).

Cadmium is released into the environment through natural activities such as volcanic eruptions and weathering and it also being released through human activities such as mining, smelting and incineration of municipal waste (Jaishankar et al., 2014). According to Järup (2003), a huge part of the cadmium pollution is caused by dumping and burning waste that are polluted with cadmium even though, some products containing cadmium can be recycled.

Humans are exposed to this metal by inhalation and ingestion and they can suffer from acute and chronic intoxications (Jaishankar et al., 2014). Shortness of breath, destruction of mucous membrane and lung edema are the effect of inhaling cadmium-contaminated air to the respiratory system (Seidal et al., 1993). According to Nordberg (2004), ingestion of food that were polluted with cadmium can cause vomiting and diarrhea as reported in 1942.

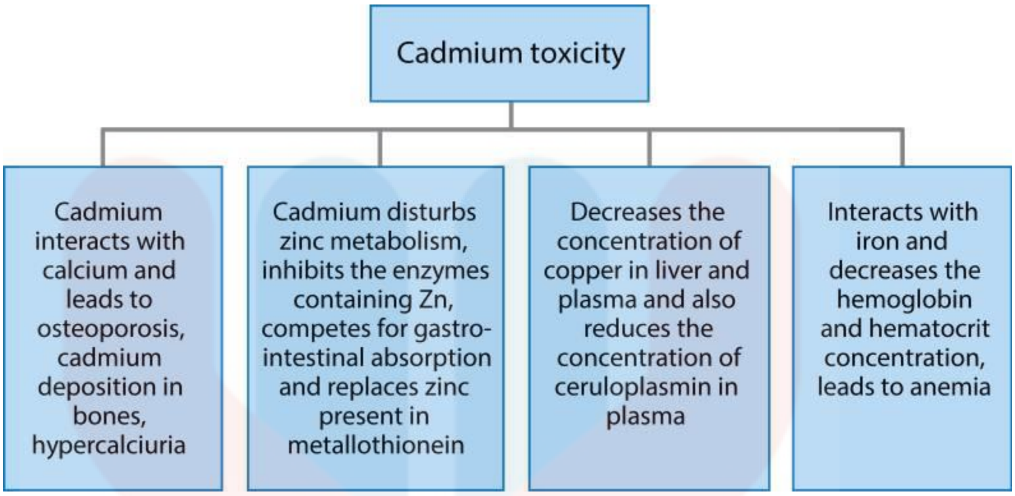

Figure 2.1: Values of cadmium toxicity (Flora et al., 2008)

2.2 Effects of Heavy Metal on Human Health

Essential metals and non-essential metals are the categories for heavy metals. Lead, mercury and cadmium are non-essential metals and in small amount; it can be toxic and harmful to organisms over a long period (Jyothirmayi & Madhusudhana Rao, 2014). According to Järup (2003), lead, cadmium, mercury and arsenic are heavy metals that are associate as main threats to human health. International bodies such World Health Organizations (WHO) have been regularly reviewed the effects of these metals on human health.

Lead is very toxic to human, especially to children because children have less effective renal excretion and greater absorption of gastrointestinal and compared to adult's brain, fetal brain have better sensitivity to the effects of Pb (Schnaas et al., 2006). For cadmium, the main source of exposure is from food consumption. Cd is known as substance that disturb endocrine and it can cause breast and prostate cancer in human (Saha & Zaman, 2013). Emami Khansari et al. (2005) stated that Hg is a human toxicant and become primary sources of human by eating fish. Toxicity of mercury (Hg) in human can cause the development of fetus to destroy and it also considered as carcinogen (Jyothirmayi & Madhusudhana Rao, 2014). There are also heavy metals that are important to human health. Manganese (Mn) have the ability to prevent heart attack and stroke as it is an element of low toxicity thus, it is considerable biological significance (Fazureen Azaman et al., 2015). However, at high concentrations, it become dangerous and toxic and may lead to the neurologic and psychologic (Saha & Zaman, 2013).

According to Hashim et al. (2014), heavy metals can be neurotoxic, carcinogenic and mutagenic if it is being consumed above the permissible limit that had been set by international organizations. Even though human have antibody that help to fight disease and health problems, continuous exposure of heavy metals could lead to serious illnesses or can cause death (Järup, 2003).

2.2 Heavy Metal in Aquatic Organisms

In fish, it is varies from element to element for natural background concentration of heavy metals and anthropogenic heavy metals. Fish carried natural burden of heavy metal concentration in unpolluted area while, concentrations of heavy metal are exceeding the natural concentration in heavily polluted area (Kalay et al., 1999). Khayatzadeh & Abbasi (2010) stated that, depending on age, developmental stage and other physiological factors, the presence of metals varies between fish species. Fish increase in both body length and mass under optimum conditions, at optimum temperature and at sufficient quantities of food, however, in the polluted water, fish growth may be inhibited. Therefore, the growth of fish can be indications for environmental conditions (Khayatzadeh & Abbasi, 2010).

In the aquatic environment, aquatic organisms easily take up heavy metals in dissolved form where they are strongly bound with sulfhydryl groups of proteins and accumulate in their tissues. Fish absorb dissolved or available metals and can therefore serve as a reliable indication of metal pollution in an aquatic ecosystem. Trench is considered a good test organism for heavy metal contamination because of its feeding behavior and bottom feeding habits (Shah & Altindağ, 2005).

2.4 Fish as A Bio-Indicator to Trace Heavy Metal

Toxicant accumulation in water suggests that fish serve as useful indicators for contamination in aquatic systems because fish respond to changes in the environment with greater sensitivity compared to invertebrates and it also tend to accumulate some metals concentrations several times higher than in ambient media (Dhanakumar et al., 2015; Zhao et al., 2015). According to Ural et al. (2012), toxicants can accumulate to toxic concentrations and cause ecological damage under certain environmental conditions such as season, pH, and hardness and biotic factors which are fish species, age and tissue.

Heavy metals are taken up through different organs of the fish because of the affinity between them and during this process, in different organs of the fish, metals will concentrate at different levels (Yancheva et al., 2015). Therefore, gills, liver, muscle, kidney and muscles are the tissue that most frequently utilized in ecological, toxicological and pathological studies (Heier et al., 2009). Jovičić et al. (2015) stated that studies on metal accumulation in fish are mainly focused on the muscle, while other tissues have been largely neglected.

Fish species has been used as bio-indicators in many studies to study heavy metal pollution in the environment (Baharom & Ishak, 2015; Hashim et al., 2014). According to Rashed (2001) fish is consider as one of the most indicative factors for pollution studies in freshwater system. Heavy metals from food that were took up by fish and other aquatic animals enter the organs through their gills. Moreover, fish is notorious for its potential to bio concentrate heavy metals in the muscles and other organs (Nor Hasyimah et al., 2011).

Abdullah et al. (2007) stated that because mollusk have high concentration factors (BCFs) values, it is possible for the mollusk to act as bio-indicator for Cd and Zn contamination in sediment and water of an estuarine environment. Moreover, the species resisted the absorption of other metal from the surrounding and only accumulated certain metals in its tissue.

2.5 *Monopterus Albus*

Monopterus albus or Asian swamp eel also known as the rice eel or rice paddy eel, is a three-foot long fish that is native to Central and Southeast Asia, East Asia, South America, and Africa. The habitat of the *Monopterus albus* includes swamps, rice fields, muddy ponds, and canals (Bob, 2009). It can breathe air and is tolerant to contaminate water or low oxygen levels. They have also been known to survive months without food or water (Yin et al., 2012). Swamp eel likes muddy environments and they spend more hours during the daytime hiding under stones or burrowing depth in muddy paddies area.

They are well known in having unique adaptations to its habitats and high survival rate (Ai Yin et al., 2018). A vital role in their survival includes environmental factors and water quality. For better growth and development of swamp eel, the temperature range should fall between 25ºC to 31ºC *Monopterus albus* is a hermaphrodite species female during their early life and turn to males thereafter. This enables small populations of swamp eel to breed after drought seasons (Liem, 1963).

Swamp eel provides protein source to consumers and there are some people believed that eel have many beneficial and medicinal values. For example, bone of the eel is being used as milk-frozen agents, while the slime from the eel's body can be used for cataract medication. To capture the swamp eel, it depends on the paddy season in which, in Malaysia, there are four paddy seasons. However, farmers and eel catchers often faced difficulties to catch the swamp eel because of their habitats complexities, behavior and life cycle (Ai Yin et al., 2018) .

There are studies that support the use of Asian swamp eel as a reliable bio-indicators in detecting heavy metal concentrations and the tissue as an indicator to the environment exposure to pollutants (Wahid, Prasarnpun, & Yimtragool, 2017; Yin et al., 2012).

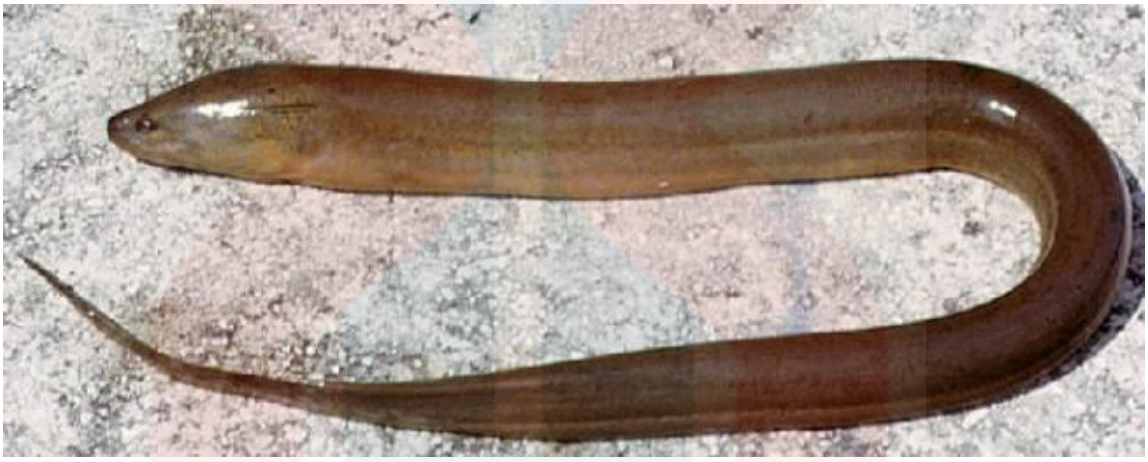

Figure 2.2: Asian swamp eel (*Monopterus albus*)

Source:
http://www.columbia.edu/itc/cerc/danoffburg/invasion_bio/inv_spp_summ/Monopterus_albus.html

CHAPTER 3

METHODOLOGY

3.1 Study Area

The study are were located at the paddy fields in Pasir Mas (N 06º 6’36.2484” E 102º 7’ 5.4876”) and Bachok (N 6º 2’28.226” E 102º21’27.1728”), Kelantan. Pasir Mas and Bachok are well known as one of the main areas in Kelantan that produce crops. To improve the production of crops, farmers tend to use agrochemical fertilizer and pesticides. The usage of the chemicals could be the factors that contribute to heavy metals contamination to the aquatic organisms that live at the paddy field particularly the Asian swamp eel.

The location of the sampling site in Pasir Mas was far from the main streets and heavy industrial activities. Along the paddy field, a small river supplied water into the field. The residential areas near the field are scattering and few. At paddy field area in Bachok, there is Sungai Kemasin that also supplied water for the paddy field. There are also housing area near the field and a school nearby the study area.

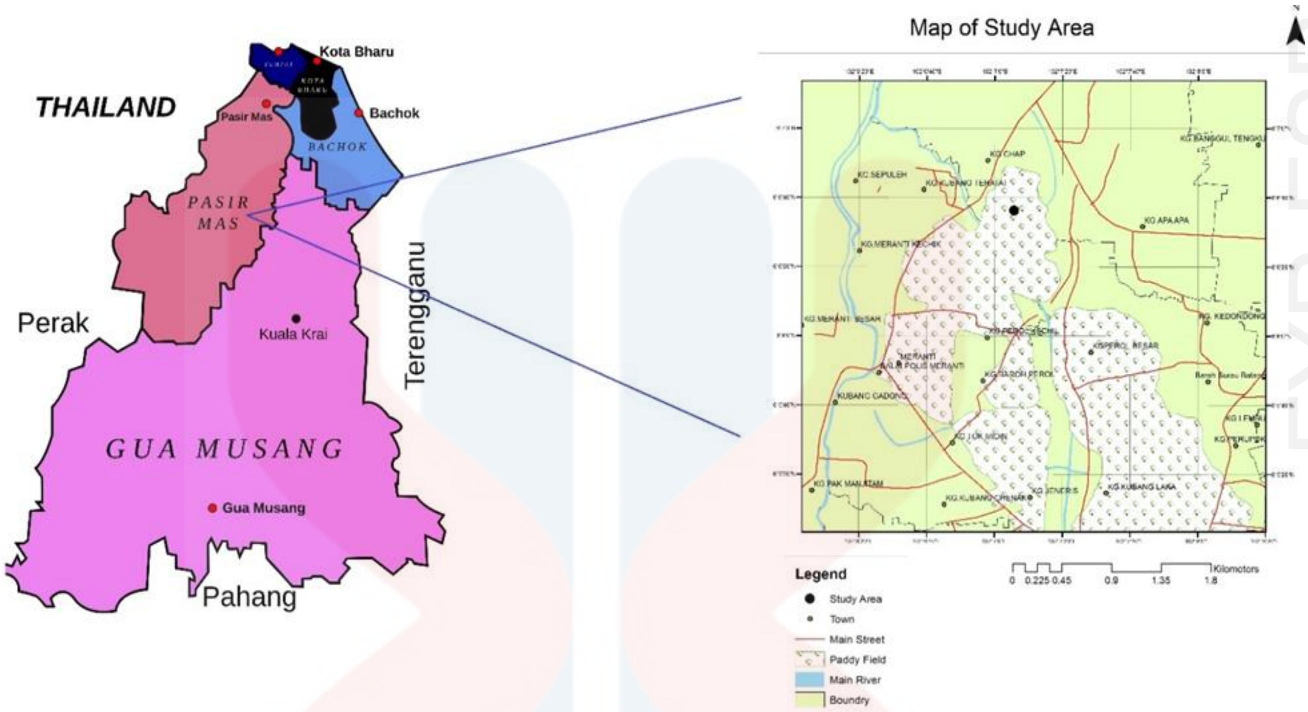

Figure 3.1: Map of study area in Pasir Mas, Kelantan

Figure 3.2: Paddy field area in Pasir Mas, Kelantan

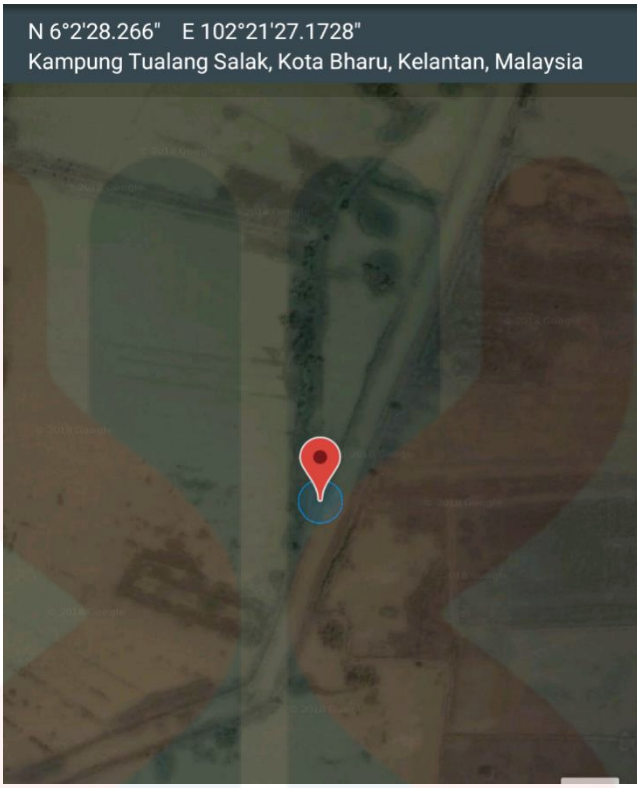

Figure 3.3: Coordinates of paddy field area in Bachok, Kelantan

Figure 3.4: Paddy field area in Bachok, Kelantan

3.2 Sample Collection and Preservation

The Asian swamp eel was collected using a tool called "*tukil*" (Figure 3.5). "*Tukil*" is a cylindrical tube that made from PVC pipe with length of 36 inches and the breadth is 2 inches (Yin et al., 2012). "*Tukil*" is commonly used by the farmers or eel catchers to collect eels. Prior to the collection of the eels, the "*tukil*" was positioned at ten selected points in the paddy field for a day and to attract the eels, bait such as cooked fish was inserted inside "*tukil*".

The trapping activities of Asian swamp eel was conducted in the late afternoon because they were active at night time to look for food. The trap was checked for the presence of eels for the next morning.

The collected Asian swamp eels were stored in polyethylene plastic bags and brought back to the laboratory. The eels then were stored in the freezer at the temperature of -20ºC until the next analysis was performed.

For the water sample, water from the paddy field was collected at the place where "*tukil*" was being placed. Then, the samples were filtered through filter paper and the sample was acidified with concentrated hydrochloric acid (HCl) to a pH less than 2. The purpose of acidification process is to preserve trace metal elements in the sample and also to reduce microbial activity. The sample was kept at 4ºC in a chiller before treatment.

Figure 3.5: "*Tukil"* that was used to trap *Monopterus albus*

3.3 Sample Preparation

The length and weight for every eel were measured and recorded. The average body weight of the eel collected from paddy field in Pasir Mas are 25.33-299.25g and for Bachok, the body weight was in the range 23.1-83.65g. For the length, eel in Pasir Mas had the length with range 31.1-69.2 cm while, for eel collected from Bachok, the length was in the range 35.5-70.1cm.

Eel was rinsed with distilled water before dissection to remove slime and dirt. Then, they were dissected by using stainless steel scalpels and forceps. Bone, muscle, heart, liver, gill, stomach content, gonad, skin, esophagus and kidney were removed from eel body and dried in an oven at 60º until a constant weight was obtained. The dried sample were then grounded into powder by using pestle and mortar (Yin et al., 2012).

To avoid any contamination, all the glassware and apparatus were washed with 10% nitric acid (HNO_3) solution for overnight, rinsed with distilled water before used (Mohamad et al., 2017).

Each organ of individual eel was weighed and placed into a beaker with the addition of 10 ml of concentrated HNO_3. Then at room temperature, the reaction was allowed to proceed. Next, the tubes was placed on the hot plate at 40 ºC for 1 hour and gradually increased up to 140 ºC for at least 3 hours. After the completion of the digestion procedure, distilled water was added into the beaker to a certain volume (40 ml) and then, through a filter paper, the extracts were filtered in a funnel (Yin et al., 2012). The filtered solution was collected in a volumetric flask. The entire process was done in the fume hood. The metal concentration in the solution was determined by Atomic Absorption Spectrophotometer (AAS) machine.

For water sample, the acidified water was undergone dilution process to get up to four dilutions. Dilution is the process of reducing concentration of a substance in a solution. A 15 mL stock solution, which is a solution with a known concentration of solute, was prepared and will act as a constant. Then, 1.5 mL of a stock solution was taken and placed into 13.5 mL of distilled water that will produces 15 mL of the dilute solution. The process was repeated until it gets up to four dilutions.

3.4 Metal Determination

All digested samples were analyzed by using Atomic Absorption Spectrophotometer (AAS) machine to check for the concentrations of Cd and Pb.

3.5 Data Analysis

Statistical analyses were performed using SPSS software. Data were analyzed by one-way of analysis of variance (ANOVA). Means were compared by Tukey multiple comparison test ($p<0.05$). The data was presented as mean ± SD.

3.6 Health Risk Assessment (HRA)

A complex parameter that is represent by target hazard quotient (THQ) is commonly used to assess the potential of non-carcinogenic risks that associate with long term exposure to heavy metals from food source such water and fish. In addition, THQ only shows a risk level that associate with exposure to pollutants but does not evaluate the risks (Agusa et al., 2005). As published by United States Environmental Protection Agency (2016), the exposed population do not have obvious risk and is safe if the ratio is less than 1. If, however, when the ratio is greater than 1, there is a possible risk for the studied metal to cause adverse health effects.

To calculate the THQ, the general formula that being used is as equations (1);

$$THQ = \frac{EFr \times ED \times FIR \times C}{RfD \times WAB \times TA} \times 10^{-3} \quad (1)$$

The EFr represents the frequency of the exposure, which was in this study (365 days/year) (Azmi et al., 2009). The ED represents the exposure duration; in this study, it was (65 years). Then, the IR represents the daily fish ingestion where in this study it was 0.17 kg/day/persons for the Malaysian adults (Azmi et al., 2009). The C represents the concentrations in wet weight of the trace element in the tissue of fish samples. The oral reference dose (RfD) of the trace element in µg/g/day. The average body weight (BW) for the Malaysian is 63 kg (Azmi et al., 2009) was used in this study. TA is the average exposure time for non-carcinogens 365 days/year × ED.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Distribution of Heavy Metals in Organs of *Monopterus albus*

The comparison of the concentration of cadmium (Cd) and lead (Pb) in the organs of *Monopterus albus* that were collected from both paddy fields in Pasir Mas and Bachok, Kelantan were summarized in Table 4.1 and Table 4.2 respectively. From Table 4.1-4.2, it showed that the accumulation patterns of Cd and Pb were different between the different organs and sites. The ranges for mean concentration of Cd and Pb in *Monopterus albus* from different paddy fields were 0.016-0.0462 and 0.0264-0.1584 µg/g respectively.

The highest concentration of Cd was observed in the bone of *Monopterus albus* from paddy field in Pasir Mas with mean concentration of 0.0462±0.0413 µg/g which is significantly higher ($p<0.05$) compared to heart, kidney, skin, muscle and gonad. Meanwhile, the lowest concentration of Cd was found in muscle of the eel that was collected from Bachok with mean concentration of 0.016±0.0012 µg/g. The concentration sequence of Cd in the organs of eel collected from Pasir Mas in decreasing order was bone> liver> gills> stomach content> esophagus> gonad> kidney> skin> heart> muscle. Meanwhile, for eel collected from paddy field in Bachok, the sequence of the concentration was from bone> gills> liver> esophagus>

stomach content> gonad> heart> kidney> skin> muscle. From Figure 4.1, it showed that the concentration of Cd also was the highest in the bone of *Monopterus albus* from paddy field in Bachok and the level of Cd in muscle also showed the lowest value in Pasir Mas

Meanwhile, the concentration of Pb in this study was ranged from 0.0264 to 0.1584 µg/g. The highest concentration of Pb was recorded in bone of *Monopterus albus* from paddy field in Pasir Mas with the mean concentration of 0.1302±0.1857 µg/g which is significantly higher ($p<0.05$) than that in kidney, skin, muscle, esophagus, liver, gonad and gills. The minimum concentration of Pb was observed in the muscle of the swamp eel collected from Bachok with the range of 0.0264±0.042 µg/g. The concentration sequences of Pb found in all tested organs used in this study were shown in Figure 4.2. From the figure, the sequence of the concentration of Pb in eel collected from Pasir Mas showed in decreasing order was from the bone> heart> stomach content> kidney> gills> skin> esophagus> gonad> liver> muscle. For the concentration of Pb in eel collected from paddy field in Bachok, the sequence in decreasing order was from bone> heart> stomach content> skin> gills> esophagus> kidney> liver> gonad> muscle.

Table 4.1: Concentration of Cd (mean±SD) in organs of M.albus from Pasir Mas and Bachok, Kelantan, Malaysia

Element	Organs	Location	
		Pasir Mas	Bachok
		mean±SD (μg/g)	mean±SD (μg/g)
Cd	Heart	0.020±0.026	0.019±0.003
	Kidney	0.022±0.019	0.016±0.004
	Skin	0.022±0.019	0.016±0.002
	Muscle	0.018±0.018	0.016±0.001
	Stomach content	0.031±0.017	0.021±0.004
	Esophagus	0.028±0.031	0.023±0.004
	Liver	0.037±0.028	0.027±0.006
	Gonad	0.022±0.024	0.021±0.006
	Bone	0.046±0.041	0.035±0.001
	Gill	0.031±0.024	0.029±0.006

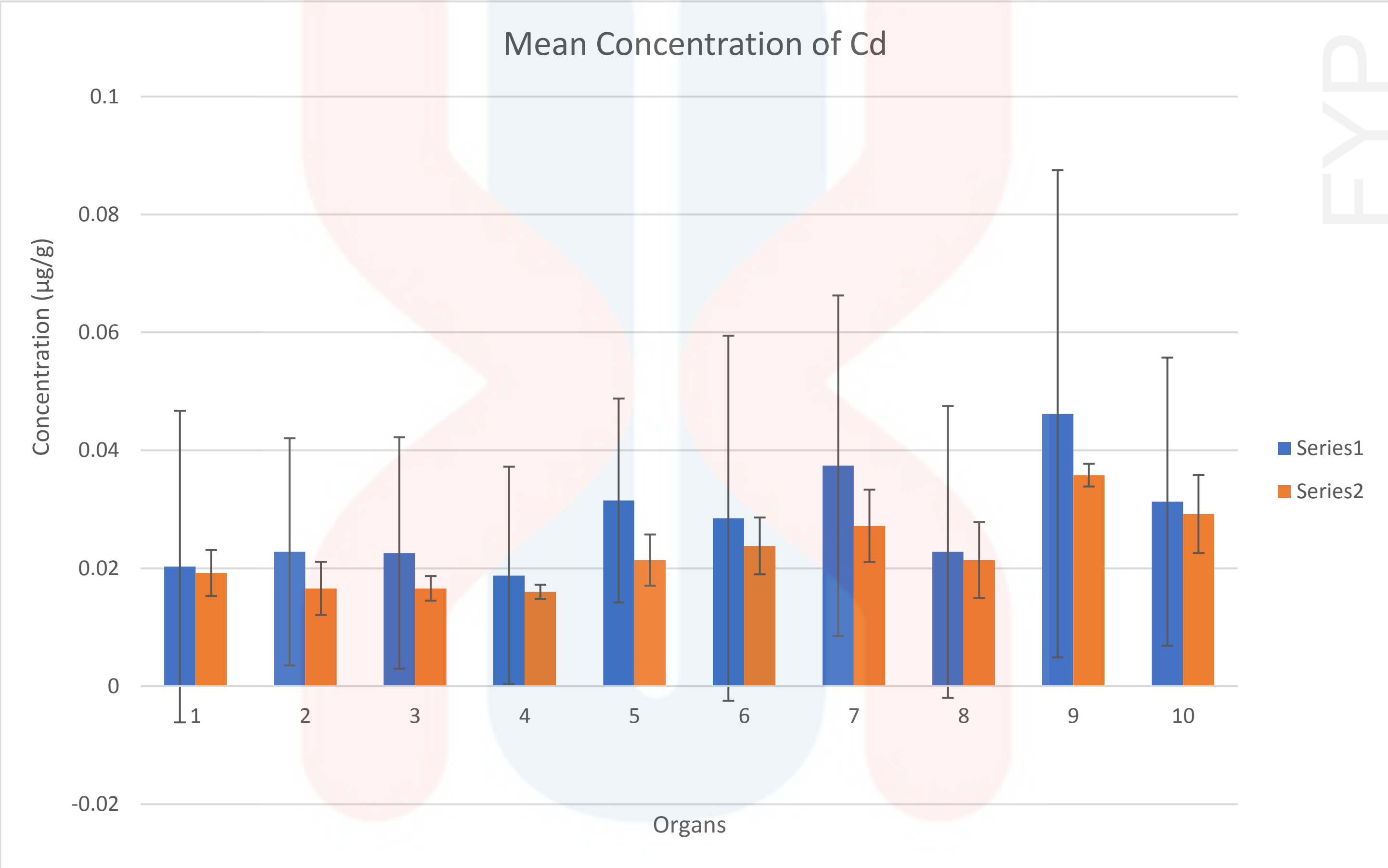

Figure 4.1: Distribution (mean±SD) of Cd in organs of M. albus collected from paddy fields in Pasir Mas and Bachok, Kelantan

Table 4.2: Concentration of Cd (mean±SD) in organs of M.albus from Pasir Mas and Bachok, Kelantan, Malaysia

Element	Organs	Location	
		Pasir Mas	Bachok
		mean±SD (µg/g)	mean±SD (µg/g)
Pb	Heart	0.121±0.1265	0.123±0.150
	Kidney	0.089±0.1185	0.062±0.083
	Skin	0.081±0.0868	0.082±0.0974
	Muscle	0.031±0.050	0.026±0.042
	Stomach content	0.110±0.1027	0.108±0.119
	Esophagus	0.055±0.0828	0.062±0.069
	Liver	0.043±0.0706	0.042±0.058
	Gonad	0.052±0.1158	0.031±0.051
	Bone	0.158±0.1580	0.130±0.186
	Gill	0.088±0.0870	0.075±0.158

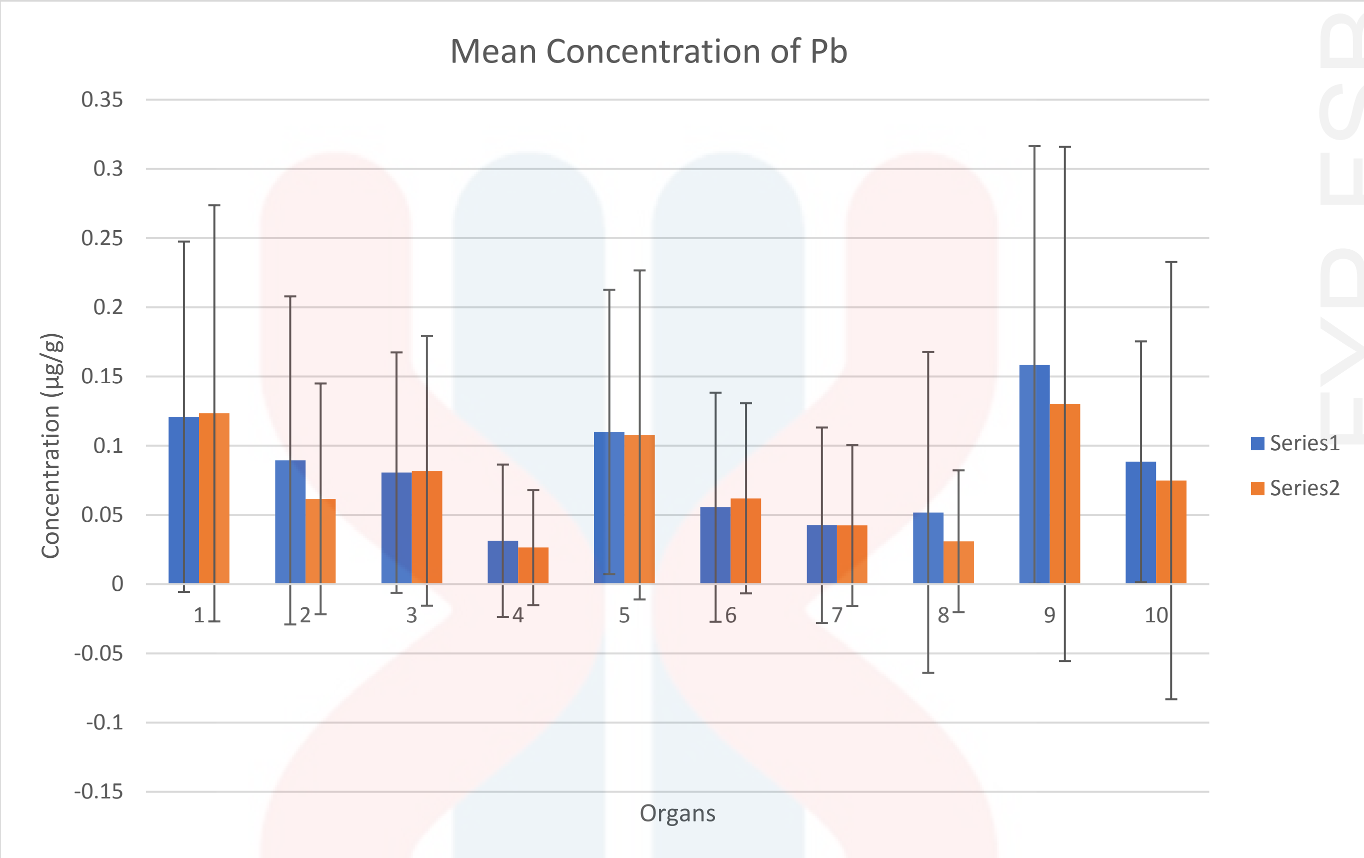

Figure 4.2: Distribution of the concentrations (mean±SD) of Pb in the organs of M. Albus collected from paddy fields in Pasir Mas and Bachok, Kelantan

Results of this study revealed that *Monopterus albus* collected from Pasir Mas and Bachok have the highest concentration of Cd and Pb in bone. According to previous studies, it showed that the concentration of Cd and Pb were the highest in gills and not in the bone (Al-Mahaqeri, 2015; Yin et al., 2012). According to Akan et al. (2012), organs such as the gonads and bones may show high metal levels because the affinity of various metals may be vary to every fish organ. There were no studies on the concentration of Cd and Pb in stomach content, thus, there are no proof to explain the high concentration that was observed in the stomach content of eel form this studies. However, Akan et al. (2012) stated that in the digestive tract, the high metal concentrations in the organ are linked to the dietary uptake route. Studies by Heath

(1987) and Ney & Van Hassel (1983) stated that high metal levels in the digestive area was because fish can accumulate heavy metals from food.

From the study by Silva (2004), fish's gills indicates the heavy metals concentration in the surrounding where the fish breathes in. For Asian Swamp Eel, gills were the target organ for the indication of accumulation for metals in the paddy field because gills are directly exposed to the surrounding water body. Tendency and capacity for gills to accumulate Cd is higher compared to liver and muscle tissues (Heath, 1987). During the process of osmoregulation and respiration, filaments and lamellae in fish gills provide large surface area to facilitate the adsorption of metals onto the surface of gills (Nor Hasyimah et al., 2011). Moreover, according to Heath (1987), metal complexing with mucus in the lamellae is impossible to be removed completely before analysis and this explained the reason why heavy metals accumulation is higher in gills. Also, there were study where three parts of tissue of catfish were compared and the results showed that the highest concentration of accumulated heavy metals was found in the gill of catfish compared to the liver and muscle (Mohamad et al., 2017).

Kidney and liver are the organs that are primarily accumulate cadmium and it may reach high concentrations in the gill, digestive tract, and spleen. The same goes to lead where it accumulate in numerous organs such as liver, kidneys and spleen, and gills (Akan et al., 2012). From the Figure 4.2, it showed that the concentration of Pb is higher compare to the concentration of Cd in the liver of *Monopterus albus*. Nor Hasyimah et al. (2011) state that liver has high tendency to bio accumulate heavy metal as it has metallothioneins binding proteins and free-protein thiol group content that makes strong fixation with heavy metals. Furthermore, since liver is active in the uptake, storing and detoxification of metal and the concentration of metal in the liver is relative to those that are exist in the environment, liver is considered as a possible bio-monitoring for heavy metal pollution in the water (Yap et al., 2015).

Then, the lowest concentration of Pb and Cd that can be observed from the Figure 4.1-4.2 is in the muscle of *Monopterus albus*. This result agrees with other studies where the lowest metal concentration was observed in muscle of fish compared to other organs (Jovičić et al., 2015; Yin et al., 2012). According to Yap et al. (2015), the reason for the low concentration of metals in the muscle compared to other organs is because low binding proteins is presence there.

The presence of Pb and Cd in the organs of *Monopterus albus* from the studied paddy fields might because of the use of chemicals in paddy farming. Chemical fertilizers are commonly use in agriculture industry in order to promote growth, increase rice yield and for weed control. At Mekong Delta, most of the farmers used more than one pesticide at the same time or apply pesticides in combination so that the working time in the farm can be reduced and at the same time to prevent, several crop diseases (Tam, 2016). Another factor that contributes to high level of Pb and Cd in the organs of *Monopterus albus* is the location of paddy fields that located near to the residential area and main roads where activities that involved the released of heavy metals into the water body that will then reached to the paddy fields.

4.2 Comparison of Pb and Cd Concentration with Permissible Limit in Food

Since there should be limits in the presence of heavy metals in fish because human is consuming them, permissible limits in fish have been set in many developed countries in order to safeguard public health. For example, Malaysian Food Act (MFA, 1983) has recommended permissible limit in Malaysia in order for maximum levels of contamination such as Cd and Pb can be set. For this study, the Pb and Cd concentration was compared with the Malaysian Food Regulation and WHO/FAO.

From the study, it showed that the level of Pb in liver and gill of *Monopterus albus* from paddy field in Pasir Mas and Bachok were higher than recommended levels set by Malaysian Food Regulation (1985) and WHO/FAO (Wood, 1974). To compare with the previous studies in Table 4.3, concentration of Pb in liver and gill of *Clarius Gariepinus*, and *Monopterus albus* from Tumpat also above the limit set by both guidelines. *Clarius Gariepinus* had the highest mean concentration of Pb, which is 55.650 mg/kg in the liver and for gill, the highest concentration was in *Monopterus albus* with value of 68.690 mg/kg. However, only *S. glanis* showed concentration of Pb that are still in the range of acceptable limits with the value of 0.067 and 0.236 mg/kg for liver and gill respectively. When inhaled or consumed in high doses, Pb can be toxic to human that it will interfere with essential nutrients that have the same characteristics such as calcium (Ca) and zinc (Zn) (Salem et al., 2000)

Then, for the concentration of Cd, the highest value was observed in the gill compare to the other two organs for the same species, which is *Monopterus albus* that were collected from different sampling site in Kelantan with the highest value is 4.45 mg/kg. The values are all exceeded the permissible limit and the results also showed the same with the concentration of Cd in the liver of the swamp eel. Muscle of

Monopterus albus that was collected from paddy field in Bachok and Pasir Mas was detected with the lowest value in which, the values were not exceeded the permissible level. However, the concentration value in in studies by Yin et al., (2012) showed that it exceed the guideline by Malaysian Food Regulation but, not exceed the limit set by WHO/FAO. Therefore, this indicates that the muscle which is an edible organ have the potential to cause low toxicity. This may be because of the presence of pesticides and fertilizers that are used by farmers to paddy fields to get rid pest and yield high quality crops.

Table 4.3: Comparison of heavy metals contents in *Monopterus albus* with literature data and food safety guidelines

Location	Organ	Element (mg/kg)		Reference
		Cd	Pb	
Pasir Mas	Liver	1.842	2.100	This study
	Gill	2.232	6.300	
	Muscle	0.700	1.169	
Bachok	Liver	1.749	2.727	This study
	Gill	2.25	5.754	
	Muscle	0.624	1.029	
Catfish (*Clarius Gariepinus*)(Sarawak, Malaysia	Liver	-	55.650	(Mohamad et al., 2017)
	Gill	-	40.050	
	Muscle	-	17.500	
Swamp Eel (*Monopterus albus*)(Tumpat, Kelantan, Malaysia)	Liver	1.78	29.550	(Yin et al., 2012)
	Gill	4.45	68.690	
	Muscle	1.61	22.730	
Wels Catfish (*S. glanis*)(Danube River, Serbia)	Liver	-	0.067	(Jovičić et al., 2015)
	Gill	-	0.236	
	Muscle	-	0.006	

Malaysian Food Regulation	-	1	2	Malaysian Food Regulation (1985)
WHO/FAO	-	2	2	Wood (1974)

According to Bowen (1979), with metallothioneins, cadmium can be accumulated with an uptake of 3.0-330.0 mg/day is deadly and 1.5-9.0 mg/day is lethal to human. The organs in human body that will be affected by the cadmium is kidney and causes symptoms of chronic toxicity such as the impairment of kidney function, hypertension and tumors (Waalkes, 2000).

4.3 Heavy Metals Contents in Water Sample and Comparison with Permissible Limit

The concentration of Pb and Cd in water samples that were collected from paddy fields in Bachok and Pasir Mas, Kelantan are was presented in Table 4.4.

Table 4.4 Concentration of Pb and Cd (mean±SD) in water sample from Pasir Mas and Bachok, Kelantan, Malaysia and comparison with permissible limit

Location	Elements (mg/L)	
	Pb (Mean±SD)	Cd (Mean±SD)
Pasir Mas	0.0683±0.0744	0.0015±0.00067
Bachok	0.2611±0.1295	0.0016±0.0007
NWQS	-	0.01
WHO	0.01	0.003

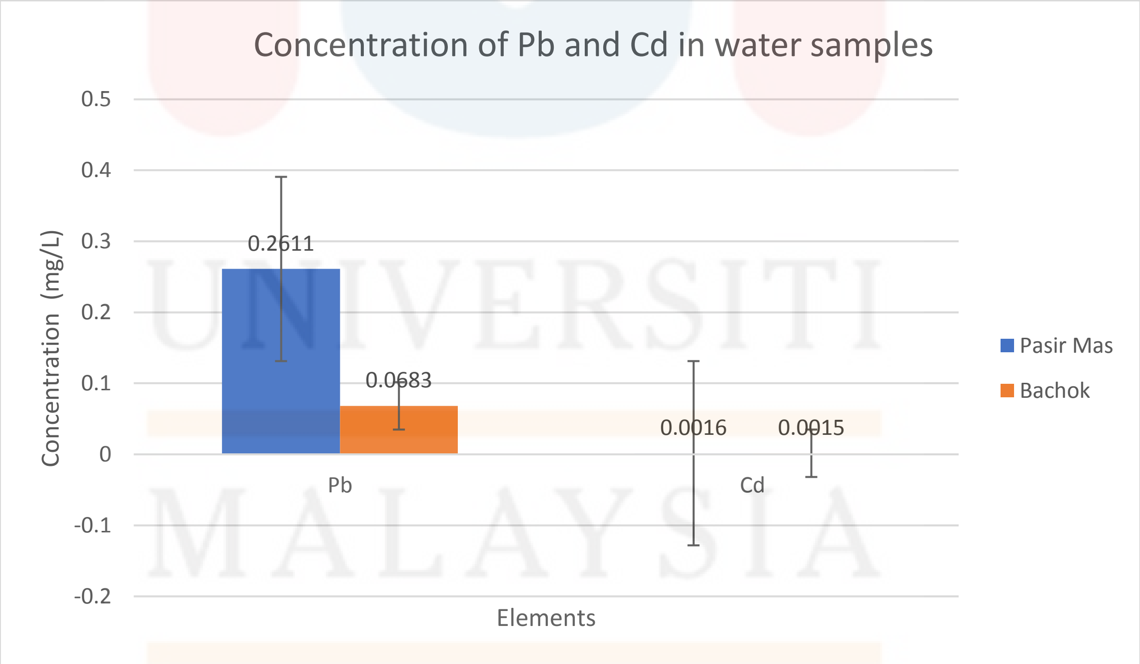

Figure 4.3: Distribution of mean concentration of Pb and Cd in the water samples collected from paddy fields in Pasir Mas and Bachok

The average concentration of Pb and Cd in water samples collected from paddy fields in Pasir Mas and Bachok followed the decreasing order of Pb>Cd for both sites. Lead concentration was the highest in the water sample from paddy field in Bachok with the mean concentration 0.2611 mg/L while in Pasir Mas the average concentration is 0.0683 mg/L. The difference in the concentration of Pb between Pasir Mas and Bachok are quite high. This is because the location of paddy field in Pasir Mas is located near to the main road and residential area. The activities from the household and the traffic at the main road may contribute to the high concentration of Pb in the water at the paddy field. There were also tractors at the paddy field that may cause the concentration of Pb to increase. However, the concentrations of Cd in both water samples were very low compared to Pb. The difference in the concentration of Cd between the two paddy fields is only 0.0001 mg/L.

The concentration of Cd that was observed in water sample from both study area showed that the values were below the permissible limit set by WHO, meanwhile for the concentration of Pb, it had exceeded the limit set.

4.5 Health Risk Assessment for Eel Consumption

Muscle is known as a non-active site for metal biotransformation and accumulation (Elnabris et al., 2013). The concentration of metals in fish muscle can exceed the permissible limit for human consumption if it live in polluted aquatic habitats and this may cause severe health threats. Thus, to assess public health risk of the *Monopterus albus* consumption, metal levels in muscles (Table 4.5) were compared with the permissible limit established by many organizations for human consumption.

THQ is used where it is the ratio between the exposure and reference dose. If the dose is equal to or greater than the RfD, the exposed population will experienced health risk and conversely, if the ratio of less than 1, it signifies non-obvious risk. As THQ below 1 indicate the level of exposure is smaller than reference dose; a daily exposure at this level can cause adverse effects during person's lifetime (Amirah et al., 2013).

Table 4.5: THQ of Pb and Cd in muscle of *Monopterus albus* collected from paddy field in Pasir Mas and Bachok, Kelantan

Location	Element	Concentration (mg/kg)	RfDo	THQ
	Cd	0.700	0.001	0.0018
Pasr Mas	Pb	1.169	0.004	0.0008
	Cd	0.624	0.001	0.0016
Bachok	Pb	1.029	0.004	0.0006

The calculation for potential health effects that related with the consumption of eel contaminated with Pb and Cd for an individual adult is presented in Table 5. The Cd concentrations in the *Monopterus albus* muscle have the highest potential as a health risk for consumption followed by Pb. The highest values of THQ were recorded in swamp eel sample collected from paddy field located near to the main roads and housing area. As a conclusion, from this study, the THQ of each metal is generally below than 1 for both sampling sites and this mean that, appreciable health risk would not be experienced by the people who consume the swamp eel that contaminated with the heavy metals.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

It can be concluded that heavy metal accumulations were varies in different organs of *Monopterus albus* regardless of the sampling location. This is because different fish have different metabolic level on heavy metals. Overall, Pb was the highest accumulated heavy metals in the organs of *Monopterus albus*. Bone of the swamp eel from both sites in Pasir Mas and Bachok was found to accumulate the highest concentrations of Pb and Cd, whereas the lowest concentration was observed in the muscle. Between the two sampling sites, the highest concentration of Pb and Cd was found in *Monopterus albus* collected from paddy field in Pasir Mas. Moreover, the highest concentration Pb and Cd also was observed in water collected from paddy field in Pasir Mas

The level of Pb and Cd in the muscle of swamp eel is lower than permissible limit set by both WHO/FAO and Malaysian Food Regulations but for liver and gills, it exceeds the limits set by both organizations. For water in paddy field from site, lead was found to be exceeded the permissible limit while for cadmium, the level was below the level set by WHO. Therefore, to avoid any serious health problem causes by heavy metals, the consumption of the swamp eel should be monitored.

5.2 Recommendations

Based on the studies that been carried out, there are a few recommendations that can be made;

1. Health hazards that involved with the consumption of aquatic organism taken from paddy field should enlightened and informed to the public.
2. Proper actions should be taken by notifying the ministry of health and environment about the excessive use of chemical fertilizers among the farmers.
3. To ascertain about the result of this study, more research should carried out with different species of organisms that live in paddy fields.
4. Conduct continuous monitoring for the use of fertilizers to ensure that the concentration of metal are within the permissible limit that had been set.
5. More research should be conducted on other organs besides muscle, gills and liver such as bones, gonad and esophagus to know the effect of heavy metals on these organs.

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APPENDICES

APPENDIX A

Sample Preparation

Figure 1A: Dissecting the eel to take out the organs

Figure 1B: Organs of eel

APPENDIX B

Sample Collection

Figure 1B: Eel was collected from the "*tukil*"

Figure 2B: *Monopterus albus* that had been collected from the paddy field

APPENDIX C

ANOVA Table

ANOVA

Concentration of Cadmium

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	9	.000	5.100	.009
Within Groups	.000	10	.000		
Total	.001	19			

Figure 1C: ANOVA analysis for the concentration of cadmium

ANOVA

Concentration of Plumbum

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.025	9	.003	24.954	.000
Within Groups	.001	10	.000		
Total	.027	19			

Figure 2C: ANOVA analysis for the concentration of plumbum

APPENDIX D

Post-Hoc Tests Table

Multiple Comparisons

Dependent Variable: Concentration of Cadmium
Tukey HSD

(I) organs	(J) organs	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Heart	Kidney	.0000500	.0046161	1.000	-.018223	.018323
	Skin	.0001500	.0046161	1.000	-.018123	.018423
	Muscle	.0023500	.0046161	1.000	-.015923	.020623
	Stomach Content	-.0067000	.0046161	.884	-.024973	.011573
	Esophagus	-.0064000	.0046161	.907	-.024673	.011873
	Liver	-.0125500	.0046161	.279	-.030823	.005723
	Gonad	-.0023500	.0046161	1.000	-.020623	.015923
	Bone	-.0212500*	.0046161	.020	-.039523	-.002977
	Gills	-.0105000	.0046161	.471	-.028773	.007773
Kidney	Heart	-.0000500	.0046161	1.000	-.018323	.018223
	Skin	.0001000	.0046161	1.000	-.018173	.018373
	Muscle	.0023000	.0046161	1.000	-.015973	.020573
	Stomach Content	-.0067500	.0046161	.880	-.025023	.011523
	Esophagus	-.0064500	.0046161	.903	-.024723	.011823
	Liver	-.0126000	.0046161	.276	-.030873	.005673
	Gonad	-.0024000	.0046161	1.000	-.020673	.015873
	Bone	-.0213000*	.0046161	.020	-.039573	-.003027
	Gills	-.0105500	.0046161	.466	-.028823	.007723
Skin	Heart	-.0001500	.0046161	1.000	-.018423	.018123
	Kidney	-.0001000	.0046161	1.000	-.018373	.018173
	Muscle	.0022000	.0046161	1.000	-.016073	.020473
	Stomach Content	-.0068500	.0046161	.872	-.025123	.011423
	Esophagus	-.0065500	.0046161	.896	-.024823	.011723
	Liver	-.0127000	.0046161	.268	-.030973	.005573
	Gonad	-.0025000	.0046161	1.000	-.020773	.015773
	Bone	-.0214000*	.0046161	.019	-.039673	-.003127
	Gills	-.0106500	.0046161	.455	-.028923	.007623
Muscle	Heart	-.0023500	.0046161	1.000	-.020623	.015923

	Kidney	-.0023000	.0046161	1.000	-.020573	.015973
	Skin	-.0022000	.0046161	1.000	-.020473	.016073
	Stomach Content	-.0090500	.0046161	.638	-.027323	.009223
	Esophagus	-.0087500	.0046161	.673	-.027023	.009523
	Liver	-.0149000	.0046161	.141	-.033173	.003373
	Gonad	-.0047000	.0046161	.984	-.022973	.013573
	Bone	-.0236000*	.0046161	.010	-.041873	-.005327
	Gills	-.0128500	.0046161	.257	-.031123	.005423
	Heart	.0067000	.0046161	.884	-.011573	.024973
	Kidney	.0067500	.0046161	.880	-.011523	.025023
	Skin	.0068500	.0046161	.872	-.011423	.025123
	Muscle	.0090500	.0046161	.638	-.009223	.027323
Stomach Content	Esophagus	.0003000	.0046161	1.000	-.017973	.018573
	Liver	-.0058500	.0046161	.941	-.024123	.012423
	Gonad	.0043500	.0046161	.990	-.013923	.022623
	Bone	-.0145500	.0046161	.157	-.032823	.003723
	Gills	-.0038000	.0046161	.996	-.022073	.014473
	Heart	.0064000	.0046161	.907	-.011873	.024673
	Kidney	.0064500	.0046161	.903	-.011823	.024723
	Skin	.0065500	.0046161	.896	-.011723	.024823
	Muscle	.0087500	.0046161	.673	-.009523	.027023
Esophagus	Stomach Content	-.0003000	.0046161	1.000	-.018573	.017973
	Liver	-.0061500	.0046161	.923	-.024423	.012123
	Gonad	.0040500	.0046161	.994	-.014223	.022323
	Bone	-.0148500	.0046161	.144	-.033123	.003423
	Gills	-.0041000	.0046161	.993	-.022373	.014173
	Heart	.0125500	.0046161	.279	-.005723	.030823
	Kidney	.0126000	.0046161	.276	-.005673	.030873
	Skin	.0127000	.0046161	.268	-.005573	.030973
	Muscle	.0149000	.0046161	.141	-.003373	.033173
Liver	Stomach Content	.0058500	.0046161	.941	-.012423	.024123
	Esophagus	.0061500	.0046161	.923	-.012123	.024423
	Gonad	.0102000	.0046161	.504	-.008073	.028473
	Bone	-.0087000	.0046161	.679	-.026973	.009573
	Gills	.0020500	.0046161	1.000	-.016223	.020323
	Heart	.0023500	.0046161	1.000	-.015923	.020623
Gonad	Kidney	.0024000	.0046161	1.000	-.015873	.020673
	Skin	.0025000	.0046161	1.000	-.015773	.020773
	Muscle	.0047000	.0046161	.984	-.013573	.022973

	Stomach Content	-.0043500	.0046161	.990	-.022623	.013923
	Esophagus	-.0040500	.0046161	.994	-.022323	.014223
	Liver	-.0102000	.0046161	.504	-.028473	.008073
	Bone	-.0189000*	.0046161	.041	-.037173	-.000627
	Gills	-.0081500	.0046161	.742	-.026423	.010123
	Heart	.0212500*	.0046161	.020	.002977	.039523
	Kidney	.0213000*	.0046161	.020	.003027	.039573
	Skin	.0214000*	.0046161	.019	.003127	.039673
	Muscle	.0236000*	.0046161	.010	.005327	.041873
Bone	Stomach Content	.0145500	.0046161	.157	-.003723	.032823
	Esophagus	.0148500	.0046161	.144	-.003423	.033123
	Liver	.0087000	.0046161	.679	-.009573	.026973
	Gonad	.0189000*	.0046161	.041	.000627	.037173
	Gills	.0107500	.0046161	.444	-.007523	.029023
	Heart	.0105000	.0046161	.471	-.007773	.028773
	Kidney	.0105500	.0046161	.466	-.007723	.028823
	Skin	.0106500	.0046161	.455	-.007623	.028923
	Muscle	.0128500	.0046161	.257	-.005423	.031123
Gills	Stomach Content	.0038000	.0046161	.996	-.014473	.022073
	Esophagus	.0041000	.0046161	.993	-.014173	.022373
	Liver	-.0020500	.0046161	1.000	-.020323	.016223
	Gonad	.0081500	.0046161	.742	-.010123	.026423
	Bone	-.0107500	.0046161	.444	-.029023	.007523

*. The mean difference is significant at the 0.05 level.

Figure 1D: Post-Hoc Tests analysis for the concentration of cadmium

Multiple Comparisons

Dependent Variable: Concentration of Plumbum

Tukey HSD

(I) organs	(J) organs	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Heart	Kidney	.04670*	.01064	.027	.0046	.0888
	Skin	.04100	.01064	.058	-.0011	.0831
	Muscle	.09330*	.01064	.000	.0512	.1354
	Stomach Content	.01330	.01064	.945	-.0288	.0554
	Esophagus	.06340*	.01064	.003	.0213	.1055
	Liver	.07970*	.01064	.001	.0376	.1218
	Gonad	.08080*	.01064	.000	.0387	.1229
	Bone	-.02210	.01064	.575	-.0642	.0200
	Gills	.04060	.01064	.061	-.0015	.0827
Kidney	Heart	-.04670*	.01064	.027	-.0888	-.0046
	Skin	-.00570	.01064	1.000	-.0478	.0364
	Muscle	.04660*	.01064	.027	.0045	.0887
	Stomach Content	-.03340	.01064	.160	-.0755	.0087
	Esophagus	.01670	.01064	.836	-.0254	.0588
	Liver	.03300	.01064	.168	-.0091	.0751
	Gonad	.03410	.01064	.146	-.0080	.0762
	Bone	-.06880*	.01064	.002	-.1109	-.0267
	Gills	-.00610	.01064	1.000	-.0482	.0360
Skin	Heart	-.04100	.01064	.058	-.0831	.0011
	Kidney	.00570	.01064	1.000	-.0364	.0478
	Muscle	.05230*	.01064	.013	.0102	.0944
	Stomach Content	-.02770	.01064	.323	-.0698	.0144
	Esophagus	.02240	.01064	.559	-.0197	.0645
	Liver	.03870	.01064	.079	-.0034	.0808
	Gonad	.03980	.01064	.068	-.0023	.0819
	Bone	-.06310*	.01064	.003	-.1052	-.0210
	Gills	-.00040	.01064	1.000	-.0425	.0417
Muscle	Heart	-.09330*	.01064	.000	-.1354	-.0512
	Kidney	-.04660*	.01064	.027	-.0887	-.0045
	Skin	-.05230*	.01064	.013	-.0944	-.0102
	Stomach Content	-.08000*	.01064	.000	-.1221	-.0379
	Esophagus	-.02990	.01064	.248	-.0720	.0122

	Liver	-.01360	.01064	.938	-.0557	.0285
	Gonad	-.01250	.01064	.961	-.0546	.0296
	Bone	-.11540*	.01064	.000	-.1575	-.0733
	Gills	-.05270*	.01064	.012	-.0948	-.0106
	Heart	-.01330	.01064	.945	-.0554	.0288
	Kidney	.03340	.01064	.160	-.0087	.0755
	Skin	.02770	.01064	.323	-.0144	.0698
Stomach Content	Muscle	.08000*	.01064	.000	.0379	.1221
	Esophagus	.05010*	.01064	.017	.0080	.0922
	Liver	.06640*	.01064	.002	.0243	.1085
	Gonad	.06750*	.01064	.002	.0254	.1096
	Bone	-.03540	.01064	.123	-.0775	.0067
	Gills	.02730	.01064	.338	-.0148	.0694
	Heart	-.06340*	.01064	.003	-.1055	-.0213
	Kidney	-.01670	.01064	.836	-.0588	.0254
	Skin	-.02240	.01064	.559	-.0645	.0197
	Muscle	.02990	.01064	.248	-.0122	.0720
Esophagus	Stomach Content	-.05010*	.01064	.017	-.0922	-.0080
	Liver	.01630	.01064	.852	-.0258	.0584
	Gonad	.01740	.01064	.806	-.0247	.0595
	Bone	-.08550*	.01064	.000	-.1276	-.0434
	Gills	-.02280	.01064	.539	-.0649	.0193
	Heart	-.07970*	.01064	.001	-.1218	-.0376
	Kidney	-.03300	.01064	.168	-.0751	.0091
	Skin	-.03870	.01064	.079	-.0808	.0034
	Muscle	.01360	.01064	.938	-.0285	.0557
Liver	Stomach Content	-.06640*	.01064	.002	-.1085	-.0243
	Esophagus	-.01630	.01064	.852	-.0584	.0258
	Gonad	.00110	.01064	1.000	-.0410	.0432
	Bone	-.10180*	.01064	.000	-.1439	-.0597
	Gills	-.03910	.01064	.075	-.0812	.0030
	Heart	-.08080*	.01064	.000	-.1229	-.0387
	Kidney	-.03410	.01064	.146	-.0762	.0080
	Skin	-.03980	.01064	.068	-.0819	.0023
	Muscle	.01250	.01064	.961	-.0296	.0546
Gonad	Stomach Content	-.06750*	.01064	.002	-.1096	-.0254
	Esophagus	-.01740	.01064	.806	-.0595	.0247
	Liver	-.00110	.01064	1.000	-.0432	.0410
	Bone	-.10290*	.01064	.000	-.1450	-.0608
	Gills	-.04020	.01064	.065	-.0823	.0019

Bone	Heart	.02210	.01064	.575	-.0200	.0642
	Kidney	.06880*	.01064	.002	.0267	.1109
	Skin	.06310*	.01064	.003	.0210	.1052
	Muscle	.11540*	.01064	.000	.0733	.1575
	Stomach Content	.03540	.01064	.123	-.0067	.0775
	Esophagus	.08550*	.01064	.000	.0434	.1276
	Liver	.10180*	.01064	.000	.0597	.1439
	Gonad	.10290*	.01064	.000	.0608	.1450
	Gills	.06270*	.01064	.003	.0206	.1048
Gills	Heart	-.04060	.01064	.061	-.0827	.0015
	Kidney	.00610	.01064	1.000	-.0360	.0482
	Skin	.00040	.01064	1.000	-.0417	.0425
	Muscle	.05270*	.01064	.012	.0106	.0948
	Stomach Content	-.02730	.01064	.338	-.0694	.0148
	Esophagus	.02280	.01064	.539	-.0193	.0649
	Liver	.03910	.01064	.075	-.0030	.0812
	Gonad	.04020	.01064	.065	-.0019	.0823
	Bone	-.06270*	.01064	.003	-.1048	-.0206

*. The mean difference is significant at the 0.05 level.

Figure 1D: Post-Hoc Tests analysis for the concentration of plumbum