# Nickel (Ni), copper (Cu) and zinc (Zn) determination in Asian swamp eel, *Monopterus albus* collected from paddy fields in Pasir Mas and Bachok, Kelantan, Malaysia.

by

**NISRINA NADIA BINTI MAIZATUL @ MAIZATU**

A report submitted in fulfilment of the requirements for the degree of

Bachelor of Applied Science (Sustainable Science) with Honours

**FACULTY OF EARTH SCIENCE**
**UNIVERSITI MALAYSIA KELANTAN**

**2019**

## DECLARATION

I declare that this thesis entitled Nickel (Ni), copper (Cu) and zinc (Zn) determination in Asian swamp eel, *Monopterus albus* collected from paddy fields in Pasir Mas and Bachok, Kelantan, Malaysia 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 candidature of any other degree.

Signature :

Name : Nisrina Nadia Bt Maizatul @ Maizatu

Date :

## ACKNOWLEDGEMENT

First and foremost, I would like to express my gratitude to my supervisor, Dr Nik Raihan Bt Nik Yusoff for giving the opportunity to conduct this research and gives much attention, advice and guidance in conducting this research. Not to forget, I also want to thank my assistant supervisor, Dr Sow Ai Yin for being supportive, patient and always have answers to my questions. Thus, their professionalism in giving advices and comments has enabled me to finish the research.

Moreover, I sincerely appreciate the help from Faculty of Earth Sciences' staffs. I humbled and grateful for their helping hands in assisting me to complete my research works.

Last but not least, my deepest thanks to my beloved family members, especially my supportive parents that always motivates me when I am feeling down and depressed with the lab works. Besides, I would like to thanks my lab mate, Farah Amalin Bt Mahhadi for always be understanding and never refuse to lend her hand for me in completing my projects. I sincerely appreciate everyone who direct or indirectly helps me in accomplishing my final year project.

**Nickel (Ni), copper (Cu) and zinc (Zn) determination in Asian swamp eel, *Monopterus albus* (Zuiew, 1793) collected from paddy fields in Pasir Mas and Bachok, Kelantan, Malaysia.**


## ABSTRACT

Eel, *monopterus albus* is one of the common fish and widely available throughout the year in paddy field in Malaysia. However, most people do not know types and concentration level of heavy metals contain in the eel and it could be toxic to the consumers. The objective of the study is to determine the heavy metals concentration of Cu, Zn and Ni, which accumulate in eel from paddy field in Bekelam, Bachok and Pasir Mas using Atomic Absorption Spectrometer (AAS) and to assess on human health using health risk assessment (HRA) toward eel consumption. A total of ten organs of the eel which were heart, kidney, skin, muscle, stomach content, liver, gonad, gill, oesophagus and bone were digested using acid digestion method. Meanwhile, a total of 300 water samples were collected at Bachok (150 samples) and Pasir Mas (150 samples) went through dilution process before analysed using AAS. Result obtained showed that, the water samples in Bachok had the highest concentration of Cu (0.07 mg/L), followed by Zn (0.06 mg/L), and Ni (0.01 mg/L). Meanwhile, the result obtained in Pasir Mas showed that Ni (0.04 mg/L) had the highest concentration followed by Cu (0.02 mg/L) and Zn (0.02 mg/L). Besides, the estimated daily intake (EDI) had been calculated to assess the human health risk. EDI were determined in the edible part of eel (muscle and skin) and were compared with maximum level intake (MLI). EDI had calculated to be lower than MLI, thus, Asian swamp eel at Bachok and Pasir Mas were still safe to be consumed. Moreover, the quality of water in cultivation area complied with Class IV, National Water Quality Standards for Malaysia (standard for irrigation water), where in general, all the measured elements were under permissible limits for aquatic consumption, hence the bio accumulation of heavy metals in aquatic organism could be low.

**Penentuan nikel (Ni), kuprum (Cu) dan zink (Zn) dalam belut sawah padi, *Monopterus albus* (Zuiew, 1793) di Pasir Mas dan Bachok, Kelantan, Malaysia.**

## ABSTRAK

Belut sawah padi, *Monopterus albus* adalah spesis ikan yang boleh didapati secara meluas sepanjang tahun di sawah padi di Malaysia. Walau bagaimanapun, kebanyakan orang tidak tahu jenis dan tahap kepekatan logam berat yang terdapat di belut dan boleh menjadi toksik kepada pengguna. Objektif kajian ini adalah untuk menentukan kepekatan logam berat Cu, Zn dan Ni, yang terkumpul di dalam belut dari sawah padi di Bekelam, Bachok dan Pasir Mas menggunakan Spektrometer Penyerapan Atom (AAS) dan menilai kesihatan manusia menggunakan penilaian risiko kesihatan (HRA). Sebanyak 10 organ belut yang terdiri daripada jantung, buah pinggang, kulit, otot, kandungan perut, hati, gonad, insang, esofagus dan tulang di analisis dengan menggunakan kaedah penghadaman asid. Sementara itu, sebanyak 300 sampel air dikumpulkan di Bachok (150 sampel) dan Pasir Mas (150 sampel) melalui proses pencairan sebelum dianalisis menggunakan AAS. Hasil yang diperoleh menunjukkan bahawa sampel air di Bachok mempunyai kepekatan tertinggi Cu (0.07 mg/L), diikuti oleh Zn (0.06 mg/L), dan Ni (0.01 mg/L). Sementara itu, hasil yang diperoleh di Pasir Mas menunjukkan bahawa Ni (0.04 mg/L) mempunyai kepekatan tertinggi diikuti Cu (0.02 mg/L) dan Zn (0.02 mg/L). Di samping itu, pengiraan anggaran pengambilan harian (EDI) telah dilakukan untuk menilai risiko kesihatan manusia. EDI ditentukan dalam bahagian yang boleh dimakan dari belut (otot dan kulit) dan dibandingkan dengan pengambilan tahap maksimum (MLI). Keputusan EDI telah dikira dan menunjukkan nilai EDI otot dan kulit belut sawah lebih rendah daripada MLI, oleh itu belut sawah di Bachok dan Pasir Mas masih selamat untuk dimakan. Selain itu, kualiti air di kawasan penanaman mematuhi dengan Kelas IV, Standard Kualiti Air Kebangsaan untuk Malaysia (standard untuk air pengairan), di mana secara amnya, semua elemen yang diukur adalah di bawah had yang dibenarkan untuk penggunaan akuatik, oleh itu pengumpulan bio logam berat dalam organisma akuatik berkemungkinan rendah.

## TABLE OF CONTENT

## CHAPTER 3 METHODOLOGY



## CHAPTER 4 RESULT AND DISCUSSION

## LIST OF TABLE

## LIST OF EQUATION



## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AAS | Atomic Absorption Spectrometer |
| Cu | Copper |
| Zn | Zinc |
| Ni | Nickel |
| HQ | Health Quotient |
| HRA | Health Risk Assessment |
| EPA | Environmental Protection Agency |
| ICP-MS | Inductively Coupled Plasma Spectrometry |
| HCl | Hydrochloric acid |
| $HNO_3$ | Nitric acid |
| $H_2SO_4$ | Sulphuric acid |
| SD | Standard Deviation |
| EF | Exposure Frequency |
| ED | Exposure Duration |
| FIR | Food Ingestion Rate |
| C | Heavy metal concentration in eel |
| RFD | Oral Reference Dose |
| WAB | Average Body Weight |
| TA | Average exposure time for non-carcinogen |
| *Et al.,* | And others |
| EDI | Estimated daily intake |
| MLI | Maximum level intake |

# LIST OF SYMBOLS

| | |
|---|---|
| mg/L | Milligram per litre |
| & | And |
| % | Percentage |
| mL | Millilitre |
| ºC | Degree Celsius |
| < | Lower than |
| mg | Milligram |
| ppm | Concentration |
| kg | Kilogram |
| µm | Micrometre |
| ± | Plus minus |
| SD | Standard Deviations |
| µg/g | Microgram/gram |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of study

Pollution is a great environmental issue which lead to unsolved solution and concern to human being. Daily anthropogenic activities are one of the top list factors of the accumulation of heavy metals in hydro systems that caused potential threat to aquatic organism and human health by encouraging broad range of adverse impacts (Arai, Chino, & and Ismail, 2012). Study in The Langat River in Negeri Sembilan by M. Shazili, (2006) reported that copper (Cu), zinc (Zn), Nickel (Ni) and other heavy metals were contributed from anthropogenic activities such as manufacturing and agriculture. Moreover, these activities induced pollution to the water and the aquatic organisms through runoff and sewage (M. Shazili, 2006).

Heavy metals are related to any metallic chemical element that has a high density and is toxic at low concentration. This heavy metal can easily diffuse or enter into human body through the consumption of food that is contaminated by heavy metal especially fish. The examples of heavy metals are Cu, Zn and Ni. Besides, the term heavy metal also called as "meaningless and misleading" and it also lack of the coherent scientific basis (Duffus, 2002).

Moreover, environmental pollution by heavy metals may occur naturally and catalyse by the human made activities (anthropogenic) such as the consumption of agrochemical fertilizers, pesticides, insecticides and herbicides are heavily used in the agriculture sector (Alloway, 1990). Thus, heavy metal contamination in aquatic food chain, soil, and water are one of the global environmental issues that are a growing threat to the environment day by day.

The rice-paddy eel, *monopterus albus* greatly found in tropical paddy field and it has becoming the favourite delicacy to Malaysian, especially Eastern-Malaysian that lives in the village area. Moreover, the rice-paddy eel is also believed that it may contain high protein source which will be very helpful in sustaining and providing good health (Zuiew, 1793). Furthermore, eel also one of the fish species which is an integral component and also contain calcium, magnesium, potassium, sodium and also has a good source of vitamins such as vitamin C, K, B-6 and B-12. Besides, eel also provide a healthy energy source and high nutrient to our body (FAO, 2010). The benefits that we can get from consuming this type of fish are healthy heart, good skin condition, reducing weight and strengthening teeth (Swanson Al, 2012).

The rapid development of modern agricultural and industrial has effecting human health and also influence the increasing of metal pollution in aquatic ecosystem which is harmful for aquatic organism and lead to bioaccumulation. Heavy metal contaminated water and soil may influence the heavy metal uptake in the eel as it will absorb or enter into the organism and therefore will accumulate in certain organs and tissues such as heart, liver, oesophagus and stomach contain, gonad, kidney, bone, muscle, skin, and gill and consequently cause the increasing of heavy metal concentration in the eel (Kumagai Akiko, Ryoko Ando & Hideyuki Miyatake, 2013). Moreover, Asian swamp eel is a species of fish that is long lived organism in paddy field, hence it is easily exposed to the pollutants such as heavy metals (Yin, 2016). Generally, the heavy metals uptake in fish fundamentally is the gills or intestinal wall absorbed the metals and distributed to different tissues in the body via circulation, or bound to transport proteins and whilst the metals are essential for life functions within the tissues, however metals also toxic to the body at high level intake (Olsson, 1998). Hence, it will create an adverse impact toward environment and also human health. The exposure of heavy metals could be either direct or indirect ways. For instant, air may be the medium of direct exposure of heavy metal to human health. On the other hand, the consumption of heavy metal contaminated food may be the medium on how the heavy metal introduce into the human body.

In this study, the regular application of fertilizers and pesticides on each paddy season might accumulate slowly from time to times which could cause the heavy metals pollution in the paddy cultivation area. Consuming food that has heavy metal contamination is very dangerous and can be fatally resulting from extreme dietary accumulation of heavy metal such as Cu, Zn,  and Ni (Arantes, Salvassi, & Santos, 2015). Therefore, the level of risk posed by water and eel with heavy metal can be trace by using several indices such as health quotient (HQ), health risk assessment (HRA) (Adeel Mahmood, 2008) and comparison with the permissible limits for human consumption based on the guidelines set by the Food and Agriculture Organization/World Health Organization (1989) and Malaysian Food Regulation (1985).

## 1.2 Problem statement

The application of aggressive agrochemical fertilizers and pesticides in paddy cultivation areas would increase the accumulation of pollutants (heavy metals) in water, thus bio accumulate in soil and eel (Yin, 2016). Since Asian swamp eel is a long-lived fish in paddy fields, the tendency of this eel to accumulate heavy metals is higher. Eel, *monopterus albus* is one of the common fish and widely available throughout the year in paddy field in Malaysia. Besides, eel has becoming one of the favourite delicacy among Malaysian especially East-Malaysian who lives in village area. Moreover, eel also type of fish that is popular with its tasty taste and tender meat. However, most people do not know types and concentration level of heavy metals contain in the eel and still enjoy eating the tasty and tenderly eel meat. Furthermore, the rapid development of agriculture and industrialization and also the introduction of technology have altered the traditional agriculture approaches to modern agriculture approaches among the farmer. The modern approaches include widely use of chemicals such as pesticides or herbicides which would pollute the water. Significant amount of pollutants discharge into the paddy field may accumulate and biomagnified along water and disrupt the aquatic food chain and the bioaccumulation process could occur in eel and certain level of metals could be hazardous for human consumption. Hence, this approach has resulted in increasing of metal pollution in the aquatic ecosystem, which significantly has adverse impacts towards environment, fish and human as well.

Therefore, the purpose of this research is to investigate the heavy metal concentration of Cu, Zn and Ni, in the rice-paddy eel, *monopterus albus*, and water collected from paddy field in Bekelam, Bachok and Pasir Mas and further investigate whether the metals concentration consists in eel are within the permissible limits for human consumption.

## 1.3 Objectives

1. To determine heavy metals concentration of Cu, Zn and Ni, which accumulate in eel from paddy field in Bekelam, Bachok and Pasir Mas using Atomic Absorption Spectrometer (AAS).
2. To assess on human health using health risk assessment (HRA) toward eel consumption.

## 1.4 Scope of study

The aim of this study is focusing on determining the heavy metals Cu, Zn and Ni in eels using Atomic Absorption Spectrometer (AAS). Eels were collected from paddy field in Bekelam, Bachok and also Pasir Mas. All parts or organs of the eel were dissected and investigated for further analysis. The organs or parts of the eel are heart, liver, oesophagus and stomach contain, gonad, kidney, bone, muscle, skin, and gill. Furthermore, this studies also focusing on the accumulation of heavy metals in paddy water in Kelantan. Therefore, the sample collection will be collected every month for four months which were on March, April, May and also June. Moreover, the scope of the study also to provide baseline data on the level of heavy metals concentration in the eel are within the permissible limits for human consumption based on the guidelines set by the Food and Agriculture Organization/World Health Organization (1989) and Malaysian Food Regulation (1985).

## 1.5 Significance of study

This study is important since in Malaysia there is only few researchers' attempted to study and determine the concentration of heavy metals in the eel. Particularly, this study was conducted to detect and monitor and to confirm the presence of the heavy metals in the eel and paddy water. Hence, identifying the heavy metal is crucial in order to maintain the environment and to sustain human health. Moreover, the results obtained throughout this experiment could be very useful in creating awareness among consumers on the health hazards that might occur from consuming eel that has higher level of heavy metals concentration within the permissible limits for human consumption based on the guidelines set by the Food and Agriculture Organization/World Health Organization (1989) and Malaysian Food Regulation (1985).

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Heavy metal

The term "Heavy metals" is used to describe more than a dozen elements that are metals or metalloids (Duffus, 2002). Few examples of heavy metals are cadmium, lead, copper, zinc, nickel and etc. Heavy metals are natural components of the Earth's crust. Heavy metals cannot be degraded or destroyed, and they also are persistent in all parts of the environment. Organism also needs heavy metals in the body for certain reasons such as Iron, cobalt, copper, manganese, molybdenum, and zinc are all required by humans. However, Excessive levels of these heavy metals can be damaging to the organisms (Reena Singh, Neetu Gautam & Anurag Mishra, 2011). Hence, heavy metals contamination is detrimental for human health and also environment. Heavy metals are very high in density, at least five times higher than water. Once, diffused or liberates into the environment through air, water, food, and others medium such as skincare products, it can be transferred into human body via inhalation, ingestion, and skin contact (Safety, 2002).

## 2.2 Source of heavy metal

Heavy metals can occur naturally and anthropogenic (human-made activities). Human activities affect the natural geological and biological distribution of heavy metals through diverse of environmental pollution such as pollution of water, air and soil. According to (Vig, Megharaj, Sethunathan, & Naidu, 2003) heavy metal exist naturally in soil from the soil forming processes of disintegration of parent resources at rare levels (<1000mgkg$^{-}$1) and not often poisonous. Nowadays, rapid development in agriculture and the modern approach that has been being introduced to the world has resulted in the alteration of chemical forms of heavy metal. Hence, the alterations often affect a heavy metal's toxicity by allowing it to bio accumulate in animals, bio concentrate and disrupt the food chain, or attack certain organs of the body. The use of chemicals in farming such as pesticide, fertilizers, industrial waste and mining and others has resulting in high amount of heavy metals. Therefore, certain heavy metals are essential for healthy life but it is needed in small quantities, for example iron, copper, manganese, and zinc.

## 2.3 Toxicity of heavy metal

Toxicity can be defined as the chemical ability to cause a detrimental effect toward human health once being exposed to them (Brian SO, 2014). According to Environmental Protection Agency (EPA) and the International Agency for Research on Cancer, heavy metal toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. Moreover, toxicity and carcinogenicity of heavy metal involves many mechanistic aspects as each metal is known to have unique features and physic-chemical properties referring to its specific toxicological mechanisms of action (Tchonwou PB, Yedjou CG, Patlolla AK, 2012)

Higher concentration of heavy metal can cause the metal to be highly toxic and some of the metals also fatal at very low concentration. Moreover, metals can be categorized into two categories which are hazardous and non-hazardous. Non-hazardous metals are required for healthy body meanwhile hazardous group of metals are the most dangerous as it can harm human health at a slightest exposure and must be kept under observation (Reena Singh, Neetu Gautam & Anurag, 2011).

## 2.4 Heavy metal regulation

Heavy metals are toxic to human health and also the environment. Hence, what are the permissible limits of heavy metals that are safe for human consumption in the fish? According to Heavy Metals Regulations Legal Notice No 66 (2003), the permissible limit of the copper for certain processes in human body such as tissue respiration and hence the limit of copper uptake in human is from 1.5 to 2.7 mg/kg per person (Desai & Kaler, 2008). Meanwhile for zinc is 100 mg/kg concentration and nickel 10mg/kg (Besada, Gonzalez, & Schultze, 2006) .

## 2.5 Heavy metals and health hazard associated with heavy metal

Humans are gifted with antibody that can always help the body to fight with diseases and humans are frequently exposed to the natural levels of trace elements. Hence, with the significance amount of this metal, humans' antibody still can control and fight with the metals that can bring sickness and harm to the body. However, elevated levels of metals and the continuous exposure of the metal itself could cause serious illness or death (Järup, 2003). There are several pathways of heavy metal uptake in human body such as it may occur through inhalation, ingestion of the contaminated food by absorption through the skin (Safety, 2002).

Moreover, heavy metals also can reach and harm unborn child in pregnant woman as heavy metal compound accumulate in the body tissues or selected organs and can cross the placenta through the mother. Furthermore, children are the most likely and easily influence to health problems caused by heavy metals, because their bodies still are growing and developing and also lack with antibody that is needed to protect the body and fight with illness (Landrigan & Goldman, 2011). The health hazards presented by heavy metals depend on the level and the length of exposure

(Thomson, 2005). In some cases, the health effects are acute and others are in long-terms. Besides, the excessive levels of toxic metals settled in body tissues and also organs and then will eventually move in the brain and hence will cause developmental delay and neurological damage, including depression, increased irritability, anxiety, insomnia, hallucination, memory loss, aggression and many other disorders.

### 2.5.1 Copper (Cu) and health effects

Copper, from the outside view is a reddish metal that occur naturally in water, rock, sediment, soil and also air and could be catalyst by the usage of chemicals such as pesticides that is commonly used among the farmers (Environmental Protection Agency (EPA), 2013). Thus, copper might accumulate in the eels mainly liver as eel lives in the soil and also in the water. According to Jalal *et al.*, (2013) copper mostly accumulates in the liver as liver has the detoxifying ability of copper. Once the copper level in the liver is exceed the safe limits, human body will be reacted such as gastrointestinal distress disease will appear. Basically, copper has lot of usage such as and commonly found in coins, electrical wiring, and pipes. Moreover, a small amount of copper also essential to human body to ensure a good dietary and health. Copper aids in the formation of red blood cells, promotes healthy connective tissue and support the immune system (Christopher Nwani & Naresh Nagpure, 2010). However, too much of copper uptake will lead to adverse impact of human health such as vomiting, diarrheal, stomach cramps, and nausea and worst liver damage and kidney diseases (Environmental Protection Agency, 2013).

### 2.5.2 Nickel (Ni) and health effects

One of the dangerous metals that could give significant health hazard is nickel which is a metal that commonly used in producing coins, stainless steel, magnet, jewellery and others (Edward, 2015). Despite the shines that this metal produces, it also toxic to environment and also human health. Nickel is carcinogenic metals and one of the environmental pollutants. The effects of nickel exposure are detrimental such as risk of lung cancer, cardiovascular disease, neurological deficits, and developmental deficits in childhood, and high blood pressure. Moreover, nickel also associated as a toxin that can harm pregnant women such as severely damages reproductive health and can lead to infertility, miscarriage, birth defects, and nervous system defects.

Nickel is widely used in industry such as welding and electroplating. Nickel may pollute certain places through runoff and also improper burning of waste that contain this metal. This metal could occur in soil as the result from the metal runoff and hence effected the living surrounds it (Abd Aziz. Roslaili, 2015). Fishes like eel that lives in soil are highly exposed with the contaminant and once the eel is contaminated by the metal, it may have biomagnified along the food chain and could give adverse impact toward

### 2.5.3 Zinc (Zn) and health effects

Zinc also one of the metals that are essential to human body and commonly found in nutritional supplements but it will be save to consume zinc in a small amount as taking too much zinc into the body also caused detrimental and adverse impacts. According to Environmental Protection Agency, (2013) zinc just like copper also occur naturally and the most common elements in the Earth's crust and it can be found in soil, air, water and also presents in all foods.

Besides, zinc also can be used for coat steel and iron as well as other metals to prevent rust and corrosion. However, once zinc enters the environment, it will give a bad effect to human health such as stomach cramps, nausea, and vomiting. Zinc enters the water, soil and air as a result of both natural and anthropogenic activity such as mining and can affect other places through runoff. Moreover, fish can collect zinc in their bodies from the water they swim in and from the food they eat. Hence, human may be exposed by the zinc contamination through digestion of the contaminated fish and also inhalation of the dust that contaminated by the zinc (Agency for Toxic Substances and Disease Registry (ATSDR)., 2005).

Furthermore, according to the Environmental Protection Agency, (2013), ingesting high amount of zinc lead to several health issues such as anaemia, damage the pancreas, and decrease levels of high-density lipoprotein (HDL) cholesterol and also makes human infertile. However, human body needs to consume little amount of zinc in order to be healthy as without enough zinc in the diet, people might be getting experiences of losing of appetite, the sense of taste will be decreased, low immune function, skin sores and slow wound healing. Least amount of zinc would not be harmful for human as long as the amount is still under permissible limit.

### 2.6 Eel as bio-indicator to trace heavy metal

A number of studies supported the use of rice-paddy eels *monopterus albus* as reliable bio-indicators of detecting level of heavy metal contaminant concentration and the eel tissue are suitable indicators regarding to the environmental exposure to pollutants (Amiard Triquet, 1987). Besides, rice-paddy eels are widely consumed by

Malaysian especially East-Malaysian who lives in village. On the other hand, eels are also popular in Asian countries such as China, Japan and Taiwan. The market value of the eels is depending on the species and varies between different countries. Currently prices range from RM18/kg which ranks the eel among the most precious species of food fish.

Concerning with the issue, little attention has been given to this species and also their resource management in Malaysia. Many researchers have been conducted on heavy metal monitoring using oyster and mussel (Yap *et al.*, 2003) but not for eels. Thus, this study aimed to trace the heavy metals such as Cu, Zn and Ni in the eel. Besides, rice-paddy eel's also an abundant population that can be found in paddy field and eels also have a long life-span with an optimum size for analysis.

## 2.7 Bioaccumulation of selected metals in fish from Malaysia's paddy fields

A study has been conducted on the bioaccumulation of selected metals in fresh water Haruan fish (*Channa striatus*) collected in Pahang River, Malaysia (Jalal, John, Habab, Mohd, & Kamaruzzaman, 2013). Certain organs of the Haruan fish has been dissected out such as liver, muscle and intestine and several parameters of heavy metals such as Cu, Zn and Pb have been analysed by using Inductively Coupled Plasma Spectrometry (ICP-MS). From the experiment, it is found out that liver is the organ that content highest concentration of Cu and Zn. Meanwhile, highest content of Pb is in the intestines. Hence, the study shows that the concentration of heavy metals might be different according to different tissues of the fish.

A study also has been conducted on the investigation on the concentration of pesticides residues in fish samples that has been collected from irrigation canals in paddy fields in Tanjong Karang and Sekinchan, Selangor and Seberang Perak, Perak, Malaysia (Abdul Rahman, Nur Azhani & Omar, 2012). Several tissues of the fishes such as skin, internal organs and head have been dissected and have been extracted using QuEChERS extraction method. From the experiment organochlorine residues has been analysed using mass spectrometry. There are three types of fish samples which are puyu (*Anabas testudineus*), haruan (*Channa striatus*) and sepat (*Tricogaster pectoralis*). This study comprises the effect of widely and excessively use of pesticides will contaminate the fishes in the paddy field. This study reveals the presence of heavy metals from pesticides in the fishes. The bioaccumulation of the heavy metals also will disrupt different tropic level of the food chain (Chen et al., 2000) thus, these harmful pesticides will give adverse impact for human consumption. Hence, it is true that pesticides are one of the sources that contribute to the heavy metal contamination in fish species.

Moreover, this study also shows the relation between the eel and also other fish species as all of them are come from paddy field. Therefore, this study also might be a strong evidence to support the presence of heavy metal in the eel.

# CHAPTER 3

# METHODOLOGY

## 3.1 Study area and sampling location

This study was conducted at paddy fields located in Kg Bekelam, Bachok, Kelantan (N06º02'30.9" E102º21'28.9") and Pasir Mas, Kelantan (N 06º 6' 36.2484" E 102º 7' 5.4876"). The selection of paddy field area based on the field area since the consumption of agrochemical fertilizers, pesticides, insecticides and herbicides are heavily used in the cultivation of paddies by farmers and the paddy field located at nearby residential area and far from heavy industrial activity. Moreover, there was a river along the paddy field and the river will become the water resources for farmers for the purpose of water supply to the paddy field. However, the difference between these two places are, paddy field in Bekelam was not so far from the road meanwhile, paddy field in Pasir Mas was more rural than Bekelam and quiet far from the road. The sampling of rice-paddy eels, *monopterus albus* were conducted and collected every month for four months which were on March, April, May and also June. The major source that leads to heavy metal contamination was from the anthropogenic materials which is pesticides and fertilizer that were used in the paddy field. There were ten stations (S1-S10) for collecting the samples. The water samples were collected along the collection of rice paddy eel. Figure 3.1and Figure 3.2 below shows sampling location of rice-paddy eel, *monopterus albus*, and water in paddy fields in Kg Bekelam, Bachok and Pasir Mas, Kelantan.

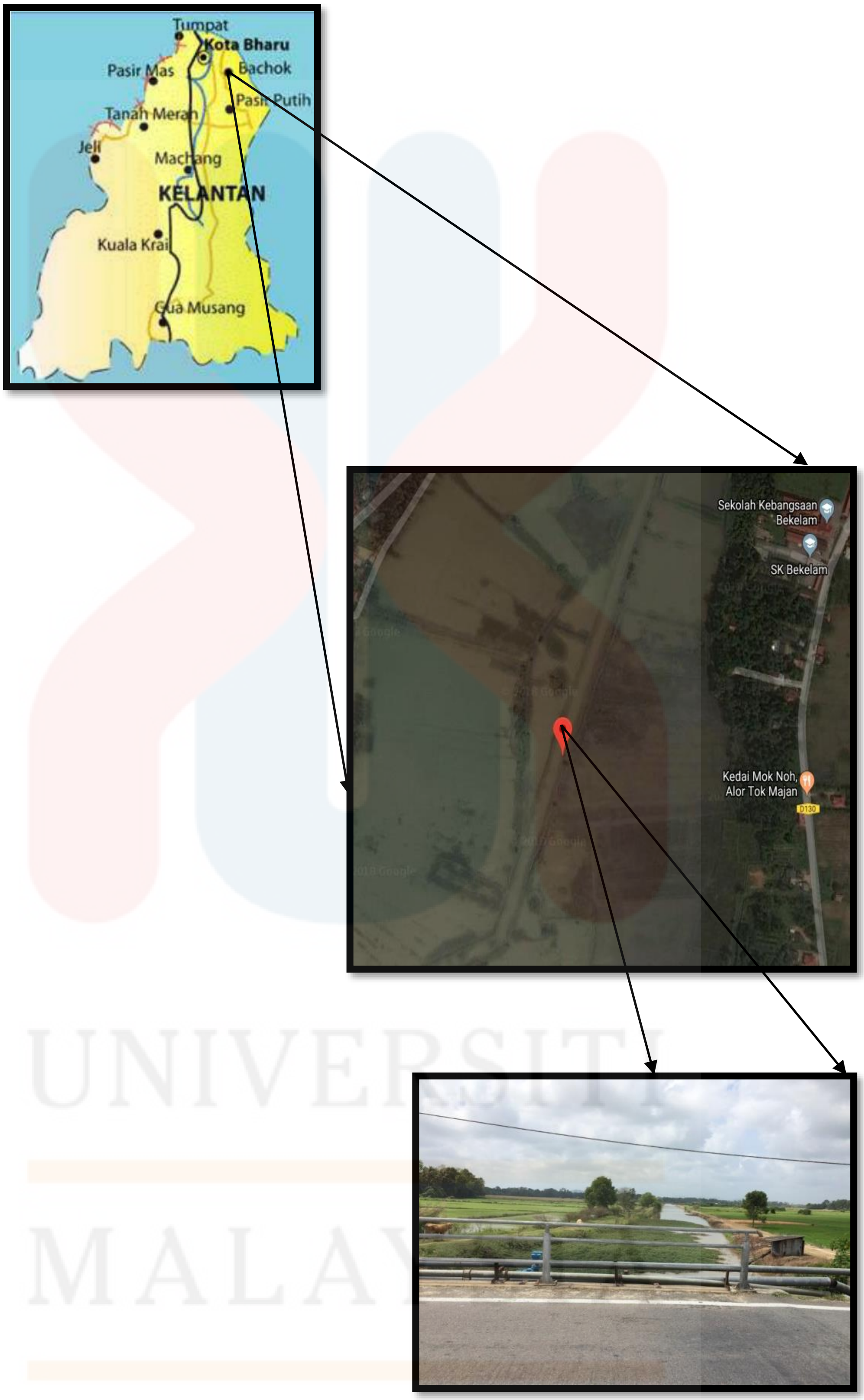





Figure 3.1: Sampling location of rice-paddy eel, *monopterus albus*, and water in paddy fields in Kg Bekelam, Bachok, Kelantan.

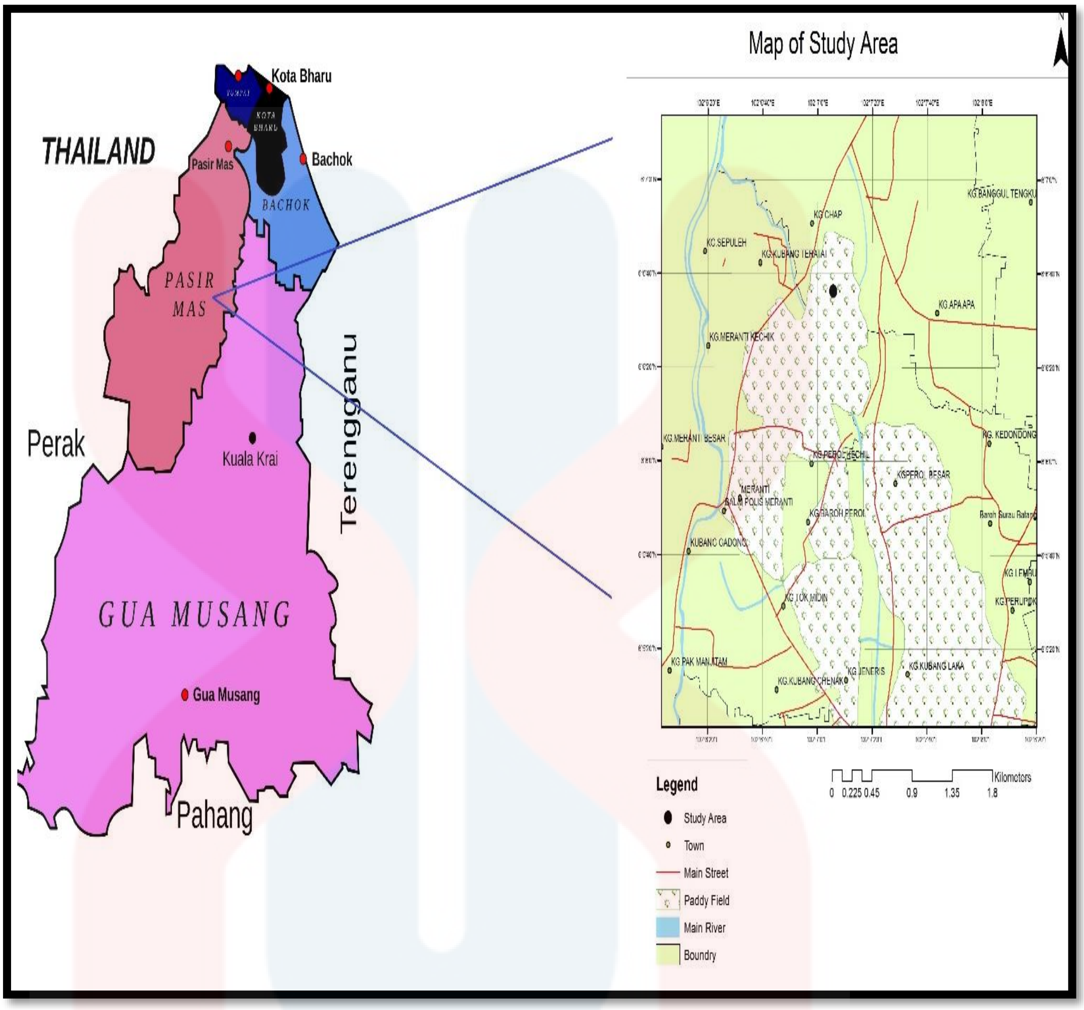



Figure 3.2: Sampling location of rice-paddy eel, *monopterus albus*, and water in paddy fields in Pasir Mas, Kelantan.

## 3.2 Sample collection

The sampling of water and rice-paddy eel was carried out on March, April, May and June, throughout the year. There were 10 points of eel and water samples with three replicates at each point for water samples respectively. Table 3.2 below shows the analysis of sample collection.

**Table3.2**: Analysis of sample collection

| MONTH | FREQUENCY | LOCATION | POINT | EEL QUANTITY | GPS |
|---|---|---|---|---|---|
| March | 2X | Bekelam and Pasir Mas | 10 | | N06º02'30.9" E102º21'28.9" N06º6'36.2484" E102º7'5.4876" |
| Apr | 2X | Bekelam and Pasir Mas | 10 | | N06º02'30.9" E102º21'28.9" N06º6'36.2484" E102º7'5.4876" |
| May | 2X | Bekelam and Pasir Mas | 10 | | N06º02'30.9" E102º21'28.9" N06º6'36.2484" E102º7'5.4876" |
| June | 2X | Bekelam and Pasir Mas | 10 | | N06º02'30.9" E102º21'28.9" N06º6'36.2484" E102º7'5.4876" |

### 3.2.1 Paddy water

The water samples were collected using 500ml washed polypropylene bottle and were securely labelled also with a unique sample number and then the water samples were directly filtered and acidified with hydrochloric acid (HCl) for the purpose to preserve most trace metal and reduce the microbial activity.

### 3.2.2 Biological sample (rice paddy eel)

The eel, *monopterus albus* were collected using a famous tool used by Malaysian farmer, known as *tukil*. *Tukil* is a cylindrical long tube made from PVC with several holes at the bottom and a cone bamboo on top of it. Small amounts of baits usually smoked fish were inserted into the tool to attract the eel. This *tukil* were placed at certain points in the paddy field area and let it sat overnight and were collected on the next day. The eel was placed in rice sack bag as the rice sack bag will help the eel to have air circulation in order to keep it alive rather than plastic bag. All the samples were securely labelled and placed for further analysis.

**Figure 3.3: The example of *tukil***

**Figure 3.4: Cone bamboo that was put on top of the tukil**

### 3.3 Water sample preparation

The water samples were in-situ preserved by using 37% concentrated hydrochloric acid (HCl). The water sample from 500ml washed polypropylene bottle were filtered and transferred into 50ml Falcon tube. Hydrochloric acid (HCl) was added to the sample until pH<2 to avoid the microbial activity. Then, the water samples were preserved in the 4$^{o}$C chiller. The filtered and acidified water sample in 50ml Falcon tube were diluted through dilution process. Five of the 15ml Falcon tubes were needed. One of them was filled with 15ml water sample as a control for analysis. Then, each four of 15ml Falcon tube were filled with 13.5ml of distilled water respectively and the tube were labelled as 1, 2, 3 and 4 for four dilutions. 1.5ml from the water sample stock was transferred into first Falcon tube and mix homogenously with the distilled water. Then, 1.5ml from first (1) Falcon tube was transferred into the second tube and mix homogenously with the distilled water. 1.5ml from second (2) Falcon tube was transferred into the third Falcon tube and mix homogenously with the distilled water. Lastly, 1.5ml from the third (3) Falcon tube then was transferred into the last tube, (4). Then, the diluted water samples were stored in the chiller for further analysis using AAS. Diagram 3.1 below shows the dilution process of the water sample.

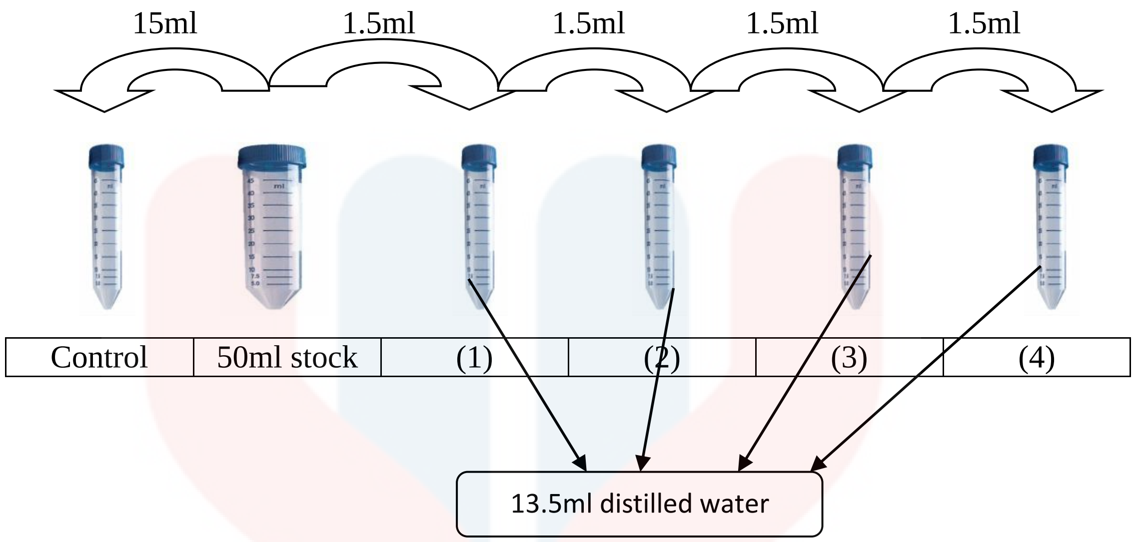



**Diagram 3.1: Dilution process of the water sample**

## 3.4 Eel sample preparation

Frozen eel were let to defrost overnight in the freezer thus it will be convenience to dissect the eel on the next day. The length and weight of the eel were recorded before dissection. The rice paddy eel was rinsed several times with distilled water before the dissection took place. All parts or organs of the eel were dissected which are heart, liver, oesophagus and stomach contain, gonad, kidney, bone, muscle, skin, and gill and each of the organ were weighed. The eels were dissected using dissecting set with highly safety awareness. After that, the organs were placed on small-sized folded aluminium folds and securely labelled. The sample then were dried in oven at least 72 hours at $60^{o}$c. Subsequent to the drying process; the samples were ground into fine powder using pestle and mortar (Min *et al.*, 2011). Figure 3.5 and figure 3.6 shows the dissecting process of the eel and also the small size folded aluminium foils that were used to place the eels' organs respectively.

**Figure 3.5: Dissecting process of the eel**

**Figure 3.6: Small size of aluminium foils that will be used to place the eels' organs**

## 3.5 Eel digestion method

First and foremost, each organ of the eels was weighed. 1 gram of each organ was put into the beaker. 10ml of concentrated nitric acid ($HNO_3$) (69%) were added into the samples and heated slowly at low temperature (40°C) for an hour. Then, after one hour, the temperature was increase up to 80°C on second hour. Later, the temperature was increased at 100°C on the third hour and lastly the temperature was increased to 140°C on the fourth hour which made the total of four hours heating on the hot plates. Furthermore, after getting a clear solution, the digested samples were diluted with distilled water up to 40ml and cooled up for 15 minutes approximately. Next, the samples were filtered using Whatman No. 42 filter paper and also syringe filter and the filtrate were stored in the chiller (4°C) until metal determination is carried out using AAS.

## 3.6 Analysis of heavy metals using Atomic Absorption Spectrometer (AAS)

The concentration of Cu, Zn and Ni in the final solutions was determined by using the PinAAcle™ 900T type of Atomic Absorption Spectrometer (AAS). This machine weighs 141 kg with width 95.0 cm. Moreover, this PinAAcle 900T AAS model is controlled by the new Syngistix™ for AA Software and this machine is manufactured by Perkin Elmer Inc. (Perkin Elmer Inc., 2018). Furthermore, AAS is a spectro analytical procedure for the determination of heavy metal or the chemical elements using the absorption of optical radiation (light) by the free atoms in the gaseous states. Moreover, standard was required with known analytic content in order to get the relation between the measured absorbance and the analytic concentration and relies. Besides, in AAS analysis, the samples were heated by a hot flame until the element atomizes and the result was read out in the computer. Figure 3.7 below shows the AAS machine.

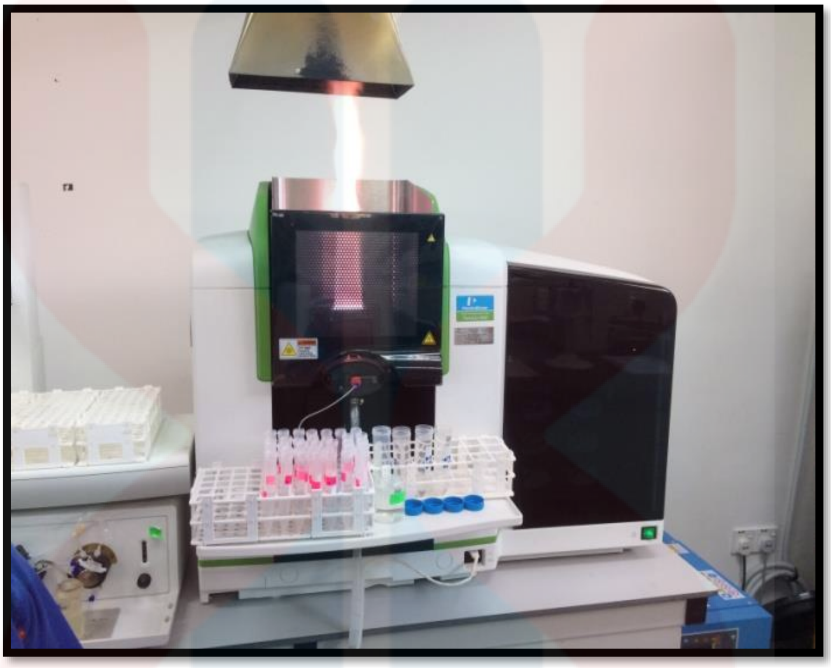


**Figure 3.7: The AAS machine**

### 3.7 Statistical analysis

The data was shown as mean ±SD (standard deviation) and Analysis of Variance (ANOVA, $p < 0.05$) was interpreted for association between pairs of variables identification.

### 3.8 Health Risk Assessment (HRA)

There are numbers of methods that have been used in order to estimate the potential health risk from heavy metal exposure. Common estimated daily intake (EDI) is frequently use to assess the limit of heavy metal consumption in human body (USEPA, 2000). If the calculated EDI are above the maximum level intake (MLI) of heavy metals for daily consumption, hence the possibility to cause adverse impacts in human health is concerning USEPA (2000). The model for estimation the EDI are:

$$EDI = DFC \ x \ MC \tag{3.1}$$

Where,

DFC = Daily food consumption (g) (6g/day)

MC = Mean metal concentration (µg/g)

### 3.9 Quality control

In order to avoid contamination, all glassware and equipment were acid-washed with 10% HCl first before used.

RESEARCH FLOW CHART

Figure 3.8 and Figure 3.9 are the research flowchart that summarize overall processes of the project, starting from the eel and water samples sampling until analysis using AAS machine.

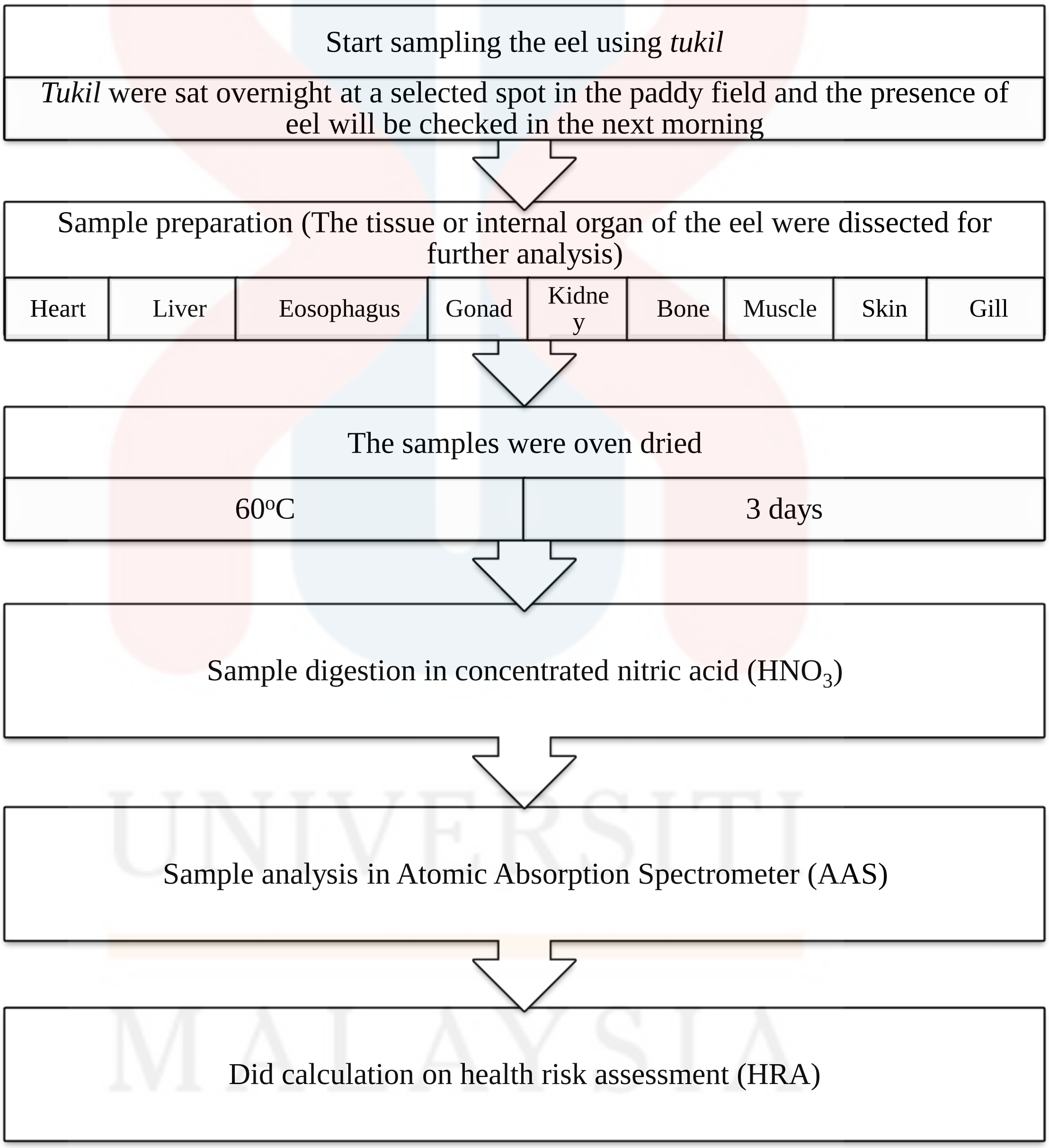



**Figure 3.8 Research flow chart for eel samples analysis**

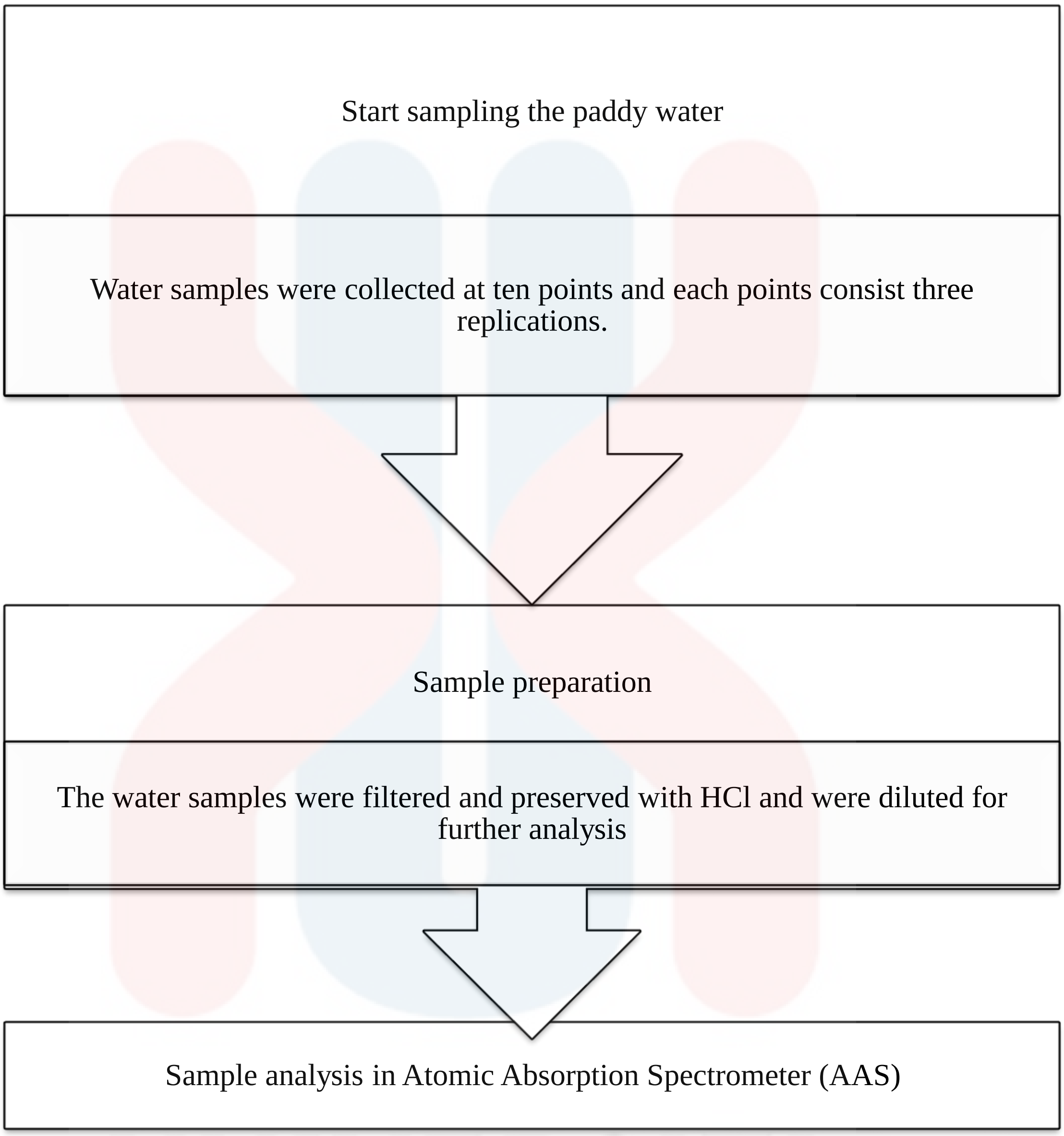



**Figure 3.9 Research flow chart for water samples analysis.**

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Result

### 4.1.1 Cu, Ni and Zn in water sample from Bachok and Pasir Mas, Kelantan.

The results obtained showed that the average concentration of the water samples in Bachok had the highest concentration of Cu (0.07 mg/L) followed by Zn (0.06 mg/L) and Ni (0.01 mg/L). Meanwhile, the result achieved in Pasir Mas showed that Ni (0.04 mg/L) had the highest concentration of heavy metal, followed by Cu (0.02 mg/L) and Zn (0.02 mg/L). Overall, the accumulation of heavy metals in water in Kg Bekelam, Bachok in sequence were Cu>Zn>Ni. On the other hands, the overall accumulation of heavy metals in Pasir Mas in sequence were Ni>Zn>Cu. Table 4.1 below shows the heavy metals concentration of Cu, Zn, Ni in Kg Bekelam, Bachok, and Pasir Mas, Kelantan.

**Table 4.1**: Heavy metals concentration (mg/L) with standard deviation (SD) of Cu, Zn and Ni in Bachok and Pasir Mas, Kelantan.

| Sampling month | Location | Cu | Ni | Zn |
|---|---|---|---|---|
| March | Bachok | 0 | 0.02 | 0.02 |
| | Pasir Mas | 0 | 0.04 | 0.05 |
| March | Bachok | 0.02 | 0.01 | 0.08 |
| | Pasir Mas | 0.01 | 0.04 | 0.02 |
| April | Bachok | 0.10 | 0 | 0.05 |

| | | | | |
|---|---|---|---|---|
| | Pasir Mas | 0 | 0.06 | 0.02 |
| May | Bachok | 0.14 | 0 | 0.07 |
| | Pasir Mas | 0.04 | 0.03 | 0.01 |
| June | Bachok | 0.09 | 0.04 | 0.06 |
| | Pasir Mas | 0.05 | 0.02 | 0.02 |
| **Mean concentration** | **Bachok** | **0.07±0.06** | **0.01±0.02** | **0.06±0.02** |
| | **Pasir Mas** | **0.02±0.02** | **0.04±0.02** | **0.02±0.01** |

### 4.1.2 Asian swamp eel, *Monopterus albus* in Kg Bekelam, Bachok, Kelantan.

Table 4.2 shows the heavy metal concentration (Cu, Zn and Ni) accumulation in Asian swamp eel organs collected in Kg Bekelam, Bachok, Kelantan in descending order. The result obtained show that stomach content (448.34 µg/g), (14.50 µg/g) accumulates the most heavy metals (Zn and Ni) compared to other organs. Meanwhile, liver had the highest accumulation of Cu metal.

**Table 4.2:** Heavy metal concentration of Asian swamp eel in Bachok, Kelantan in descending order

| Metals | Descending order sequences |
|---|---|
| Cu | Liver>Oesophagus>Heart>Skin>Muscle>Gonad>Kidney>Stomach contents>Gill>Bone |

| | |
|---|---|
| Zn | Stomach content>Heart>Gill>Bone>Kidney>Oesophagus>Skin>Liver>Gonad>Muscle |
| Ni | Stomach content>Liver>Kidney>Gonad>Gill>Oesophagus>Heart>Bone>Skin>Muscle |

Furthermore, lowest concentration of Cu (7.11 µg/g) was found in bone of the eels at Bachok. Besides, concentration of Zn (57.24 µg/g) and concentration of Ni (1.27 µg/g) were recorded lowest in muscle compared to other tissues in Asian swamp eel.

### 4.1.3 Asian swamp eel, *Monopterus albus* in Pasir Mas, Kelantan.

Table 4.3 shows the concentration of heavy metals (Cu, Zn and Ni) in Asian swamp eel in descending order. From the result attained, it shows that Cu accumulates highest in kidney (25.34 µg/g), Zn accumulates highest in liver (362.73 µg/g) and Ni (94.37 µg/g) accumulates highest in heart. Meanwhile, muscle recorded the lowest accumulation of Cu (1.22 µg/g), Zn (55.5 µg/g) and Ni (0.83 µg/g)

**Table 4.3:** Heavy metal concentration of Asian swamp eel in Pasir Mas, Kelantan in descending order

| Metals | Descending order sequences |
|---|---|
| Cu | Kidney>Heart>Liver>Gonad>Oesophagus>Stomach content>Skin>Gill>Bone>Muscle |

| | |
|---|---|
| Zn | Liver>Kidney>Stomach content>Gonad>Gill>Bone>Oesophagus>Heart>Skin>Muscle |
| Ni | Heart>Kidney>Liver>Gill>Gonad>Bone>Oesophagus>Stomach content>Skin>Muscle |

## 4.2 Discussion

### 4.2.1 Heavy metals content in paddy water from different cultivation area.

Water is known as universal solvent which uniquely vulnerable to pollution because of its ability to dissolve much substances than any other liquid on the earth (Denchak, 2018). Furthermore, Adebayo (2007) stated that the irrigation water led to the accumulation of heavy metals in aquatic organism that lives in the same area as it will bio magnified along the water (IA, 2007).

Furthermore, water pollution is derived from two types of sources that were known as point source (single source) for example chemical spill and non-point source (diffuse sources) such as agriculture (Denchak, 2018).

Cu, Zn and Ni metals are mainly discharged from electroplating and metalworking industries which in their effluent omnipresent in the water as a major problem to human health and aquatic life (Thakur, 2013). However, in this research, the study areas in Figure 3.1 for Bachok and Pasir Mas were far from the industrial area. Moreover, the usage of pesticide and herbicides is commonly used by the farmer at paddy field for the purpose of combating the fungi as it will bring damage to the crop. Besides, the fungicide and herbicide has high Zn, Ni and Cu content as these heavy metals are the best metals in order to withstand the fungi (Cezar, 1999). Thus, in this research the heavy metals accumulation in the water might be contributed from the chemicals input such as pesticide from the farmer.

In this study, Bachok had higher concentration of Zn and Cu. However, Pasir Mas had higher concentration of Ni. The comparison data of heavy metal concentration in water from Bachok and Pasir Mas are shown in table 4.4. The possibility that led to different result of heavy metals concentration accumulation was demographic area. The paddy field at Bachok was located at nearby residential area and not so far from the road. Thus, apart from the pesticides, herbicides and fertilizers that were used by farmers, the heavy metals influent might come from the domestic waste that is contributed from the residential. Meanwhile, the paddy field at Pasir Mas was more rural and far from residential area. Hence, the concentration of heavy metals influence in the water was lower than Bachok as there were little pollutants sources.

**Table 4.4**: Mean concentration mg/L with standard deviations (SD) in water from Bachok and Pasir Mas, Kelantan.

| Locations | Zn | Cu | Ni |
|---|---|---|---|
| Bachok | 0.06±0.02 | 0.07±0.06 | 0.01±0.02 |
| Pasir Mas | 0.02±0.01 | 0.02±0.02 | 0.04±0.02 |

### 4.2.2 Heavy metals content in different part of organs of Asian swamp eel, *Monopterus albus.*

Fish is one marvellous source that rich with high quality proteins and omega-3 fatty acids that is required for the growth of tissues in organism. In addition, fish also has numerous of essential minerals and vitamins (Chu, 2015). However, consuming fish nowadays has growing concerns towards consumers as fish could have high intake of heavy metals accumulation from water or sediments (Pooter, 2018).

Generally, the accumulation of heavy metals in fish occurs because of the continuous flow of water through gills and skin and hence, fish are continuously exposed to waterborne and particulate heavy metals (Fatima & Usmani, 2013). According to Mahino Fatima (2014), the heavy metals could accumulate in the fish through metal ion exchange from the gills and skin, ingestion of particles or food and also suspended particulate matter. In addition, blood then will transport the heavy metal ions to other tissues (Mahino Fatima, 2014). Moreover, one of the pathways of heavy metal accumulation in human body is the consumption of fish. Hence, excessive accumulation of dietary heavy metals such as Cu, Zn and Ni lead to acute health problems such as cancer and other detrimental damages to the tissues (Adeel Mahmood, 2008) . Therefore, from the result it shows that there are heavy metals (Cu, Ni and Zn) were exist in the tissues of the fish and hence could potentially give adverse impacts for consumption at certain level.

### 4.2.3 Variations in organs ability to accumulate metals

In this study, liver, kidney and stomach content of Asian swamp eels have the highest accumulation of Cu and Zn and became the target organ of accumulation for heavy metals in both study areas. The plot of the mean heavy metal concentration score for each combination of groups of organs and heavy metals are plotted in a line graph, as shown in Appendix 3. Table 4.7 below show the mean concentration and its standard error of heavy metals (Cu, Zn and Ni) in Pasir Mas and Bachok. Besides, a lot of studies had been conducted reported that pesticides will have high accumulation in liver, kidney, stomach content and gonad (Stoner, 2017) Cu and Zn are the essential heavy metals that are needed for survival as it will form a healthy tissues and organs and control metabolism (Tremblay, 2018). Thus, target organs such as gonads, liver, kidney, gill and intestines that are known as metabolically active tissues will accumulates high level of heavy metals (Mohamed, 2014). Meanwhile, Ni concentration was recorded accumulates the highest in stomach content and heart in the eels from both study areas respectively. Aside from Cadmium (Cd) and Lead (Pb), Ni is one of the non-essentials heavy metals that are needed for metabolic processes and hence, these non-essential metals accumulates higher in brain, bone, heart and blood (Crafford, 2010). Ni also metal that is common to be found in the animal tissues at low concentrations. However, it is toxic at higher level of concentration (Tchonwou, Yedjou & Patlolla, 2012).

Moreover, from the result obtained shows that Zn element was the richest in all of the eels' organs collected in both sites, Pasir Mas and Bachok. Zn is one of the essential elements required for numerous protein structure and also catalytic function and also plays critical roles in many cellular processes such as immune function, enzymatic activity, DNA repair and others (Mudipalli, Anuradha & Zelikoff, 2017). Zn element cannot be produced naturally within an organism. Therefore, Zn need to be obtained through diet such as consuming meat, seafood, dairy products, beans or dietary supplements (Guynn, 2017). However, very large doses of the Zn element could be toxic to both humans and organisms which can cause damage to tissue and damage to DNA (Pooter, 2018).

Furthermore, many studies recorded that muscle always had the lowest concentration of heavy metals. The study conducted by Sow Ai Yin (2016) showed that many heavy metals such as Cd, Pb, Zn, Cu and Ni concentration were found lowest in muscle which indicates the richness of contractile proteins presents in the tissues (Yin, 2016). In this study, most of heavy metals concentration (Cu, Zn and Ni) were found lowest in muscle both at Bachok and Pasir Mas.

The two-way *ANOVA* table (table 4.5) below shows the independent variables (Heavy_Metals*Organ) and their interaction (Heavy_Metal) have statistically significant effect on the dependent variable (concentration of heavy metal). Overall, the result obtains appeared the accumulation of heavy metals in the tissues was significant ($p<0.05$). It indicates that the heavy metals do accumulate in the tissues and hence proving that fish are one of the potential organisms to have uptake on heavy metals. Moreover, the mean concentration of heavy metals and its standard error in Bachok and Pasir Mas was shown in table 4.6

**Table 4.5:**Tests of Between-Subjects Effects

Dependent Variable: Concentration

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 424867.142[a] | 30 | 14162.238 | 8.499 | .000 |
| Intercept | 210408.873 | 1 | 210408.873 | 126.269 | .000 |
| Heavy_Metals * Organ | 104013.802 | 18 | 5778.545 | 3.468 | .001 |
| Heavy_Metals | 261914.080 | 2 | 130957.040 | 78.589 | .000 |
| Organ | 58043.896 | 9 | 6449.322 | 3.870 | .003 |
| Place | 895.365 | 1 | 895.365 | .537 | .469 |
| Error | 48324.347 | 29 | 1666.357 | | |
| Total | 683600.363 | 60 | | | |
| Corrected Total | 473191.489 | 59 | | | |

a. R Squared = .898 (Adjusted R Squared = .792)

**Table 4.6:** Mean concentration of heavy metals and its standard error in Bachok and Pasir Mas.

Dependent Variable: Concentration

| Heavy_Metals | Organ | Place | Mean | Std. Error |
|---|---|---|---|---|
| Cu | Heart | Bachok | 18.960 | 29.342 |
| | | Pasir Mas | 11.235 | 29.342 |
| | Kidney | Bachok | 20.038 | 29.342 |
| | | Pasir Mas | 12.312 | 29.342 |
| | Skin | Bachok | 12.349 | 29.342 |
| | | Pasir Mas | 4.623 | 29.342 |
| | Muscle | Bachok | 9.567 | 29.342 |
| | | Pasir Mas | 1.841 | 29.342 |
| | Stomach content | Bachok | 12.020 | 29.342 |
| | | Pasir Mas | 4.294 | 29.342 |
| | Oesophagus | Bachok | 19.536 | 29.342 |
| | | Pasir Mas | 11.810 | 29.342 |
| | Liver | Bachok | 30.980 | 29.342 |

| | | | | |
|---|---|---|---|---|
| | | Pasir Mas | 23.254 | 29.342 |
| | Gonad | Bachok | 15.735 | 29.342 |
| | | Pasir Mas | 8.009 | 29.342 |
| | Bone | Bachok | 9.441 | 29.342 |
| | | Pasir Mas | 1.715 | 29.342 |
| | Gill | Bachok | 10.053 | 29.342 |
| | | Pasir Mas | 2.327 | 29.342 |
| Ni | Heart | Bachok | 58.190 | 29.342 |
| | | Pasir Mas | 50.464 | 29.342 |
| | Kidney | Bachok | 27.479 | 29.342 |
| | | Pasir Mas | 19.754 | 29.342 |
| | Skin | Bachok | 7.978 | 29.342 |
| | | Pasir Mas | .252 | 29.342 |
| | Muscle | Bachok | 4.916 | 29.342 |
| | | Pasir Mas | -2.810 | 29.342 |
| | Stomach content | Bachok | 13.914 | 29.342 |
| | | Pasir Mas | 6.188 | 29.342 |
| | Oesophagus | Bachok | 8.730 | 29.342 |
| | | Pasir Mas | 1.004 | 29.342 |

| | | | | |
|---|---|---|---|---|
| | Liver | Bachok | 12.137 | 29.342 |
| | | Pasir Mas | 4.411 | 29.342 |
| | Gonad | Bachok | 10.086 | 29.342 |
| | | Pasir Mas | 2.360 | 29.342 |
| | Bone | Bachok | 11.709 | 29.342 |
| | | Pasir Mas | 3.983 | 29.342 |
| | Gill | Bachok | 13.456 | 29.342 |
| | | Pasir Mas | 5.730 | 29.342 |
| Zn | Heart | Bachok | 109.800 | 29.342 |
| | | Pasir Mas | 102.074 | 29.342 |
| | Kidney | Bachok | 169.784 | 29.342 |
| | | Pasir Mas | 162.058 | 29.342 |
| | Skin | Bachok | 78.875 | 29.342 |
| | | Pasir Mas | 71.149 | 29.342 |
| | Muscle | Bachok | 60.059 | 29.342 |
| | | Pasir Mas | 52.333 | 29.342 |
| | Stomach content | Bachok | 306.317 | 29.342 |
| | | Pasir Mas | 298.591 | 29.342 |
| | Oesophagus | Bachok | 116.382 | 29.342 |

| | | | | |
|---|---|---|---|---|
| | | Pasir Mas | 108.656 | 29.342 |
| | Liver | Bachok | 339.947 | 29.342 |
| | | Pasir Mas | 332.221 | 29.342 |
| | Gonad | Bachok | 141.238 | 29.342 |
| | | Pasir Mas | 133.512 | 29.342 |
| | Bone | Bachok | 109.058 | 29.342 |
| | | Pasir Mas | 101.332 | 29.342 |
| | Gill | Bachok | 133.706 | 29.342 |
| | | Pasir Mas | 125.980 | 29.342 |

## 4.3 Guidelines and human consumptions

The levels of Cu, Zn and Ni in paddy water in both study area, Bachok and Pasir Mas were complied with the permissible limit that sets by Class IV, National Water Quality Standards for Malaysia (standard for irrigation water) (Wepa, 2006). Table 4.7 below shows the comparison of heavy metals concentration in paddy water at both sites using standards. Table 4.8 below shows the comparison of heavy metal concentrations in the edible parts (muscle and skin) of the Asian swamp eel at Bachok and Pasir Mas with other guidelines and study

**Table 4.7**: Comparison of heavy metals concentration in paddy water at both sites using standards.

| **Sample** | **Standard** | **Cu** | **Zn** | **Ni** |
|---|---|---|---|---|
| Water | Class IV, National Water Quality Standards for Malaysia (standard for irrigation water) (mg/L) | 0.2 | 2.0 | 0.2 |
| | **Location** | | | |
| | Bachok | 0.07 | 0.06 | 0.01 |
| | Pasir Mas | 0.02 | 0.02 | 0.04 |

**Table 4.8:** Comparison of heavy metal concentrations in the edible parts (muscle and skin) of the Asian swamp eel at Bachok and Pasir Mas with other guidelines and study.

| Description | Elements (µg/g) | | | | References |
|---|---|---|---|---|---|
| | **Tissue** | **Cu** | **Zn** | **Ni** | |
| Permissible limits of heavy metals set by Malaysian Food Regulation (1985) (µg/g) | | 30.0 | 100.00 | NA | (Regulations, 1985) |
| Permissible level set by FAO/WHO (µg/g) | | 30 | 100 | 0.05 | (Yunus*, 2014) |
| Heavy metal uptake by Asian swamp eel from paddy fields of Kelantan (other research) | Muscle<br>Skin | 0.84<br>1.13 | 59.31<br>69.34 | 20.84<br>22.98 | (Yin, 2016) |
| Bachok | Muscle<br>Skin | 10.19<br>11.27 | 57.24<br>80.52 | 1.27<br>5.28 | This study |
| Pasir Mas | Muscle<br>Skin | 1.22<br>5.70 | 55.15<br>69.50 | 0.83<br>4.95 | This study |

Overall, from the table of comparison above, this study obtains that, Cu and Zn in both study areas are within the permissible limits set by the guidelines. However, Ni was slightly beyond the safety limits for both sites. This might be caused of the usage of herbicide, fungicides, pesticides and fertilizers by the farmers for the purposed of withstanding the snails and other pests from damaging the crops (Christopher Didigwu Nwani & Naresh Sahebrao Nagpure, 2010). This indicates that, the edible parts of Asian swamp eel in Bachok and Pasir Mas are potentially to cause toxicity for the consumers.

In addition, although Ni concentration in Bachok and Pasir Mas were above safe limits set by the guideline, the accumulation of metals in edible parts of the eel based on estimated daily intake (EDI) (Equation 3.1) were calculated; in Bachok, Cu (128.76 µg/day), Ni (39.3 µg/day) and Zn (826.56 µg/day); in Pasir Mas Cu (41.52 µg/day), Ni (34.68 µg/day) and Zn (747.9 µg/day) were lower compared to the maximum level intake (MLI). Therefore, the Asian swamp eel located at cultivation area in Bachok and Pasir Mas were safe to consume.  Hence, table 4.9 below show the comparison of the estimated daily intake of edible tissue (muscle and skin) of Asian Swamp eel with the recommended metal intake values (µg/day).

**Table 4.9**: Comparison of the estimated daily intake of edible tissue (muscle and skin) of Asian Swamp eel with the recommended metal intake values (µg/day).

| **Location** | **Elements** | **EDI (muscle)** | **EDI (skin)** | **EDI (total)** | **MLI** |
|---|---|---|---|---|---|
| Bachok | Cu | 61.14 | 67.62 | 128.76 | 3200 |
| | Ni | 7.62 | 31.68 | 39.3 | 450 |
| | Zn | 343.44 | 483.12 | 826.56 | 17000 |
| Pasir Mas | Cu | 7.32 | 34.2 | 41.52 | 3200 |
| | Ni | 4.98 | 29.7 | 34.68 | 450 |
| | Zn | 330.9 | 417 | 747.9 | 17000 |

*Notes: EDI: Estimated daily intake, MLI: Maximum level intake without detriment to health* (Yin, 2016)

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

High accumulation of heavy metals such as Cu, Ni and Zn in water will lead to high accumulation of heavy metals in aquatic organism. This is because, the heavy metals in the water biomagnified in the organism as it lives in the water (Chen et al., 2000). Practically, Bachok accumulates high Cu and Zn concentration compared to Pasir Mas. However, Pasir Mas accumulates higher Ni concentration than Bachok. Meanwhile, the quality of water in cultivation area complied with Class IV, National Water Quality Standards for Malaysia (standard for irrigation water), where in general, all the measured elements were under permissible limits for aquatic consumption. Thus, the investigation indicates that all heavy metals analysed in water at cultivation area in both site is safe and complied with the Class IV, National Water Quality Standard for Malaysia. Hence, the possibility of the aquatic living organism to have high accumulation of heavy metals could be low.

Overall, the highest concentration of Zn in all organs of Asian swamp eel collected in Bachok and Pasir Mas. Meanwhile, Cu concentration was found highest in the liver of Asian swamp eel at both study areas and Ni concentration was found highest in the bone of the eel also at both study areas. Besides, Cu and Zn in the edible parts of the Asian swamp eel (muscle and skin) were under permissible limit of the guidelines from Malaysia Food Regulations (1985), Food and Agriculture Organization (FAO)/ World Health Organization (WHO) but Ni concentration in both sites are slightly above the safe limits. However, based on the comparison of calculated EDI with MLI, all metals in edible tissues (muscle and skin) were under safety limits to be consumed.

## 5.2 Recommendation

Asian swamp eel, *Monopterus albus* is one of delicacies that people love to eat. However, nowadays consuming the fish from paddy field is quiet concerning as there are high possibilities of heavy metals intake from chemical input such as fertilizers, pesticides and also herbicides that would pollute the area. Therefore, the farmer should cut the usage of pesticides, herbicides or fertilizers into the paddy field and hence, the organisms that live in the surrounding would not have high uptake of heavy metals from the chemical input.

Moreover, in order to have much information on heavy metals intake in the organisms, some recommendation for the future research is;

i. The study should have more than one supporting data so that the heavy metal in organism could be trace in more than one condition, for example determination of heavy metals in soil sample as well. In this research, there is only one supporting data which is water. Thus, there is limitation to trace the heavy metals intake source.

ii. The sampling of the Asian swamp eel could have been done throughout the paddy seasons. There are four seasons of the paddy which are ploughing, seedling, growing and harvesting. Thus, it is believed there is a different type of chemicals input such as fertilizers, pesticides and herbicides in each seasons. Hence, the examination of different heavy metals concentration could be more accurate.

## REFERENCES


Abdul Rahman, Nur Azhani and Omar, M. A. (2012). Detection of organochlorine compound in puyu, sepat and haruan fish caught from irrigation canals in paddy fields in Selangor and Perak, Malay, 29–33. Retrieved from http://psasir.upm.edu.my/26776/

Adeel Mahmood, R. N. M. and K. (2008). Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian Journal of Chemistry*, *7*(1), 91–99.

Agency for Toxic Substances and Disease Registry (ATSDR). (2005). Public Health Statement for Zinc. Retrieved from https://www.atsdr.cdc.gov/phs/phs.asp?id=300&tid=54

Alloway, B. J. (1990). Heavy Metals in Soils. *Journal of Encapsulation and Adsorption Sciences*, *4*, 100–124. Retrieved from http://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=1286386

Amiard-Triquet C., A. J. C. A. A. C. E. P. M. C. (1987). The eel (Anguilla Anguilla L.) as a bioindicator of metal pollution: Factors limiting its use. *Water Science and Technology*, *19*(7), 1229–1232. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-0023626664&partnerID=40&md5=d80aa71bb20f2a998b5cd3e1b56a3474

Arai, R. F., , Chino, N., & and Ismail, A. (2012). Heavy Metal Concentration in Tropical Eel, Anguilla bicolor bicolor in Peninsular Malaysia, Malaysia, *41*(1), 43–46. Retrieved from http://journalarticle.ukm.my/7423/1/41_1_6.pdf

Arantes, F. P., Savalssi, L. A., Santos, H. B., Gomes, M. V. T., & Bazzoli, and N. (2015). Bioaccumulation of mercury, cadmium, zinc, chromium, and lead in muscle, liver, and spleen tissues of a large commercially valuable catfi sh species from Brazil, *88*(1), 137–147. Retrieved from http://www.scielo.br/pdf/aabc/v88n1/0001-3765-aabc-20140434.pdf

Besada, V., Gonzalez, J. J., & Schultze, F. (2006). Mercury, cadmium, lead, arsenic, copper and zinc concentrations in albacore, yellowfin tuna and bigeye tuna from the Atlantic Ocean. *Ciencias Marinas*, *32*(2B), 439–445. https://doi.org/10.7773/cm.v32i22.1083

Cezar, P. (1999). Effects of zinc fungicides and different zinc fertilizer application methods on soluble and total zinc in potato plant shoots. *Communications in Soil Science and Plant Analysis*, *30*(13–14), 1847–1859. Retrieved from https://www.researchgate.net/publication/249073177_Effects_of_zinc_fungicides_and_different_zinc_fertilizer_application_methods_on_soluble_and_total_zinc_in_potato_plant_shoots

Chen, C. Y., Stemberger, R. S., Klaue, B., Blum, J. D., Pickhardt, P. C., & Folt, C. L. (2000). Accumulation of heavy metals in food web components across a gradient of lakes. *Limnology and Oceanography*, *45*(7), 1525–1536. https://doi.org/10.4319/lo.2000.45.7.1525

Christopher Didigwu Nwani and Naresh Sahebrao Nagpure. (2010). Lethal concentration and toxicity stress of Carbosulfan, Glyphosate and Atrazine to freshwater air breathing fish Channa punctatus (Bloch), *2*, 105–111. Retrieved from https://www.researchgate.net/publication/258932118_Lethal_concentration_and_toxicity_stress_of_Carbosulfan_Glyphosate_and_Atrazine_to_freshwater_air_breathing_fish_Channa_punctatus_Bloch_Christopher

Chu, J. (2015). Health Benefits of Fish Consumption, (108). Retrieved from https://www.cfs.gov.hk/english/multimedia/multimedia_pub/multimedia_pub_fsf_108_01.html

D Crafford, A. A.-O. (2010). Bioaccumulation of non-essential trace metals in tissues and organs of Clarias gariepinus (sharptooth catfish) from the Vaal River system – strontium, aluminium, lead and nickelNo Title, *vol 36*(no 5). Retrieved from https://www.ajol.info/index.php/wsa/article/view/61996

Denchak, M. (2018). Water Pollution: Everything You Need to Know. Retrieved from https://www.nrdc.org/stories/water-pollution-everything-you-need-know

Department of health. (2001). Food Ration Scales for Hospitals and Health Institutions. Food Service Management and Administration, 3. Retrieved from https://policyresearch.limpopo.gov.za/bitstream/handle/123456789/874/Foodration scales for hospitals v3.pdf

Desai, V., & Kaler, S. G. (2008). Role of copper in human neurological disorders. In *American Journal of Clinical Nutrition* (Vol. 88). https://doi.org/88/3/855S [pii]

Edward. (2015). Metal Toxicity: Health Dangers of Nickel. Retrieved from https://www.globalhealingcenter.com/natural-health/metal-toxicity-health-dangers-nickel/

Duffus, J. H. (2002). "Heavy metals." *Chemistry and Human Health Division Clinical Chemistry Section, Commision on Toxicology*, *74*(5), 793–807. Retrieved from https://www.iupac.org/publications/pac/74/5/0793/

Environmental Protection Agency (EPA). (2013). Copper in Drinking Water Health Effects and How to Reduce Exposure. Retrieved from http://www.health.state.mn.us/divs/eh/water/factsheet/com/copper.html

Guynn, J. (2017). Zn and Zn dependent protein in cancer and chemotherapeutics. Reyrieved from https://www.springer.com/kr/book/9783319554464

IA, A. (2007). Determination of Heavy Metals in Water, Fish and Sediment from Ureje Water Reservoir. *Environmental & Analytical Toxicology*, *7*(4). Retreived from https://www.omicsonline.org/open-access/determination-of-heavy-metals-in-water-fish-and-sediment-from-urejewater-reservoir-2161-0525-1000486.php?aid=91799

Inc., P. (2018). PinAAcle 900T Atomic Absorption Spectrometer. Retrieved from http://www.perkinelmer.com/product/pinaacle-900t-atomic-absorption-spectrometer-pinaacle900t

Jalal, K. C. A., John, B. A., Habab, M., Mohd, A. Y., & Kamaruzzaman, B. Y. (2013). Bioaccumulation of selected metals in fresh water Haruan fish (Channa striatus) collected from Pahang River Basin, Malaysia. *Oriental Journal of Chemistry*, *29*(4), 1553–1558. https://doi.org/10.13005/ojc/290436

Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*. https://doi.org/10.1093/bmb/ldg032

Kumagai Akiko, Ryoko Ando, Hideyuki Miyatake, P. G. (2013). A Bilirubin-Inducible Fluorescent Protein from Eel Muscle, *153*(7), 1602–1611. Retrieved from http://www.cell.com/abstract/S0092-8674(13)00644-2

Landrigan, P. J., & Goldman, L. R. (2011). Children's vulnerability to toxic chemicals: A challenge and opportunity to strengthen health and environmental policy. *Health Affairs*, *30*(5), 842–850. https://doi.org/10.1377/hlthaff.2011.0151

Mahino Fatima, N. U. and M. M. H. (2014). Heavy Metal in Aquatic Ecosystem Emphasizing its Effect on Tissue Bioaccumulation and Histopathology: A Review. *Environmental Science and Technology*, *7*(1–15). Retrieved from

https://scialert.net/abstract/?doi=jest.2014.1.15
Mohamed, H. . E. (2014). Bioaccumulation of some heavy metals in tissue and head of commercial nile fish in Sudan. *Internatiional Journal of Aquaculture*, *vol 4*(No.20), 118–122. Retrieved from https://www.researchgate.net/publication/280768938_Bioaccumulation_of_Some_Heavy_Metals_in_Tissues_and_Head_of_Commercial_Nile_Fish_in_Sudan
Mudipalli, Anuradha, Zelikoff, J. T. (Eds. . (2017). *Essential and Non-essential Metals*. Retrived from https://www.springer.com/us/book/9783319554464
Noor Azhar M. Shazili, Kamaruzaman Yunus, Ahmad Shamsudin Ahmad, & Norantonina Abdullah, and M. K. A. R. (2006). Heavy metal pollution status in the Malaysian aquatic environment, *9*(2), 137–145. Retrieved from http://www.academia.edu/1519753/Shazili_NAM_Kamaruzzaman_B.Y._Ahmad_S_Rashid_MKA._2006_Heavy_metal_pollution_status_in_the_aquatic_environment_of_Malaysia._Aquatic_Ecosystem_Health_and_Management_Journal_9_2_137-145
Olsson PE., Kling P., Hogstrand C. (1998) Mechanisms of heavy metal accumulation and toxicity in fish. In: Langston W.J., Bebianno M.J. (eds) Metal Metabolism in Aquatic Environments. Springer, Boston, MAPooter, D. De. (2018). Heavy metals. Retrieved from https://doi.org/10.1007/978-1-4757-2761-6_10
Reena Singh, Neetu Gautam, Anurag Mishra, and R. G. (2011). Heavy metals and living systems. *Indian Journal of Pharmacology*, *43*(3), 246–253. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3113373/
Regulations, M. F. (1985). Food Regulations 1985. Retrieved from https://extranet.who.int/nutrition/gina/sites/default/files/MYS%201985%20Food%20Regulations_0.pdf
Roslaili Abdul Aziza, S. A. R. (2015). Speciation and Availability of Heavy Metals On Serpentinized Paddy Soil and Paddy Tissue, *195*, 1658–1665. Retrieved from https://www.sciencedirect.com/science/article/pii/S1877042815037143
Safety, E. H. and. (2002). Toxicology and exposure guidelines. Retrieved from http://ehs.unl.edu/
Siobhan O Brian, D. J. H. and A. B. (2014). Social evolution of toxic metal bioremediation, *281*(1787). Retrieved from http://rspb.royalsocietypublishing.org/content/281/1787/20140858
Stoner, H. B. (2017). Toxicity of Triphenyltin, *23*(222). Retrieved from http://oem.bmj.com/
Swanson Al, W. J. and W. Y. (2012). Accurate and efficient bypass of 8,5’-cyclopurine-2’-deoxynucleosides by human and yeast DNA polymerase No Title. *Hem Res Toxicol*, *25*(8). Retrieved from https://www.yeastgenome.org/reference/S000150157#overview
Tchonwou PB, Yedjou CG, Patlolla AK, S. D. (2012). Heavy metal toxicity and the environment., *101*.
Thakur, L. S. (2013). Heavy metal Cu, Ni and Zn: Toxicity, health hazards and their removal techniques by low cost adsorbents. *International Journal of Plant Sciences*. Retrieved from https://www.researchgate.net/publication/285384234_Heavy_Metal_Removal_from_Wastewater_Using_Low_Cost_Adsorbents
Tremblay,, Sylvie. (2018) "The Effects of Zinc & Copper on Your Metabolism." Healthy Eating | SF Gate, http://healthyeating.sfgate.com/effects-zinc-copper-metabolism-6505.html. 06 December 2018.
Vig, K., Megharaj, M., Sethunathan, N., & Naidu, R. (2003). Bioavailability and

toxicity of cadmium to microorganisms and their activities in soil: A review. *Advances in Environmental Research*. https://doi.org/10.1016/S1093-0191(02)00135-1

Wepa. (2006). National water quality standrds.

Yin, S. A. (2016). *Bioaccumulation of heavy metals and metallothionein effects on reproductive biology of Asian swamp eel in paddy fields in Tumpat, Kelantan, Malaysia*.

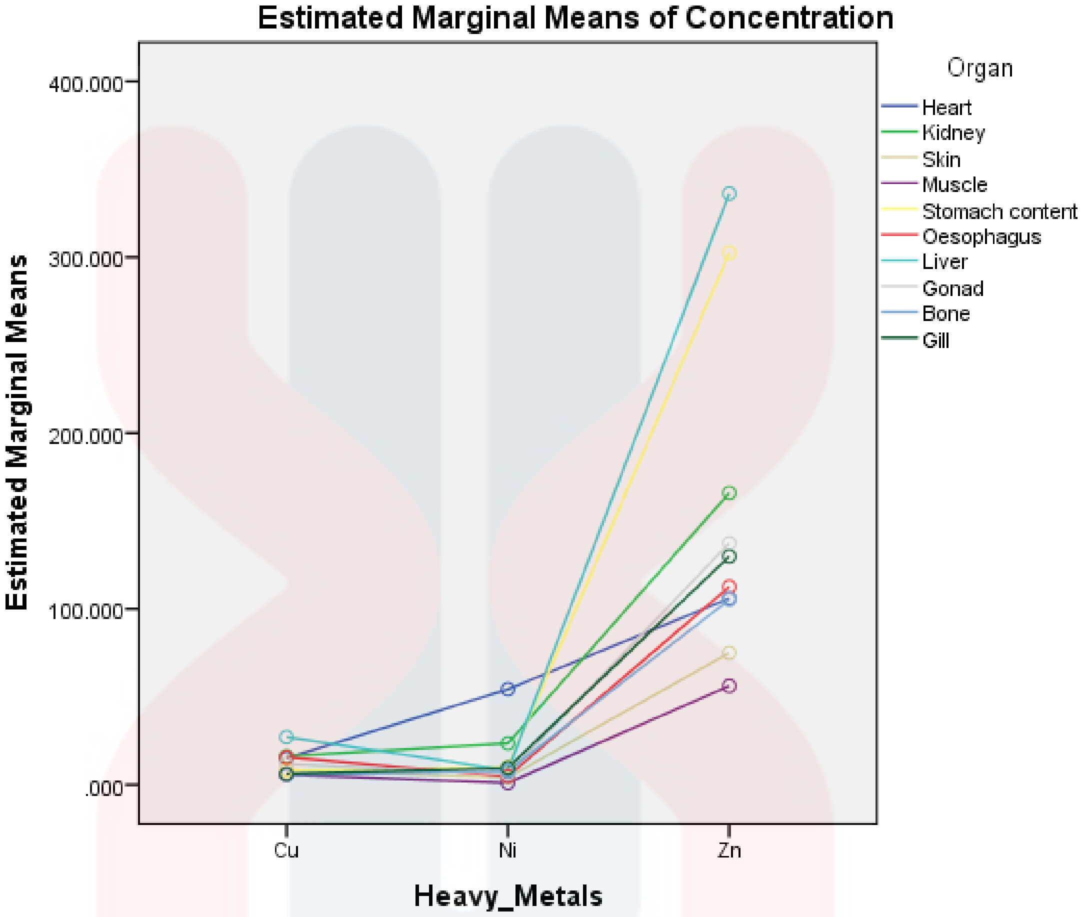
Yunus, S. M. (2014). Cadmium, Chromium, Copper, Lead, Ferum and Zinc level in the cockle, (Anadara granosa) from Kuala Selangor, Malaysia. *The Malaysian Journal of Analytical Sciences*, *18*(3), 514–521.

Zuiew. (1793). Feeding Habit and Body Composition of the Asian Swamp Eel, Monopterus albus, *4*(2). Retrieved from http://www.myanmar-education.edu.mm/wp-content/uploads/2013/05/22_Theingi_Soe_Myint.pdf

## APPENDICES

Appendix 1: Eel collection using *tukil*

Appendix 2: Eel dissection




Appendix 3: Estimated marginal means of concentration for Bachok and Pasir Mas.