



UNIVERSITI PUTRA MALAYSIA

***DIETARY HEALTH RISK ASSESSMENT AND DETERMINATION OF
HEAVY METALS IN *Anabas Testudineus* FROM PADDY FIELD IN
SABAK BERNAM, SELANGOR***

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SERDANG, SELANGOR**

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BERNAM, SELANGOR**



**BY
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**Thesis submitted in fulfilment of the requirement for the degree of Bachelor
Science (Environmental and Occupational Health) from the Faculty of Medicine
and Health Sciences, Universiti Putra Malaysia.**

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ABSTRACT

DIETARY HEALTH RISK ASSESSMENT AND DETERMINATION OF HEAVY METALS IN *Anabas testudineus* FROM PADDY FIELD IN SABAK BERNAM, SELANGOR

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Fish is important in the balance of nature and in the food chain, as it is a main source of protein for human consumption. Rapid industrialized agriculture practices have increased the usage of pesticides in the paddy field, which results in increased heavy metals in fish. Consumption of fish contaminated with heavy metals by humans causes chronic health impacts and can be toxic to the human body when it exceeds the permitted limits. The objective of this study is to determine the concentration of Pb, Cd, and As in *Anabas testudineus* and to assess the dietary health risk among respondents who consumed *Anabas testudineus* from Sabak Bernam. A set of questionnaire was used to obtain information on socio-demographic, health status, and frequency of fish intake. The heavy metals were analyzed by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to determine the heavy metal concentration contained in the flesh muscle of *Anabas testudineus*. Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR) were used to estimate the non-carcinogenic and carcinogenic effects, respectively. The concentration of As (0.00597 mg/kg) and Pb (0.05117 mg/kg) were found in the *Anabas Testudines*. When compared to the Food Regulation 1985, the concentration of heavy metals does not exceed the permitted level. Both the HQ of As and Pb for male and female showed lower than one. Whereas, the LCR of As showed the result with an unacceptable range of lifetime risk. Based on HQ estimation, no adverse health effects are expected from the dietary exposure to Pb and As in the *Anabas testudineus*. The cancer risk is likely to happen after estimating the lifetime cancer risk of arsenic intake from *Anabas testudineus*. Even at a low amount of heavy metals detected, but people still expose to these heavy metals via consumption of *Anabas testudineus*. Regular monitoring is needed to ensure the concentration of heavy metals are within the safe limit.

Keywords: heavy metals, *Anabas testudineus*, dietary health risk assessment, paddy field.

ABSTRAK

PENILAIAN PEMAKANAN RISIKO KESIHATAN DAN PENENTUAN LOGAM BERAT DALAM IKAN PUYU DARI SAWAH PADI DI SABAK BERNAM SELANGOR

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Ikan adalah penting dalam keseimbangan alam dan dalam rantai makanan, Ikan menjadi salah satu sumber utama protein dalam pemakanan manusia. Amalan pertanian industri pesat telah meningkatkan penggunaan racun perosak di sawah padi yang menyebabkan peningkatan logam berat dalam ikan. Memakan ikan yang tercemar dengan logam berat boleh menyebabkan kesan kesihatan kronik dan boleh menjadi toksik kepada tubuh manusia apabila melebihi tahap yang dibenarkan. Objektif bagi kajian ini adalah untuk menentukan kepekatan plumbum, kadmium dan arsenik dalam ikan puyu dan menilai risiko kesihatan pemakanan dalam kalangan responden yang memakan ikan puyu di sawah padi. Borang soal selidik telah digunakan untuk mendapatkan maklumat tentang sosio-demografi, status kesihatan dan kekerapan pengambilan ikan. Logam berat dianalisa menggunakan kaedah ICP-MS untuk menentukan kepekatan logam berat dalam ikan puyu. Hazard Quotient (HQ) dan Risiko Kanser Sepanjang Hayat (LCR) digunakan untuk menganggarkan kesan bukan karsinogenik dan karsinogenik. Kepekatan arsenik (0.00597 mg / kg) dan plumbum (0.05117 mg / kg) telah ditemui dalam Ikan Puyu. Apabila dibandingkan dengan Peraturan Makanan 1985, logam berat ini tidak melebihi paras yang dibenarkan. Kedua-dua HQ As dan Pb bagi lelaki dan wanita menunjukkan kurang daripada satu. Sedangkan LCR of As menunjukkan hasil dengan risiko jangka hayat yang tidak dapat diterima. Berdasarkan anggaran HQ, tiada kesan kesihatan buruk yang dijangkakan dari pendedahan makanan kepada Plumbum dan arsenik. Risiko kanser mungkin akan berlaku selepas menganggarkan risiko kanser seumur hidup pengambilan arsenik dari ikan puyu. Malah pada jumlah yang rendah logam berat dikesan, tetapi orang masih mendedahkan kepada logam berat ini melalui pengambilan ikan puyu. Pemantauan perlu dilakukan untuk memastikan kepekatan logam berat berada dalam batas yang selamat.

Kata kunci: logam berat, Ikan Puyu, penilaian pemakanan risiko kesihatan , sawah padi

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LIST OF ABBREVIATIONS

Pb	Lead
As	Arsenic
Cd	Cadmium
HQ	Hazard Quotient
LCR	Lifetime Cancer Risk

CHAPTER 1

INTRODUCTION

1.1 Background

Fish is an important food represents a major part of many natural food chains; as it is the main source of digestible protein vitamins, minerals and polyunsaturated fatty acids (PUFA). Fish are usually among the top consumers. Nevertheless, fish and people are primarily exposed to heavy metals through food ingestion, dermal contact and inhalation. However, fish can accumulate high levels of metal through water and their food. Fishes are widely used as a bio indicator of heavy metal pollution in aquatic ecosystems (Janadeleh & Kardani, 2018).

Even though metals in the human body enable the healthy function (Alissa & Ferns, 2011), exogenous heavy metals, are considered as the toxic metal that is detrimental to human health. Heavy metals intakes through ingestion of fish from the polluted aquatic environment are varies depends on the ecological requirements, fish body metabolism and other factors such as salinity, water pollution level, food and sediment.

The accumulation of metals in flesh muscle of fish through absorption has causes human to uptake residuals of heavy metals while consuming fish, particularly when the fish originated from heavy metals contaminated water sources.

Past studies have shown that heavy metals affected different body systems such as cardiovascular, respiratory, endocrine, immune and reproductive systems (Wirth, Mijal, & Wirth, 2010). Prolonged exposure to heavy metals can disturb the oxidative stress genes thus highly susceptible to disease might occur as the heavy metals can be accumulated in the body over a long period of time.

Lead (Pb), Cadmium (Cd) and mercury (Hg) are among the commonest toxic substances in as according to the Agency for Toxic Substances and Disease Registry (ATSDR, 2011). In addition, heavy metals such as Cd, Ni, As, Pb can cause harmful to humans. These metals are either potent carcinogenic or mutagenic based on (Alissa & Ferns, 2011). Based on past studies (Navas-Acien, Guallar, Silbergeld, & Rothenberg, 2007) exposure to heavy metals causes health effect to human body, such as, reduce mental and central nervous system function, lower energy levels, and damage to blood composition, lungs, liver, kidneys and other vital organs. Long term exposure to heavy metals also can lead to slowly progressing physical, muscular, and Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis (Raikwar, Kumar, Singh, & Singh, 2014).

Sabak Bernam is a district in the northern part of Selangor, covering an area of 992 square (sq) km or about 11% of Selangor land area. The population in 2010 was 105,777 and have decreased to 113,245 in 2000. The district is considered most rural as compared to other districts of Selangor State. Its population density of 106 respondents per sq km and urbanization rate of 31 percent (compared to 91.4 percent for Selangor in 2010). Paddy, coconut and palm oil are the main agricultural crops in Sabak Bernam. Paddy is considered as an important food crop, and Sabak Bernam as the rice bowl of the nation is among the main producer of rice for the country (Nghah, 2015).

The composition of the population consists of a majority Malay (74%), followed by Chinese (19%) and Indian (3.9%). About 47 percent of the total area of Sabak Bernam is under agriculture, 40.18 under forest and less than 5 percent being occupied by urban settlements (Nghah, 2015).

1.2 Problem statement

Environmental pollution is one of the major causes of environmental degradation worldwide. Heavy metal pollution not only affects the production and quality of crops, but also influences the quality of the atmosphere and water bodies, threatens health and life of animals and human beings by inclusion into food chains

Agricultural soils and water contain both heavy metals and pesticides originating from various agricultural practices (Tariq et al., 2016). For instance, laboratory analytic done by past study (Rahman et al., 2015) showed that increases mixture of synthetic chemicals pesticides and fertilizer used at paddy farmland has increased the level of heavy metals in the soil and water.

In fact, the agricultural sustainability has long been associated with the use of a broad spectrum of pesticides that control the disease causing pests and crop destroying insects. A significant portion of these pesticides find their way into the soil through spray drifts, or as wash-off from treated foliage (Rial Otero et al., 2003). From the soil and underground water, these pesticide residues-contained heavy metals may get directly vaporized along with the evaporating soil moisture or are leaked into the ground and surface waters through leaching or run-off.

While agricultural soil and water are known for their contaminated with heavy metals and pesticides, fish in the paddy farmland has served as one the main food sources for rice farmers for decades. The culture of fish in rice fields is a traditional integrated farming practice in Asia, especially among those who increased adoption of integrated pest management practices.

In fact, fish plays an important role in aquatic food chain (Singh, 2010) as it takes up a large amount of heavy metals from polluted water by ingestion, ion-exchange of dissolved metals across lipophilic membranes and absorption on tissue and membrane surface (Mendil et al, 2005 & Agbozu, 2007). For instances, cadmium resided in the aquatic ecosystems can bio accumulate in fish, mussels, oysters, shrimps and lobsters. The susceptibility to cadmium can vary greatly between aquatic organisms. Salt-water organisms are known to be more resistant to cadmium poisoning than freshwater organisms.

Consequentially, heavy metals in aquatic environments are transferred throughout the web chain into humans. It is well known that fish muscle is not an active tissue to accumulate heavy metals, but it was discovered that heavy metal levels in the edible portion (muscle) of some fish in contaminated regions exceeded and thus increase their concentrations above normal levels in the environment (Puel, 1987).

Contaminated soil with heavy metals will give the negative impacts to the ecosystem when the heavy metals taken up by the plants and animals and results in threatening the ecosystem due to its translocation and accumulation of substances such pesticides or other chemicals in an organism (Puel, 1987). Consumption of fish contaminated with heavy metals by humans cause damage to the human health (Puel et al., 1987; Luoma and Rainbow, 2008) and heavy metals can become toxic to human body when exceed a certain limits (Rainbow, 1985).

The large amount of synthetic chemicals pesticides applied at the agricultural over the past decades has gradually contaminated the soil and water, which later increase heavy metals contaminated in the environment, particularly cadmium, lead and arsenic. One of the major source of cadmium exposure is through phosphate fertilizers. On the other hand, non-essential heavy metals such as cadmium (Cd), zinc (Zn) and lead (Pb) have no known essential role in living organisms; exhibit extreme toxicity even at very low (metal) exposure levels and have been regarded as the main threats to all forms of life especially human health. If they consume that heavy metal, lead in the body is distributed to the brain, liver, kidney and bones while cadmium exerts toxic effects on the kidney, the skeletal and the respiratory systems (Wani, Ara, & Usmani, 2015) and for zinc, there is some evidence that increased levels of zinc in the body might play a role in the development of kidney stones.

Furthermore, arsenic are the most abundant in seafood especially in fish. The high intake in arsenic can be very toxic to human health in a long term exposure chronic effects which may take many years to develop the disease. The effects may depend on the level of exposure to arsenic. Arsenic can be accumulated in the body and may cause diseases such as the skin lesions, peripheral neuropathy, gastrointestinal symptoms, diabetes, renal system effects, cardiovascular disease and cancer (WHO, 2017).

1.3 Study Justification

Heavy metals are defined as metallic chemical element that has a relatively high density and are toxic or poisonous at low concentrations (Connel, 1984). Heavy metals are potential toxicants that have the ability to disrupt a number of prominent proteins and alter the expression patterns of numerous genes, thus disrupt the cellular events lead to the increase of diseases (Ademuyiwa et al., 2010, Kakkar and Jaffery, 2005, Zawia et al., 1998). On the other hand, long term exposure of cadmium may lead to health problems such as physiological problems in blood production and liver malfunction. Cadmium through the intake of food may cause metal poisoning and medical treatment may concern to avoid further damage (Baby et al., 2011).

When accumulation of heavy metal, lead reaches a substantially high level, it can become toxic (Yildirim, 2009). As above mention, lead toxicity has become very

important due to its great concern for human health (Juberg, 1997; Rossi, 2008; Healey, 2009). Fish are usually among the top consumers (Bunton, 1987). Fish and people are primarily exposed to lead by food ingestion and breathing.

Arsenic, on the other hand, are the most abundant in seafood especially in fish. The high intake in arsenic can be very toxic to human health in a long term exposure causing chronic health effects which may take many years to develop the disease. The effects may depend on the level of exposure to arsenic. Arsenic can be accumulated in the body and may cause diseases such as the skin lesions, peripheral neuropathy, gastrointestinal symptoms, diabetes, renal system effects, cardiovascular disease and cancer (WHO, 2017).

Anabas testudineus, also known by its common name, *Ikan Puyu*, is a fresh water fish that can normally be found in paddy fields. This fish is air breathing type, that is, they can survive longer than other fish without water. It is carnivorous, a voracious eater, likes to eat insect, has hard scales and spiny fins. The superficial wound healing in fish differs greatly from those of mammals and other higher vertebrates. Contrary to mammals, epithelisation in fish is a rapid process with movement of the epidermis, starting from the wound edge, within 1 to 4 hours of infliction of the wound (Banerjee and Mittal, 1999). This is achieved perhaps to prevent the heavy influx of water through the damaged skin.

To date, the study of the fish contaminated of heavy metals are few and limited, especially fish contaminated with heavy metals from paddy field since the local residents in Sabak Bernam are usually consume the fish directly caught from the paddy field. Therefore, this study is important as it examines the heavy metals contaminated inside the *Anabas testudineus* that is likely to be consumed by farmers and the villagers in a daily basis.



1.4 Conceptual Framework

The conceptual framework was constructed as shown in Figure 1.1 based on study variables. The aim of this study was to examine the concentration of heavy metals in fish and assess the dietary health risk among the respondents who consumed *Anabas testudineus* from paddy field in Sabak Bernam.

From the framework, heavy metals such as lead (Pb), arsenic (As) and cadmium (Cd) are of particular interest to examine in this study. The concentration levels of the heavy metals contained in *Anabas testudineus* were used to assess the dietary health risks among the respondents in Sabak Bernam who consumed *Anabas testudineus* from paddy field. In this context, both non-carcinogenic and carcinogenic health effects were estimated by using the standardized Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR) formula, respectively.

There are three pathways the heavy metals can enter human body; by ingestion, by inhalation and direct skin contact. However, this study focus only on the potential health risk through dietary ingestion of *Anabas testudineus*. The main objective was to determine *Anabas testudineus* consumed by the respondents in Sabak Bernam contaminated with heavy metals of interest in this study. The heavy metal concentration in *Anabas testudineus* detected was then be compared with the permitted limit in Food Regulation 1985.

1.4 Conceptual framework

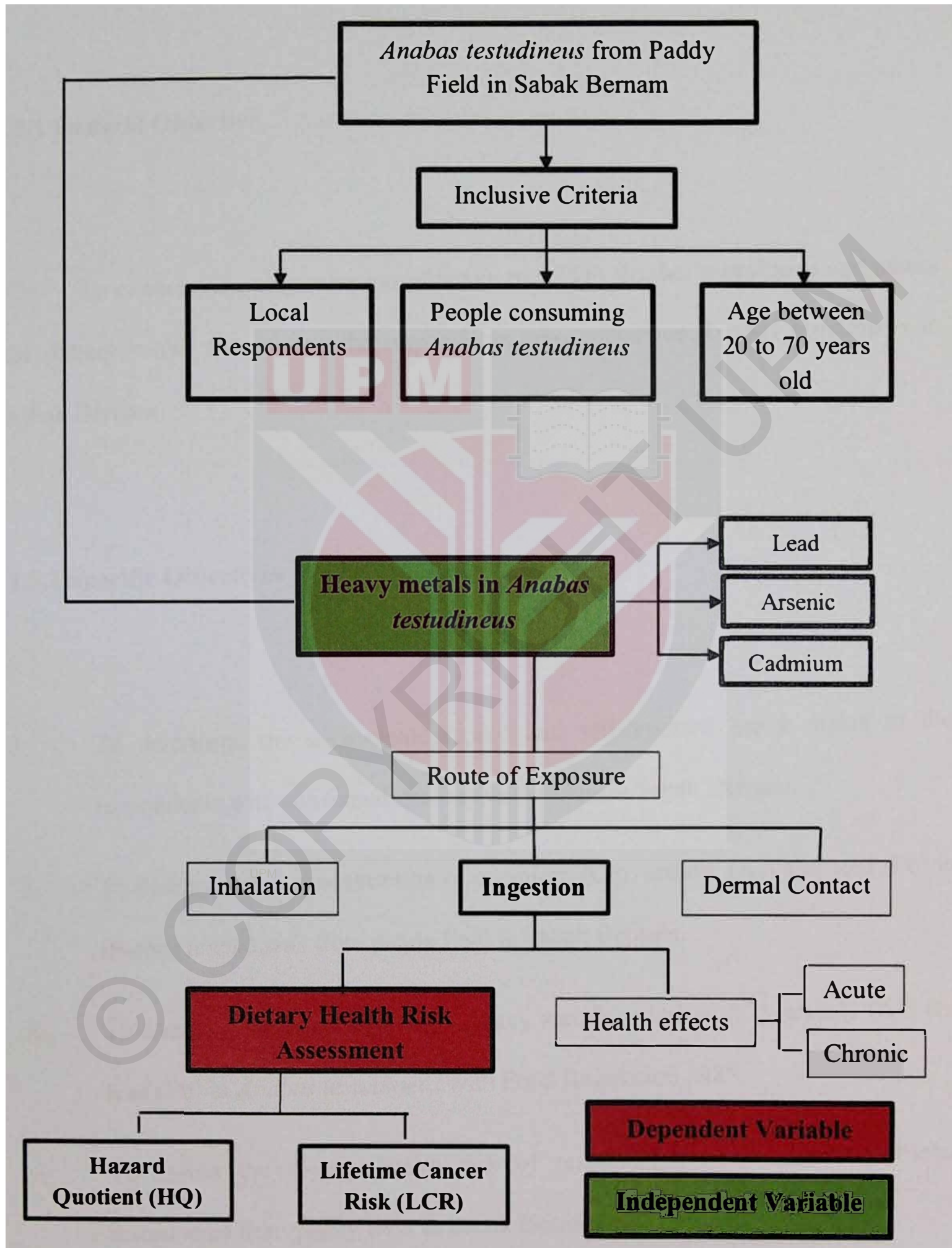


Figure 1.1: The conceptual framework of this study

1.5 Research Objectives

1.5.1 General Objective

To examine the concentration of heavy metals in *Anabas testudineus* and assess the dietary health risk among the respondents who consumed *Anabas testudineus* in Sabak Bernam.

1.5.2 Specific Objectives

- i. To determine the socio-demographic and self-reported health status of the respondents who consumed *Anabas testudineus* in Sabak Bernam.
- ii. To determine the concentration of cadmium (Cd), arsenic (As) and lead (Pb) in *Anabas testudineus* from paddy field in Sabak Bernam.
- iii. To compare the concentration of heavy metals cadmium (Cd, arsenic (As) and lead (Pb) in *Anabas testudineus* with Food Regulation 1985.
- iv. To assess the dietary health risk of respondents who consumed *Anabas testudineus* from paddy field in Sabak Bernam.

1.6 Research Hypothesis

- i. The concentration of heavy metal in *Anabas testudineus* is significantly higher as compared to Food Regulation 1985.
- ii. The higher the concentration of heavy metals cadmium (Cd), arsenic (As) and lead (Pb) in *Anabas testudineus*, the higher the dietary health risk among respondents who consumed *Anabas testudineus* in Sabak Bernam.

CHAPTER 2

LITERATURE REVIEW

2.1 General Toxicity of Heavy Metals

Heavy metals has no real definition and are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water. Heavy metals has been used as a general term for the metals and metalloids accompanied by its toxicity. Heavy metals are found naturally in earth's crust but technology-based and/or research-related activities have caused drastic changes in their geochemical cycles (Bawuro et al., 2018).

These heavy metals then accumulate in different parts of plants thus causing various harmful effects on human health. The human toxicity is depends on the total dose absorbed, the chemical form taken, the age of an individual and the route of exposure of a person. Most of the heavy metals have the potential health effects and other human biochemical process and does not have any benefit from the heavy metals.

When accumulation of heavy metal lead reaches a substantially high level, it can become toxic (Yildirim, 2009). Their toxicity depends on several factors which are the dose, route of exposure, and chemical species, as well as gender, age, genetics and nutritional status of exposed individuals. (Tchounwou, Yedjou, Patlolla, & Sutton, 2014). Also, heavy metals often form a part of the active compounds of pesticides.

Other than that, a surplus of heavy metals in soils is frequently caused by using fertilizers, metallic-pesticides and sewage sludge, or by industrial activities, and the concentration of heavy metals in soils can increase by repeated and excessive fertilizer and pesticide applications.

Heavy metals have also been discovered in paddy field waters and these have been attributed to excessive use of agrochemicals which are known to contain cadmium, copper, lead and zinc (Ismail, 1994).

2.2 Characteristics and General Toxicity of Lead (Pb)

Lead is a widely used metal, but it is simultaneously a versatile, subtle, and persistent poison. Lead is a soft metal that has known many applications over the years. It has been used widely since 5000 BC for application in metal products, cables and pipelines, but also in paints and pesticides. Significant exposure to lead is an environmental threat to optimal health and to physical development in young children that affects all socioeconomic groups (Bunton, 1987).

Lead in blood has an estimated half-life of 35 days, in soft tissue 40 days, and in bones 20 to 30 years. The biological half-life of lead may be considerably longer in children than in adults (Yildirim, 2009).

Lead can enter the human body through uptake of food (65%), water (20%) and air (15%). Blood lead concentrations reflect the intake of only the previous 3 to 5 weeks and thus cannot be used as indices of chronic exposure. Generally, lead excretion is low, with the most significant route being via the urinary tract. The use of chelating agents can enhance lead excretion in urine and this constitutes the basis of the therapeutic approach to lead poisoning.

Lead can cause several unwanted effects, such as disruption of the biosynthesis of haemoglobin and anaemia, rise in blood pressure, kidney damage, miscarriages and subtle abortions, disruption of nervous systems and brain damage.

Lead can also disrupt the children performance though their learning ability and behavioural disruption. The children tend to be aggressive and have an impulsive behaviour and hyperactive. Other than that, lead can also effect the fertility of men which it can damage the sperm. Lead can enter a foetus through the placenta of the mother. Because of this it can cause serious damage to the nervous system and the brains of unborn children.

Lead may also be excreted with bile through the gastrointestinal tract. Although minute amounts of lead are excreted through the sweat and the nails, these routes do not have any practical significance. In general, lead is excreted extremely slowly from the body, with its biological half-life estimated at 10 years, thus facilitating accumulation in the body.

Lead is found in mineral deposits and is released into the environment from natural causes as well as through human industrial activity. It does not dissipate, nor is it biodegradable. Therefore, lead in dust becomes a long-term source of lead exposure. Because of its malleability, low melting point, and ability to form compounds, it has been

used in hundreds of products such as pipes, solder, brass fixtures, crystal, paint, cable, ceramics, and batteries.

The increase of lead in the environment is not only because of the leaded gasoline, but also the human activities such as anthropogenic activities, industrial process, solid waste combustion and fuel combustion. Lead is a potent chemical where it can be accumulated in an individual organism and also the entire food chain.

When accumulation of heavy metal lead reaches a substantially high level, it can become toxic (Yildirim, 2009). Lead toxicity has become very important due to its great concern for human health (Juberg, 1997; Rossi, 2008; Healey, 2009).

Lead is a particularly dangerous chemical, as it can accumulate in individual organisms, but also in entire food chains. Lead has the tendency to accumulate in the bodies of water organisms and soil organisms. Lead can end up in water and soils through corrosion of leaded pipelines in a water transporting system and through corrosion of leaded paints. It cannot be broken down; it can only be converted to other forms.

Even in a low concentration of lead, the health effects of lead can be seen. Soil functions are disturbed by lead intervention, especially near highways and farmlands, where extreme concentrations may be present. Soil organisms then suffer from lead poisoning, too.

Fish are usually among the top consumers (Bunton, 1987). Fish and people are primarily exposed to Pb by food ingestion and breathing. Lead accumulates in the muscles, bones, blood and fat. New borns and young children are especially delicate to even low levels of lead (Elder and Collins, 1991). Consumption of lead can affect organ such as brain which is causing memory problem, heart like high blood pressure and other organs.

These studies also show that farmers themselves are exposed and gradually poisoned when they work in pesticide-sprayed fields. Heavy metals have also been discovered in paddy field waters and these have been attributed to excessive use of agrochemicals which are known to contain cadmium, copper, lead and zinc (Ismail, 1994).

2.3 Characteristics and Toxicity of Cadmium (Cd)

Cadmium is a lustrous, silver-white, ductile, very malleable metal. Its surface has a bluish tinge and the metal is soft enough to be cut with a knife, but it tarnishes in air. It is soluble in acids but not in alkalis. It is similar in many respects to zinc but it forms more complex compounds. Cadmium occurs as a minor component in most zinc ores and therefore is a by-product of zinc production (Janadeleh & Kardani, 2018).

Cadmium is released into the environment naturally about 25,000 tons per year. The cadmium has been released to the river for about half into the forest fires and volcanoes and the remaining of the cadmium is released through human activities such as manufacturing.

Cadmium is used generally as stabilizer in different products like colour pigments, several alloys and in Polyvinyl chloride (PVC)-related products. Another major source of cadmium exposure is phosphate fertilizers. Besides, cadmium also can be found in the earth crust. Cadmium also consist in the industries as the by-product of zinc, lead and copper extraction thus, after being applied the cadmium will enter the environment through the ground because later it is found mainly in manures and pesticides (Yildirim, 2009).

The route of exposure of cadmium is through the ingestion, inhalation and skin dermal. As fertilizer is one of the chemical that being use by the farmer, it has contained some cadmium. So, fish in paddy field will contaminate if they consume water that contaminated with cadmium.

Usually, the uptake of cadmium by human is through the intake of food. Food that rich in cadmium content can also increase in the human body for example liver, mushrooms, shellfish, mussels, cocoa powder and dried seaweed. Besides, cadmium can damage their lungs as human inhale it which may also cause death.

Liver is the first take place for the transportation of cadmium through the blood. Cadmium accumulates in the kidney and then damages the filtration mechanism in the kidney. The essential proteins and sugars are caused to be excreted from the body and further damage the kidney. It takes a long time for the accumulated cadmium in kidney to be excreted in the human body.

The health effects that can be caused by cadmium are diarrhoea, stomach pains, severe vomiting, bone fracture, reproductive failure and possibly even infertility. Other than that, cadmium also can cause damage to the central nervous system and damage the immune system. The psychological of a person can also be disrupted and can be possibly damage the DNA or may cause the cancer development.

Cadmium is an environmental hazards. Cadmium through the consumption of rice may develop disease Itai-Itai and renal abnormalities. Most of the researcher has been concluded cadmium is a major factor of causing Itai-Itai disease in Japan and there are research linking exposure to cadmium may also develop lung and prostate cancer.

Some members of the local agricultural communities consuming the contaminated rice developed Itai-Itai disease and renal abnormalities, including proteinuria and glycosuria. Cadmium is a major factor in the Itai-Itai disease in Japan, most researchers have concluded that it was one of several factors. There has been research linking exposure to cadmium to lung and prostate cancer.

Cadmium waste streams from the industries mainly end up in soils. The causes of these waste streams are for instance zinc production, phosphate ore implication and bio industrial manure. Cadmium waste streams may also enter the air through (household) waste combustion and burning of fossil fuels. Because of regulations only little cadmium now enters the water through disposal of wastewater from households or industries.

Another important source of cadmium emission is the production of artificial phosphate fertilizers. Part of the cadmium ends up in the soil after the fertilizer is applied on farmland and the rest of the cadmium ends up in surface waters when waste from fertilizer productions is dumped by production companies.

Cadmium can be transported over great distances when it is absorbed by sludge. This cadmium-rich sludge can pollute surface waters as well as soils. Cadmium strongly adsorbs to organic matter in soils. When cadmium is present in soils it can be extremely dangerous, as the uptake through food will increase. Soils that are acidified enhance the cadmium uptake by plants. This is a potential danger to the animals that are dependent upon the plants for survival.

Earthworms and other essential soil organisms are extremely susceptible to cadmium poisoning. They can die at very low concentrations and this has consequences for the soil structure. When cadmium concentrations in soils are high they can influence soil processes of microorganisms and threaten the whole soil ecosystem.

In aquatic ecosystems cadmium can bioaccumulate in mussels, oysters, shrimps, lobsters and fish. The susceptibility to cadmium can vary greatly between aquatic organisms. Salt-water organisms are known to be more resistant to cadmium poisoning than freshwater organisms.

2.4 Characteristics and Toxicity of Arsenic (As)

Arsenic is a toxic metal and it has its own bio active element. Arsenic appears in three allotropic forms: yellow, black and grey; the stable form is a silver-gray, brittle crystalline solid. It tarnishes rapidly in air, and at high temperatures burns forming a white cloud of arsenic trioxide.

Other than that, arsenic can be found naturally on earth in small concentrations. It occurs in soil and minerals and it may enter air, water and land through wind-blown dust and water run-off. Arsenic in the atmosphere comes from various sources which is volcanoes and microorganisms however, human activity is also responsible for the release of arsenic by the burning of fossil fuels in the environment (Yujie et al., 2012).

The bioactive element accumulated in rice of several regions of the world. Arsenic chronic exposure can cause disease such as hyperpigmentation, keratosis, lung, prostate cancer and bladder. A very high exposure to inorganic arsenic can cause infertility and miscarriages with women, and it can cause skin disturbances, declined resistance to infections, heart disruptions and brain damage with both men and women (Satpathy, Reddy & Dhal, 2014).

The effects of inorganic arsenic can damage DNA, hypertension, involuntary muscular dysfunction, diabetes, neuropathy, depression, obesity and other condition that is related to the alteration of intercellular voltage-dependent potassium channels. But organic arsenic can cause neither cancer, nor DNA damage. The high dose of arsenic exposure can affect the nerve and cause stomach ache.

The global reported issue has been arising from rice and the increase of arsenic in field soil has also been reported in various region of the world. Toxicity by Arsenic may also cause reduction in locomotors activity that ultimately disturbs the CNS (Rainbow, 1985).

Calderon et al conducted a study in children which concluded that children showing higher Arsenic concentration were found to show a low verbal intelligence. In Taiwan, high school students were compared between areas who were affected with Arsenic and those of non-affected areas. Study results showed that in children from Arsenic affected are as neurobehavioral performance were found to be low compared to areas which were not affected by arsenic.

2.5 Consumption of Fish Contaminated with Heavy Metals

Fish are often at the top of aquatic food chain and may concentrate large amounts of some metals from the water (Mansour and Sidky, 2002). Some metals are essential to human health. Metals are naturally occurring elements that become contaminants when human activities

Heavy metal pollution is a serious and widespread environmental problem due to their toxicity; heavy metals enter the environment through various natural methods and human activities, and can accumulate in fish and other organisms (Kalay and Canli, 2000).

Fish are the final organism in the aquatic food chain and a significant food source for man. Consequentially, heavy metals in aquatic environments are transferred throughout the web chain into humans. It is well known that fish muscle is not an active tissue to accumulate heavy metals, but it was discovered that heavy metal levels in the edible portion (muscle) of some fish in contaminated regions exceeded and thus increase their concentrations above normal levels in the environment (Bunton, 1987).

Fish are usually among the top consumers (Bunton, 1987). Fish and people are primarily exposed to lead by food ingestion and breathing. Heavy metal pollution is a serious and widespread environmental problem due to their toxicity; heavy metals enter the environment through various natural methods and human activities, and can accumulate in fish and other organisms (Kalay and Canli, 2000).

Consumption of fish contaminated with heavy metals by humans cause damage to the human health (Puel et al., 1987; Luoma and Rainbow, 2008) and heavy metals can become toxic to human body when exceed a certain limits (Rainbow, 1985). Fish are the final organism in the aquatic food chain and a significant food source for man. Consequentially, heavy metals in aquatic environments are transferred throughout the web chain into humans.

It is well known that fish muscle is not an active tissue to accumulate heavy metals, but it was discovered that heavy metal levels in the edible portion (muscle) of some fish in contaminated regions exceeded (Janadeleh & Kardani, 2018).

CHAPTER 3

METHODOLOGY

3.1 Study Design

A cross sectional study design was used throughout the study. This study was included *Anabas testudineus* (*ikan puyu*) collection from paddy field, a set of self-administered questionnaires and face-to-face interview on the socio-demographical background and self-reported health status among respondents who consumed *Anabas testudineus*. This study was used examine the concentration of heavy metals in *Anabas testudineus* and to assess the dietary health risk of respondents who consumed *Anabas testudineus* from paddy field at Sabak Bernam. This study was conducted on December 2018 to June 2019.

3.2 Study location

This study was conducted at Sungai Besar, Sabak Bernam. Sungai Besar provides substantial level of local economic activities involving fish production and rice irrigation. The respondents in Sungai Besar consumed *Anabas testudineus* from paddy field for more than a decade. The decimal latitude and longitude coordinates for Sabak Bernam (Malaysia) is 3.7698, 100.9879. Sabak Bernam is located in Malaysia country, in Southeast Asia continent (or region). DMS latitude longitude coordinates for Sabak Bernam are: 3°46'11.28"N, 100°59'16.44"E. Below are the map and the picture showed the study location where the study was conducted. There are five locations that were randomly selected in Sungai Besar to catch the fish which are Paket 60, Paket 100, Parit 13, Parit 14 and Parit 15.

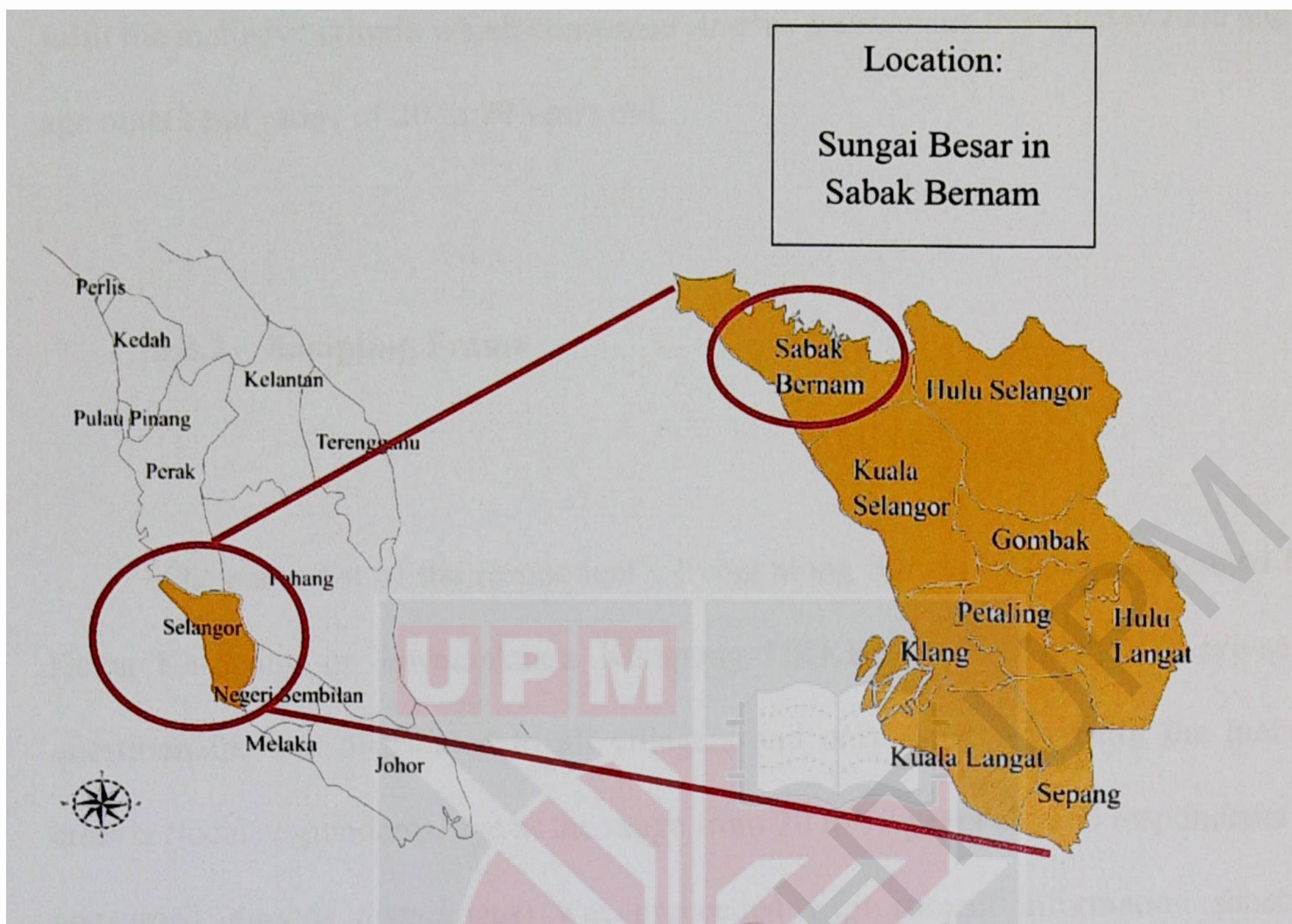


Figure 3.1: Map of the study location

3.3 Sampling

3.3.1 Study Population

One hundred and twenty residents in Sungai Besar were randomly recruited as respondents in this study. The target population of this study is the local respondents who

fulfil the inclusive criteria which consumed *Anabas testudineus* from paddy field and the age must be at range of 20 to 70 years old.

3.3.2 Sampling Frame

The name list of the respondent's living along Sungai Besar was obtained from Ketua Kampung or Jawatankuasa Kampung (JKKK) in the village. A pre-survey questionnaire was distributed to all villagers and only those who fulfil the inclusive criteria (local respondents, age at the range from 20 to 70 years old and respondents who consumed *Anabas testudineus*) was proceeded with subject information sheet and concern letter which indicated that he/she agreed to participate in this study.

3.3.3 Sampling Unit

The respondents living in Sungai Besar were chosen based on a certain criteria:

- i. Local respondents
- ii. Age between 20-70 years old
- iii. People who consumed *Anabas testudineus* from paddy field based on their dietary habit

3.3.4 Sampling Method

The respondents were selected by using simple random sampling in this study as the residents are from the name list from Ketua Kampung or Jawatankuasa Kemajuan & Keselamatan Kampung (JKKK). The *Anabas testudineus* were also randomly collected by using fish net and fishing rod at the trench near paddy field.

3.3.5 Sample size

The calculation by Fischer et al., (1991) formula for sample size estimation is shown below:

$$N = \frac{Z^2 pq}{d^2}$$

Based on Fischer et al., (1991), the proportion is equal to 0.5.

Where,

N = Desired sample size

p = Proportion in the large population estimated to consume *Anabas testudineus*

q = Expected contaminated proportion (1-p) (1-0.5) = 0.5

z = The standard normal deviation set at 1.96 of the 95% confidence interval

d = The degree of accuracy desire set at 0.05 significance

$$N = (1.96)^2(0.5)(0.5) / (0.1)^2$$

$$N = 97 + 20\% \text{ drop rate}$$

$$N = 117$$

A minimum number of population required 117 respondents however, the study was recruited 120 respondents to fulfil the study objective.

3.4 Material and Methodology

3.4.1 Questionnaires

The face to face interviews was conducted to obtain information of respondents in Sungai Besar regarding socio-demographic, self-reported health status, as well as frequency of *Anabas testudineus* intake in their dietary intake. Besides, face to face interviews also was used to obtain the information regarding the most frequent spot to catch the *Anabas testudineus* from paddy field in Sungai Besar.

A set of questionnaires was used in this research to gather information on socio-demographic data, self-reported health status, as well as, frequency of *Anabas testudineus* intake in their dietary intake from each of the respondents in Sungai Besar.

The questionnaires include:

Section A: Socio demographic information

Section B: Frequency intake of *Anabas testudieus*

Section C: Self-reported health status

The questionnaires was written in English and Malay. The survey was carried out on December 2018. The respondents were approached and asked to volunteer to participate in this study. They were interviewed face-to-face based on questions in the questionnaires. The questionnaires were divided into 3 parts. In part A, the respondents were required to provide demographic data such as age, race, sex, marital status, education level and income. Then in part B, the respondents were asked about the frequency intake of *Anabas testudineus* in their dietary habit. In part C, the respondents were asked about their self-reported health status.

3.4.2 *Anabas testudineus* sample collection

The fish sample collection was collected by using fishing rod and fishing nets at the trench near the paddy field. The fish sample was collected after the harvesting process. The collected fish was measured by using digital weighting scale around 2 kg. The weight of each fish on average is 120g to 150g. The fishes were washed by using deionized water and it was placed in a clean isolated polyethylene bags. The isolated polyethylene bags was placed in a potable ice box which was filled with ice to maintain temperature of 0°C. Next, the samples were transported to laboratory on the same day of fish sampling. During the transportation, the temperature was maintained at (0°C) in the portable ice box. The samples were then be kept in freezer (-20°C) at Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM).

3.4.3 *Anabas testudineus* sample preparation

The extractions of fishes were conducted at the Laboratory of Environmental Health, UPM. Fishes were thawed in the room temperature and removed the body parts by using stainless steel knives. The fish samples were weight and homogenized. After the flesh muscle of the fish had been extracted, the fish samples were weighted.

The fish samples were then let dried oven for 48 hours at the temperature of 80°C and placed in the acid washed petri dish. The acid washed petri dish was done before the preparation of fish sample conducted. After 48 hours, the fish samples were cool in desiccator for several minutes. After the sample had been cool in the desiccator for several minutes, the fish samples were then grounded using porcelain mortar and pestle. The fish samples were then sieved and weighted by using analytical balance. The weight of the sample was recorded and the fish samples were placed at the 15 mL centrifuge tube.

Three fish samples were weighted approximately with 0.5 g each. The acid digestion was prepared using 3mL of nitric acid (HNO₃). The instrumentation used for acid digestion was Microwave Reactive System. Three millilitres of nitric acid was mixed with the residue and slowly dissolve the residue by heating. The solution was filtered by using 0.45 um filter paper and transferred to a 10 mL of volumetric flask and made up to

volume. Ten millilitres from the solution was then transferred into centrifuge tubes and proceeded to analyse heavy metals by using ICP-MS in Faculty of Environmental, UPM.

3.4.4 Heavy metal contained in fish analytical analysis

After acid digestion was done, the fish sample was sent to Faculty of Environmental, Universiti Putra Malaysia for heavy metal analysis. The heavy metal was analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Heavy metals included in this study were lead, arsenic and cadmium. The samples were then triplicate for each of the heavy metals. The standard solution was prepared and diluted to 30 ppb, 50 ppb, 100 ppb, 300 ppb and 500 ppb. The standard solutions and blank were analysed with ICP-MS to obtain the standard curve values of ± 0.999 prior to the samples analysis.

3.4.5 Dietary Health Risk Assessment

Risk assessment is one of fastest method which is need to assess the effect of hazard in human health and also need to determine the level of contaminants that can be probable give the impact to the human health (Yujie et al., 2012). There are two formula used in this study to assess the dietary health risk of respondents. The dietary health risk of respondents was calculated by using Hazard Quotient (HQ) for non-carcinogenic effect and Lifetime Cancer Risk (LCR) for carcinogenic effect. The carcinogenic health risk

was only assessed for exposure to As because USEPA do not list the cancer slope factors for Cd and Pb although they are identified as potential carcinogens. Thus, the carcinogenic health risk of respondents due to the exposure dietary of these heavy metals was not assessed. The formula of HQ and LCR are as follows:

$$\text{Hazard Quotient (HQ)} = \text{Average Daily Dose (ADD)} \div \text{RfD}$$

Below is the formula of how ADD and HQ was calculated for a food ingestion exposure.

$$\text{ADD} = \frac{C_F \times R_i \times F_i \times EF \times ED}{BW \times AT}$$

$$\text{HQ} = \frac{\text{ADD}}{\text{RfD}}$$

Where, ADD = Average daily dose of pollutant through ingestion of *Anabas testudineus* (mg/kg-day),

RfD = Reference dose for each heavy metals (mg/kg-day)

CF = Pollutant concentration in *Anabas testudineus* (mg/kg)

R_i = *Anabas testudineus* ingestion rate

F_i = Fraction ingested from contaminated source (unitless)

EF = Frequency intake of *Anabas testudineus*

ED = Exposure duration

BW = Body weight of respondents consumed *Anabas testudineus* (kg)

AT = Averaging time (days); for non-carcinogenic effects

Below is the example of how LADD and LCR was calculated for a food ingestion exposure,

$$LADD = \frac{C_F \times R_i \times F_i \times EF \times ED}{BW \times AT}$$

Lifetime Cancer Risk (LCR) = Lifetime Average Daily Dose (LADD) x Cancer Slope Factor (CSF)

Where, LADD = Lifetime average daily dose of pollutant through ingestion of *Anabas testudineus* (mg/kg-day)

RfD = Reference dose for each heavy metals (mg/kg-day)

CF = Pollutant concentration in *Anabas testudineus* (mg/kg)

Ri = Food item ingestion rate

Fi = Fraction ingested from contaminated source (unitless)

EF = Exposure frequency

ED = Exposure duration

BW = Body weight of respondents consumed *Anabas testudineus* (kg)

AT = Averaging time (days); for non- carcinogenic effects; AT = 70 years

Table 3.1: Oral Reference Dose (RfD) and Cancer Slope Factor (CSF) in three heavy metals

Heavy metals	RfD	CSF
Lead (Pb)	0.002 ^a	N/A
Arsenic (As)	0.0003 ^b	1.5 ^b
Cadmium (Cd)	0.001 ^b	N/A

^a Ecotoxicology and Environmental Safety

^b Integrated Risk Information System (IRIS) USEPA

3.5 Quality Control

3.5.1 Fish Sampling in the Field

Fish were carefully conserved with ice in clean polyethylene bags. Fish from multiple collection sites were stored in the same portable icebox as long as each sample is stored in a clean separate plastic bag. The length and weight of the each fish were measured and recorded in the logbook. Ice was used to maintain the temperature of 0°C to prevent rapid microbial growth and minimize the tissue decay during transportation. Fish were placed in an isolated container during transportation and immediately taken to Institute of Bioscience (IBS) to deep frozen at -20°C while awaiting dissection on the following day until it is prepared for analysis.

3.5.2 Acid digestion

Standard Operating Procedure (SOP) of acid digestion is followed. The precaution steps was followed. A validated method was followed: Power Ramp (600W), Power Hold (600W), ramp time 15 minutes, temperature (280°C), cooling temperature (55°C).

3.5.3 Heavy Metal Analysis in the Laboratory

Arsenic Cadmium and Lead in fish were determined using ICP-MS (PerkinElmer Élan 9000-USA). For better operating conditions the ICP-MS was adjusted to: Nebulizer gas flow 0.91 L/min, Radio frequency (RF) 1200 W, Lens voltage 1.6 V, Cool gas 13.0 L/min, and Auxiliary gas 0.70 L/min²⁰. CRM samples were procured from NIST and EC and used for method standardization, standard graphs were drawn. The standard solution was prepared and the standard curve value was performed. The validation of methodology for chemical analysis which involved parameters of precision, selectivity, bias/recovery, ruggedness, sensitivity/linearity/working range, detection limit and quantification limit was done.

3.5.4 Questionnaire

Pre-test of questionnaire was conducted among 10 percent of the total respondent which aimed the validity and reliability of the questions.

3.6 Data Analysis

The data were analysed by using software Statistical Package for the Social Sciences (SPSS) for Windows, version 22. Descriptive statistics, including percentages, were calculated for relevant items obtained from the questionnaires, the socio demographic and self-reported health status of the respondents who consumed *Anabas testudineus*. The one sample t-test was used to compare the concentration of heavy metals detected in the *Anabas testudineus* with Food Regulation 1985. A p-value of <0.05 was considered to be statistically significant.

3.7 Ethical Consideration

Before data collection was started, permission to carry out the study was obtained from the Ethics Committee for Research involving Human Subjects Universiti Putra Malaysia (JKEUPM) (Ref No. JKEUPM-2018-361). The purposes of the study were explained to the respondents and participation consent forms were to sign by the respondents before socio-demographic and self-reported health status were collected from respondents. The respondents were given explanation about the whole of the study activities involved. The identity of the respondents including their personal information remained confidential and individual statement or description are not going to be stated in any parts of the study or publication. Standard operating procedure has been followed.

CHAPTER 4

RESULTS

4.1 Socio Demographic and Self-reported Health Status of respondents in Sabak Bernam

As shown in the Table 4.1, the respondents that participated in this study were male and female with mean age of (46.63 ± 14.41) and (44.51 ± 14.39) years old respectively, ranged from 20 to 70 years old. The mean weight of male and female in this study was (67.81 ± 8.28) kg and (59.72 ± 8.07) kg respectively. The mean height of male and female was (1.67 ± 0.06) m and (1.55 ± 0.06) m respectively. The highest education level among male and female respondents is secondary school which is (44.4%) and (35.1%). About 66.6% and 86.0% of male and female respondents respectively has income less than RM3000 per month. In the result shown below, the Body Mass Index (BMI) of female were mostly overweight which contribute to 50% of the total respondents while male were in a normal BMI range which contribute to 63.5% of the total respondents. Both overweight and obese category contributes to 1.8% for female respondents. However, male contribute to 3.2% for obese category which is higher than female which contribute to 1.8%. Based on the questionnaires and during face to face

interview, it was found out that 71.9% of respondents were male smokers while 3.6% of respondents were female smokers. None of the respondents was consumed alcohol.

Table 4.1: Socio-demographical background of respondents in Sabak Bernam (N=120)

Variable(s)		Male (n=63)	Female (n=57)
(A) Socio-demographical			
Age (Mean ± SD)		46.63 ± 14.41	44.51 ± 14.39
Weight (Mean ± SD)		67.81 ± 8.28	59.72 ± 8.07
Height (Mean ± SD)		1.67 ± 0.06	1.55 ± 0.06
Education (%)	Primary School	20.6	14.0
	Secondary School	44.4	35.1
	Diploma	20.6	21.1
	Degree	7.9	15.8
	Others	6.3	14.0
Income/season (%)	< RM 3,000	66.7	86.0
	> RM 3,000	33.3	14.0
BMI (%)	Underweight	0	1.8
	Normal	63.5	45.6
	Overweight	33.3	50.9
	Obese	3.2	1.8
Smoker (%)	Yes	69.8	3.5
	No	30.2	96.5
Alcohol intake (%)	Yes	0	0
	No	100	100

Based on Table 4.2, the highest self-reported health status among male is hypertension (32.8%), the second one leading is diabetes (27%), heart disease (9.5%),

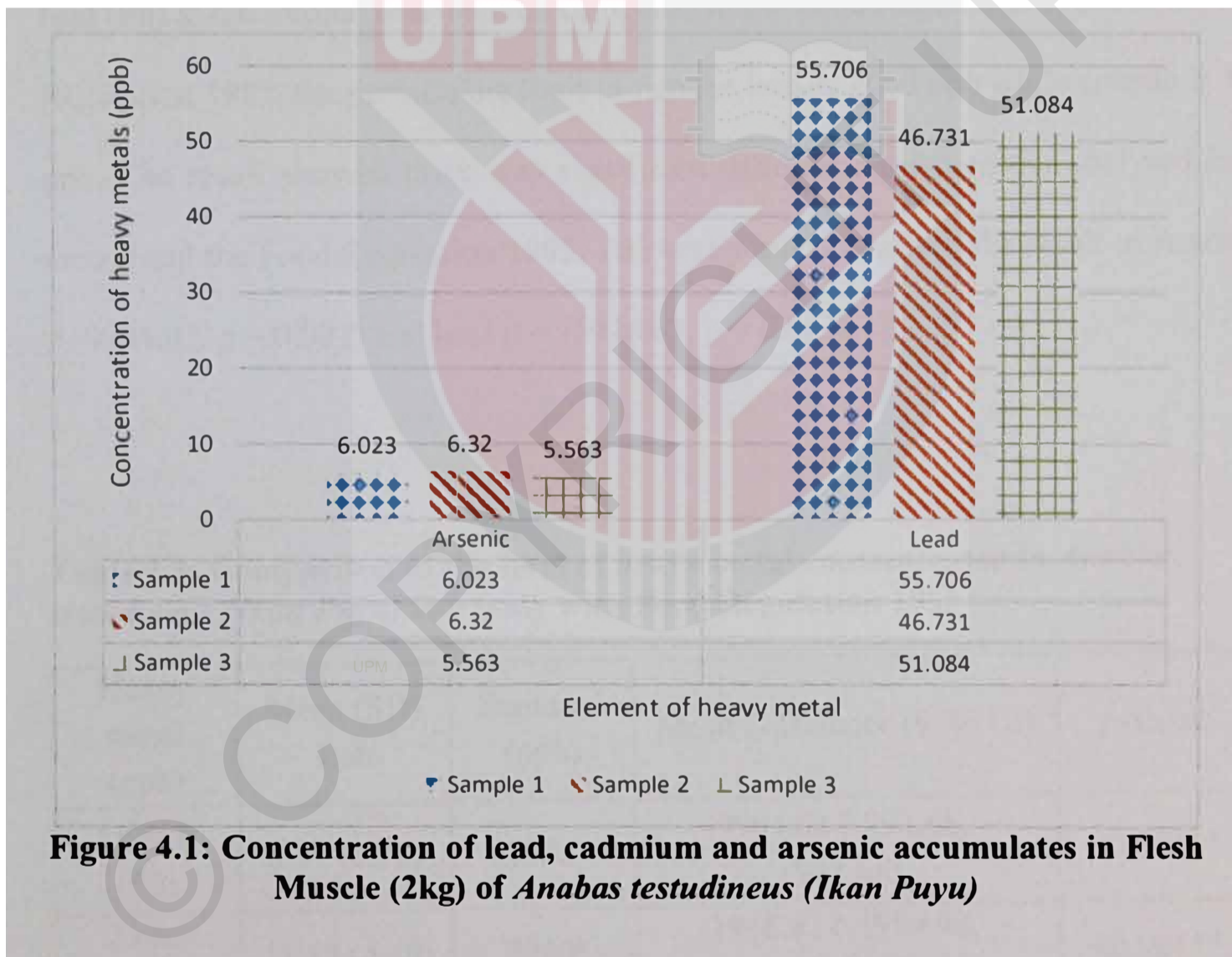
asthma (7.9%), and none of the male respondents suffer from cancer. Female respondents are mostly suffered from hypertension (19.3%), diabetes (14.0%) and heart disease (7.0%) and none of the female respondents suffered from asthma, cancer and kidney disease.

Table 4.2: Self-reported health status of respondents in Sabak Bernam (N=120)

Variable(s)		Male (n=63)	Female (n=57)
Heart Diseases (%)	Yes	9.5	7.0
	No	90.5	93.0
Asthma (%)	Yes	7.9	0
	No	92.1	100
Diabetes (%)	Yes	27.0	14.0
	No	73.0	86.0
Hypertension (%)	Yes	27.0	19.3
	No	73.0	80.7
Kidney Diseases (%)	Yes	0	0
	No	100	100
Cancer (%)	Yes	0	0
	No	100	100

4.2 Heavy metal concentration in the *Anabas testudineus* (Ikan Puyu)

Based on the Figure 4.1 below the mean concentration of lead (Pb) in fish sample showed the highest compared to arsenic (As) and cadmium (Cd). In the fish sample, cadmium is not detected. There are three samples for each of the heavy metal and the mean concentration for arsenic (As) is 5.97 ppb and lead (Pb) is 51.17 ppb.



4.3 Comparison between Contamination Levels of Heavy Metals Arsenic (As), Lead (Pb) and Cadmium (Cd) Accumulates in Flesh Muscle (2kg) of *Anabas testudineus* (Ikan Puyu) and Food Regulation 1985.

The contamination levels of heavy metals in flesh muscle (2kg) of *Anabas testudineus* and Food Regulation 1985 was determined in Table 4.3. Arsenic (As) and lead (Pb) in the sample was lowered compared to the Food Regulation 1985. In the Food Regulation 1985, the permissible limit in fish for lead is 2000 ppb while arsenic is 1000 ppb. The result showed there was significant difference between the analyzed heavy metals and the Food Regulation 1985. The heavy metals showed the result of Arsenic ($t = -9994.03$, $p = 0.001$) and lead ($t = -1948.83$, $p = 0.001$).

Table 4.3: Comparison of the level of heavy metal contaminated in *Anabas testudineus* (Ikan Puyu) fish body with Food Regulation 1985

Heavy metal (ppb)	Mean (SD) Ppb	Standard (ppb)	Mean Difference (95% CI)	p-value
Arsenic (As)	5.97 (0.38)	1000 ^a	-9994.03 (-994.98, -993.08)	<0.001**
Lead (Pb)	51.17 (4.49)	2000 ^a	-1948.83 (-1959.98, -1937.68)	<0.001**
Cadmium (Cd)	ND	1000 ^a	ND	ND

^a Food Regulation 1985 Malaysia

**p-value is significant at 0.05 level

4.4 Dietary health risk consumed *Anabas testudineus* (Ikan Puyu) from paddy field in Sabak Bernam

Table 4.4 summarized the dietary health risk calculation. In the dietary health risk calculation, the formula that was used to assess the health risk of respondents in Sungai Besar was Hazard Quotient (HQ) for non-carcinogenic effects and Lifetime Cancer Risk (LCR) for carcinogenic effects. Both the hazard quotient (HQ) of arsenic (As) and lead (Pb) for male and female showed lower than one. Whereas, the lifetime cancer risk (LCR) of arsenic (As) showed the result with an unacceptable range of lifetime risk level as shown in the Table 4.4. The health risk with lower than one of HQ indicates there is no significant to health risk towards the non-carcinogenic effects and this study also posed the unacceptable risk condition towards the carcinogenic health effects.

Table 4.4: Dietary health risk consumed *Anabas testudineus* (Ikan Puyu) from paddy field in Sabak Bernam

Gender	Hazard Quotient ^a		Lifetime cancer risk ^b
	Arsenic	Lead	Arsenic
Male	0.43	0.55	1.4 x 10 ⁻⁴
Female	0.40	0.51	1.2 x 10 ⁻⁴

^a Formula: Hazard Quotient (HQ) = Average Daily Dose (ADD) ÷ RfD, Source: Guidance Document on Health Impact Assessment (HIA) in Environmental Impact Assessment (EIA)

HQ: Acceptable (<1)

^b Formula: $ADD = \frac{C_F \times R_l \times F_l \times EF \times ED}{BW \times AT}$, Source: Guidance Document on Health Impact Assessment (HIA) in Environmental Impact Assessment (EIA)

LCR: Acceptable (10⁻⁶ to 10⁻⁴), Clearly Acceptable (<10⁻⁶), Unacceptable (>10⁻⁴)

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Socio-Demographical Background and Self-Reported Health Status of Respondents in Sabak Bernam

A total of 120 respondents who consumed *Anabas testudineus* (Ikan Puyu) in Sabak Bernam involved in this study. The respondents were selected based on the inclusive criteria and the age of the respondents was at the range from 20 to 70 years old. Most of the respondents have the secondary educational level with 44.4% for male and 35.1% for female respondents.

According to the World Health Organization (2019), low education level will lead to poor health, more stress and low self-confidence. In fact, educational level associated with health status as suggested by Steven W. (2015), who highlighted that health condition could be influenced by several factors such as income status, resources and facility availability and educational background. Majority of the respondents consumed *Anabas testudineus* (Ikan Puyu) in Sabak Bernam has income below than RM 3000.

Besides, the information about income also recorded as socio economic factors.

According to the Dole and Schroder (2001), income was considered to be as a factor that can influence health attitude as well as overall health performance among adults.

As for the lifestyle among respondents who consumed *Anabas testudineus* (*Ikan Puyu*) in Sabak Bernam, majority of smokers are male which is 71.9% and only 3.6% of female reported as smoker. According to the Action on Smoking and Health (2016), the daily consumption of smoking are highly recorded among men as compared to women. Even though past studies (Bawuro et al., 2018) indicated that the levels of heavy metals varied significantly among fish species and organs, other studies (Action on Smoking and Health, 2016a, 2016b; Royal College of Physicians and Royal College of Psychiatrists, 2013; Talati, Keyes, & Hasin, (2016) have shown that there was strong association between smoking prevalence and educational level. On the other hand, the highest self-reported health status for both male and female respondents was hypertension, diabetes and followed by heart disease. In this study, at least 10- 30% of respondents reported with hypertension, diabetes, heart disease, and asthma. These symptoms could be associated with the chronic low level heavy metals exposure via different route of exposure, including ingestion. Among all, lead is a ubiquitous environmental toxin that is capable of causing numerous chronic illness such as hypertension and cardiovascular disease (Vaziri, 2008), and arsenic exposure may also induce hypertension in humans (Rahman, et al., 1999).

Arsenic metabolism was prospectively associated with increased incidence of

diabetes (Kuo et al., 2015) and environmental exposure to lead may have a long-term effect on progressive diabetic nephropathy in type II diabetic patients (Huang et al., 2012). Based on Taweel, Shuhaimi-Othman, & Ahmad., (2013) the long term exposure of arsenic can lead to formation of skin lesion, cancers, neurological problems, pulmonary disease, peripheral vascular disease, hypertension and cardiovascular disease and diabetes mellitus.

Past study Artopoulou, Martin, & Suchko., (2012) also revealed that male respondents suffer greatly from heart disease and this can be related from the exposure to lead, which would result in mental retardation, birth defects, psychosis, autism, allergies, dyslexia, weight loss, hyperactivity, paralysis, muscular weakness, brain damage, kidney damage and may even cause death. Since the toxicity of lead exposure can cause either acute or chronic effect, the fact that most of the male and female respondents in this study complaints of having hypertension could be related to the chronic low levels to heavy metals through different route of exposure in the contaminated pesticide-treated farmland.

In addition, the toxicity of lead also can either be acute or chronic and it also can cause hypertensions which as the result of male and female respondents in Sabak Bernam are mostly suffered from hypertension that might because of the lead poisoning. Failure to control the exposure will result in severe complications in the future because of the adverse effects imposed by heavy metals.

5.2 Contamination Levels of Heavy Metals Arsenic (As), Lead (Pb) and Cadmium (Cd) Accumulates in Flesh Muscle (2kg) of *Anabas testudineus* (Ikan Puyu)

From the result, the concentration of heavy metals followed the sequence of Lead > Arsenic > Cadmium). This study result is similar to Amirah et al., (2013) which showed the average concentration of Pb>As>Cd. On average, the concentration of Pb in the flesh muscle *Anabas testudineus* was 51.17 ppb while the concentration of As was 5.97 ppb. The concentrations of heavy metals in the tissues of fishes indicate the concentrations of heavy metals in water and their accumulation in food chains (Janadeleh & Kardani, 2018)

The concentration of Pb was higher in this present study and this is supported by the previous study stated that high level of Pb found in the paddy soil compared to the normal soil. Besides, Pb was also found at the roots parts rather than shoots and grains of the paddy plants. The paddy soil was found to be compared with the worldwide soil and the Pb value for roots were 9610 ppm and 9740 ppm, indicating high plant availability of Pb in the soil. (Satpathy, Reddy & Dhal, 2014). This is also supported by the previous study stated paddy fields are likely to receive numerous amounts of anthropogenic pollutants due to overuse of chemical fertilizers and pesticides. These applications will eventually contribute to a significant accumulation of heavy metals in the paddy field soil.

In addition, the exploitation of polluted water for irrigation, animal manures, mining activities and atmospheric depositions are also factors contributing to heavy metal contamination in this type of agricultural area. According to Sridhara et al., it was stated that heavy metals derived from anthropogenic sources can highly influence their speciation and availability in the soils. Most of the heavy metals derived from anthropogenic sources are likely to accumulate in the top soil.

The cadmium was not detected in the fish sample and this is supported from the previous study by Monalisa, Taeq, & Abdullah (2012) which presented the result of no cadmium detected in the *Anabas testudineus*. Based on the previous study also had found that the Cd concentration in the paddy soil is low compared to the other paddy soil. Compared with this present study, the Cd in the fish was not detected but in the previous study the Cd had the lowest concentration detected. And this indicated that the Cd did not mobilize and accumulate in the soil and thus prevent from getting inside along the food chain with the aquatic animals present in the paddy soil. This study can be supported by Satpathy, Reddy & Dhal (2014) which the concentration of Cd was higher in shoots than roots and grains of the plant parts of the paddy. This is indicates that the probability of the Cd accumulation in the soil is lower and when moving along the food chain, low aquatic food chain will take up the Cd. When compared to the Janadeleh & Kardani (2018), this present study also had similar results based on the Cd concentration found in fish. In the previous study, it was stated that Cd showed the lowest concentration in fish

In addition in this present study, Pb showed the results of the highest concentration in the fish compared to the other heavy metals. Pb is a non-essential element and was proved that the human activities can increase the concentration of Pb (Jabeen and Chaudry, 2010b). Pb is the first in the ascending order among the other heavy metals that can damage the human health. The accumulation of lead can reduce the intellectual performance, cognitive development in children which also can increase hypertension and cardiovascular disease in adults (Al-Hossainy et al., 2012).

Lead is a non-essential element and was proved that the human activities can increase the concentration of lead (Jabeen and Chaudry, 2010b). Based on the study of Amirah et al., (2013), the average concentration of Pb was also higher than Cd. Therefore, *Anabas testudineus* inhabiting the water accumulated and elevated the level of Pb in their flesh muscle from paddy field. The bioaccumulation of heavy metals from the natural and anthropogenic sources in fish are more common rather than in water sedimentation (Luinnik and Zubenko, 2000).

The concentration of As accumulates in the flesh muscle of *Anabas testudineus* is higher than the concentration of Cd. In the sample 2, the fish sample showed the highest reading which is 6.32 ppb compared to sample 1 and sample 3 which is 6.023 ppb and 5.563 ppb respectively.

Chintaka et al., (2016) presented the concentration of As is higher than concentration of Cd and this can be supported to the fact that in this present study which also showed the concentration of As is higher than the concentration of Cd. In the previous study, it was found that concentration of As in Tanjung Karang found in the paddy water samples but it does not exceed the maximum recommended value for irrigation water by Malaysia which is National Water Quality Standard (NWQS) and by Food and Agriculture Organization (FAO) (Rudzi, Ho, & Abd Khani, 2018). The exposure of arsenic can affect most of the organs in the human body including dermatologic, cardiovascular, nervous, renal hepatobiliary, gastro-intestinal and respiratory systems (Tchounwou et al., 2013).

5.3 Comparison Between Contamination Levels of Heavy Metals Arsenic (As), Lead (Pb) and Cadmium (Cd) Accumulates in Flesh Muscle (2kg) of *Anabas Testudineus* (Ikan Puyu) and Food Regulation 1985.

Result showed the heavy metals detected in the flesh muscle of *Anabas Testudineus* was Pb (51.17 ppb) > As (5.97 ppb) respectively and it showed significant ($p = 0.001$) between the heavy metals detected in the fish sample and Food Regulation 1985 when one-sample t-test was performed. Compared to the Food Regulation 1985, Pb and As concentration was 2000 ppb and 1000 ppb respectively. Both of the heavy metals were not exceeded the permissible limits in the Food Regulation 1985. This may due to the fact that fish sampling is after the paddy harvesting season which causes low level of heavy metals residuals' effluent into the environment.

The Food Regulation 1985 sets Pb, As and Cd of 2000 ppb, 1000 ppb and 1000 ppb respectively. Although the concentration of As and Pb is below than the Food Regulation 1985, the accumulation of heavy metals in the flesh muscle of *Anabas Testudineus* could occur and thus increase the level of heavy metals in the fish and not surprisingly the concentration of heavy metals might be higher than this present study in the future. Based on Satpathy, Reddy & Dhal (2014), the low Pb concentration may not pose any potential health risk but it can cause adverse effects at a certain level of concentration.

Sungai Besar provides substantial level of local economic activities involving fish production and rice irrigation. Overall the concentration of As and Pb is below than the Food Regulation 1985 and this result is to what have been found in previous study (Saliza et al., 2014).

Nevertheless, the accumulation of heavy metals in the flesh muscle of *Anabas testudineus* could possibly occur in the long-term basis and thus increase the level of heavy metals in the fish and not surprisingly the concentration of heavy metals might be higher than this present study in the future. The presence of cumulative low level toxic heavy metals in fish can invalidate their beneficial effects (Bawuro et al., 2018). Several unfavorable effects of heavy metals to human health have been known for long time (Castro-Gonzalez et al., 2008), which includes serious threat like renal failure, liver damage, cardiovascular disease and even death (Al-Busaidi et al. 2011; Rahman et al., 2012).

However, in this study there is no Cd detected and this can be supported by a study of Monalisa et al., (2014), Cd is not detected in *Anabas testudineus*. This is maybe due to there is no indication of bio magnification of cadmium along the aquatic food chain in the paddy field. However, Cd can accumulate in human body and may cause kidney damage, skeletal damage, and reproductive impairment (Janadeleh & Kardani, 2018).

Therefore, the results may be varied among the studies as there are many factors that can affect the bioaccumulation in fish. Besides, no detection of cadmium can be because of there is no indication of bio magnification of cadmium along the aquatic food chain in the paddy field. Cd is a trace elements that cannot be chemically degraded unlike the organic pollutants (Hooda, 2010) or biodegraded by living organisms or microorganisms (Mermut et al., 1996). Hence all these chemicals can be easily taken up and stored faster than metabolized (Extension Technology network, 1993).



5.4 Dietary health risk of respondents consumed *Anabas testudineus* (Ikan Puyu) from paddy field in Sabak Bernam

Based on the dietary health risk after the calculation of Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR), findings in this study showed that HQ of arsenic and lead are both lowered than one. This result was similar to the Janadeleh & Kardani, (2018) which stated the HQs of the individual metals were below than one which indicated the daily intake of these metals would not cause any adverse effects for local residents. Both of the heavy metals indicate there is did not cause considerable human health risks. It might be due to relatively low concentration of heavy metals detected in the flesh muscle of *Anabas testudineus*. This result can be supported by the previous study which also shows the health risk below than one but compared to the previous study, the result shown that Pb concentration is exceeded the permitted limit in Food Regulation 1985 (Saliza et al., 2014).

The Lifetime Cancer (LCR) risk of As is in the unacceptable range risk which is greater than 10^{-4} . Another study reported the LCR calculated for As were 1.67×10^{-4} which was in the unacceptable risk level of LCR, indicating the carcinogenic health risk was unacceptable. Nevertheless, this present result was comparable with few similar studies, which the calculated LCR was in unacceptable range.

Hence, the carcinogenic risk faced by the respondents considered unacceptable risk from carcinogenic effects. This present study was also supported by Yujun et al. (2011) which suggested people would experience health risk from the individuals' intake of metals contaminants through the consumption of fish.

Although the present data of health risk of a heavy metals which is As and Pb is below than one, but there is still some concentration of As and Pb found in the flesh muscle of *Anabas testudineus* which may induced cumulative subtle health effect to human. The concentration of As and Pb can be accumulated and might be imposed to a certain health risk if the fish is consumed continuously and there will be no guarantee that the risk will remain safe.

The continuous intake of heavy metals contaminated fish can cause health effects. From the previous researcher had proven that the long term intake of heavy metals even at low concentration of exposure can cause several health effects. According to Lustberg et al. (2002), intake of Pb can cause detrimental on our human health and more specifically is on our cardiovascular system. Heavy metals are known to be poorly absorbed into the body but once they are absorbed to the body, they are slowly expelled and accumulated which can cause organ damage in the body.

5.5 Study Limitation

This finding in this study has limitation by only calculated the health risk of the respondents from one type of exposure only which is ingestion of fish that is contaminated with heavy metals but not considered through the inhalation and dermal contact. In this study also did not considered the environmental monitoring such as water and soil monitoring because the accumulation of heavy metals could be transferred to the water surface and absorption in the soil.

The environmental monitoring such as water and soil monitoring could be done in the future. Last but not least, this study is only considered fish as the biomarker to detect the heavy metal and not considered blood to detect the presence of heavy metals by the ingestion of fish that is contaminated with heavy metals.

5.6 Conclusion

In short, heavy metals were detected (Pb = 51.17 ppb) and (As = 5.97 ppb) in *Anabas testudineus* (*Ikan Puyu*) from paddy field in Sungai Besar, Selangor but in this study the Cd cannot be detected. However the presence of heavy metal was not exceeded the limit of Food Regulation 1985.

Although the level of Pb and As were not exceeded the standard and detected in the small amount, but the accumulation in fish can occur. There is no safe guarantee for the consumer to eat the fish entire of their life due to the several factors. The health risk is indicated by the calculation of Hazard Quotient (HQ) for non-carcinogenic effects and Lifetime Cancer Risk (LCR) for carcinogenic risk. The HQ of Pb and As was lower than one which means that the daily intake of these metals would be unlikely to cause adverse health effects for local residents.

While for the LCR showed within the unacceptable range which is greater than 10^{-4} . And this showed the carcinogenic health risk may face by the respondents can be considered as unacceptable risk.

5.7 Recommendation

It is suggested to educate the people on safe surface water to ensure people do not dispose their waste water such as pesticides waste in river or trench in order to reduce the contamination by pesticides and heavy metals.

Furthermore, introduction of such programs such as pesticide laws and regulations, handling chemical spills, storage of chemicals pesticides toxicity and health effects at the training institution especially for the famers in Sungai Besar.

Last but not least, continuous monitoring of the control the effluent of agriculture to reduce the risk of contamination of fish in the paddy field.(Raikwar, Kumar, Singh, & Singh, 2014)

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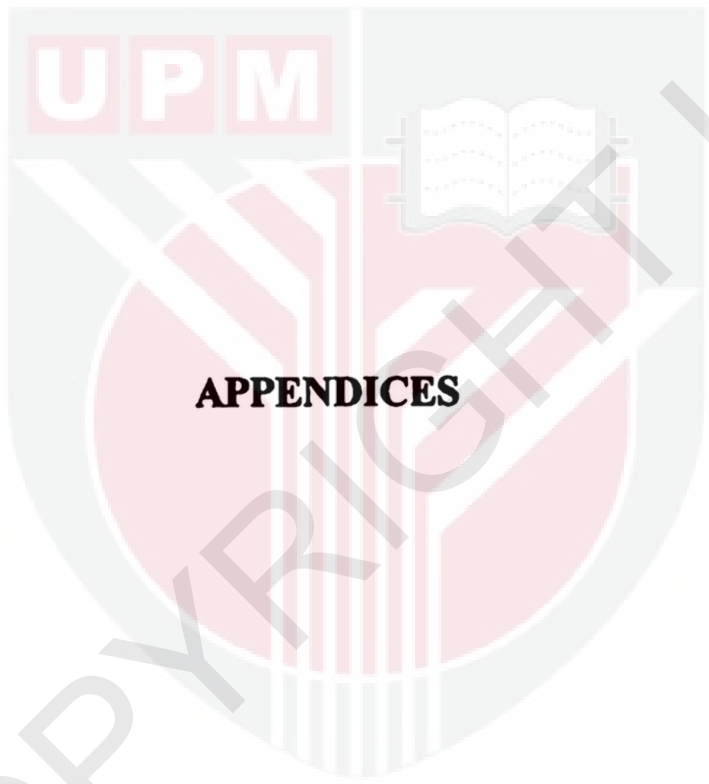
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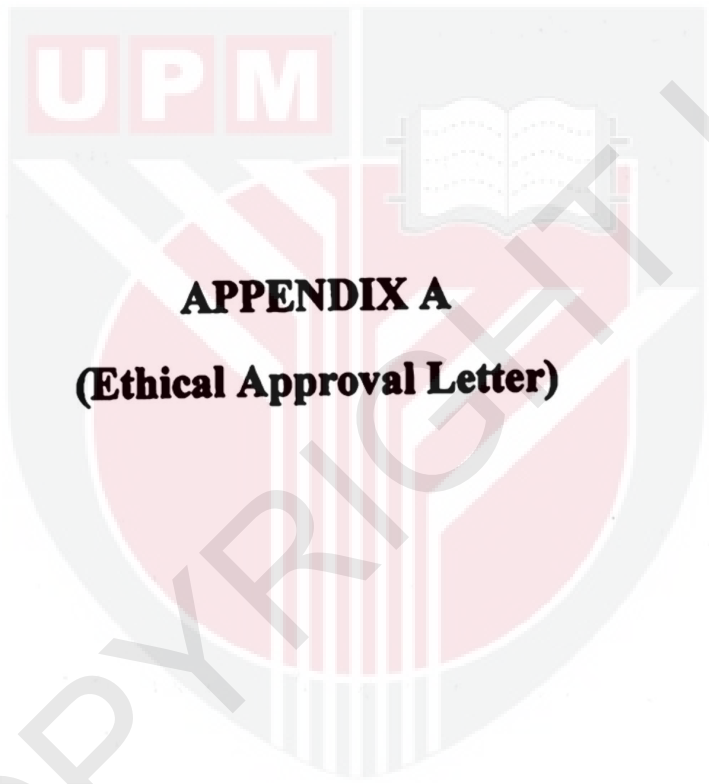
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APPENDICES



APPENDIX A
(Ethical Approval Letter)

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**ETHICS COMMITTEE FOR RESEARCH INVOLVING HUMAN SUBJECTS
(JKEUPM)
UNIVERSITI PUTRA MALAYSIA**

Research title	: Dietary Health Risk Assessment and the Determination of Heavy Metals in Fish from Paddy Field in Sabak Bernam, Selangor
Study Site	: Sabak Bernam, Selangor
JKEUPM Ref No.	: JKEUPM-2018-361
Researcher	: Nurul Nadiah binti Mohkhtar @ Mokhtar
Supervisor	: Dr. Vivien How

Documents received and reviewed with reference to the above study:

1. Ethics Application Form, Version 1 dated 29/10/2018
2. Respondent Information Sheet & Consent (English), Version 1 dated 29/10/2018
3. Proposal (English), Version 1 dated 29/10/2018
4. Questionnaires/ Interviews (English), Version 1 dated 29/10/2018
5. Curriculum Vitae of:
 - a. Dr. Vivien How
 - b. Dr. Ho Yu Bin

The University Research Ethics Committee, Universiti Putra Malaysia (JKEUPM) operates in accordance to the ICH-GCP Guidelines.

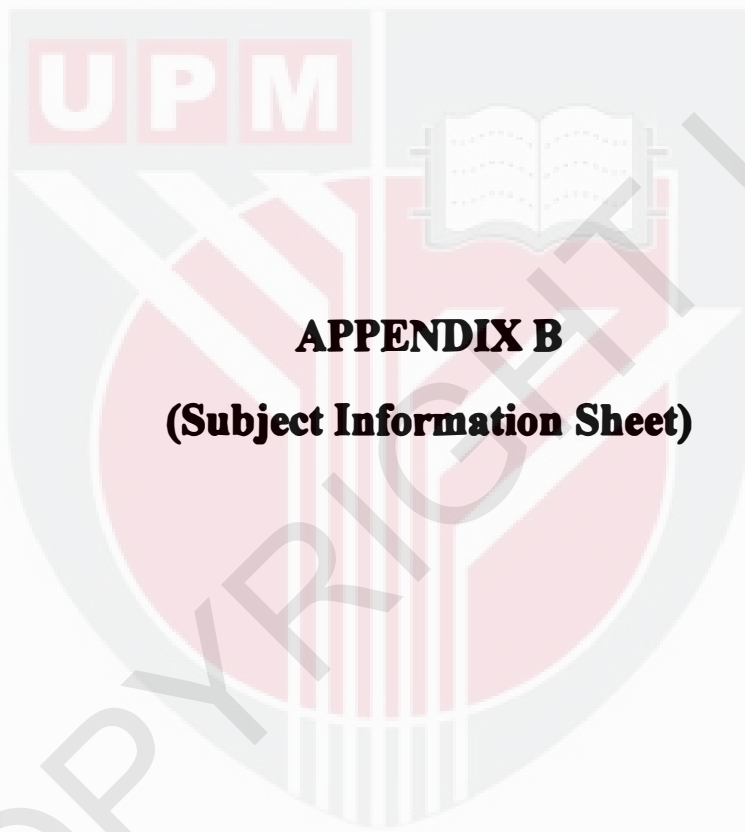
Decision by JKEUPM:

- Approved
- Permission MUST BE OBTAINED from the respective hospitals/ institutions before conducting the research**
- Disapproved

Please note that the approval is **VALID UNTIL 27 NOVEMBER 2019**

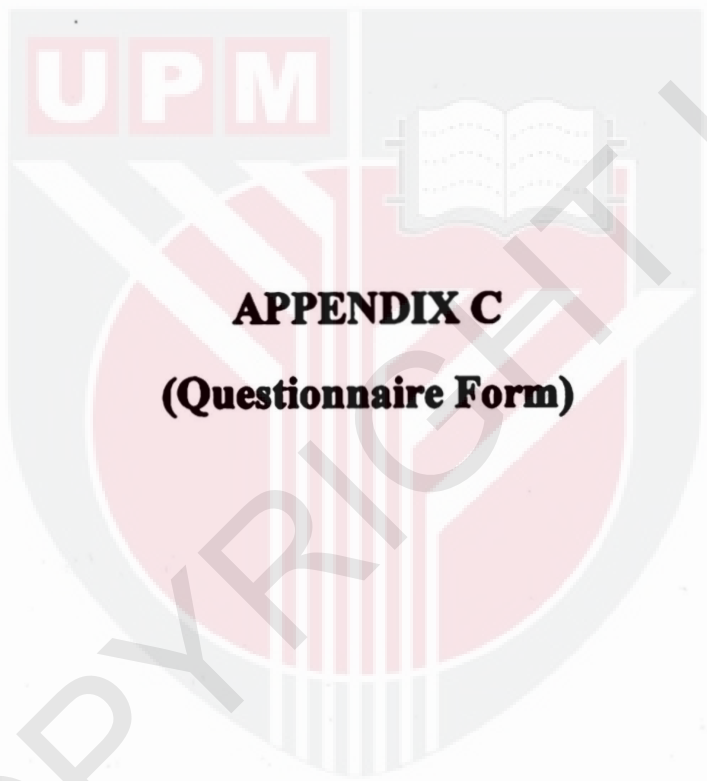
Researchers should comply with the following:

- I. Complete a Study Final Report upon study completion (Form 3.2).
- II. Ethical approval is required in the case of amendments/ changes to the study documents/ study sites/ study team.
- III. Applicable for Clinical Trial Studies and Clinical interventional Studies only: Progress Report has to be submitted to JKEUPM at every 6 months from the date of approval (Form 3.1). Report occurrences of all Serious Adverse Events (SAEs), Suspected Unexpected Serious Adverse



APPENDIX B
(Subject Information Sheet)

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APPENDIX C
(Questionnaire Form)

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BORANG 2.4: PENERANGAN DAN PERSETUJUAN RESPONDEN

Sila baca maklumat berikut dengan teliti. Sekiranya anda mempunyai sebarang pertanyaan, sila kemukakan kepada penyelidik.

1. TAJUK KAJIAN

Penilaian Risiko Kesihatan Pemakanan dan Penentuan Logam Berat dalam Ikan dari Sawah Padi di Sabak Bernam, Selangor

2. PENGENALAN

Ikan adalah makanan penting yang mewakili sebahagian besar rantai makanan semulajadi; kerana ia adalah sumber utama vitamin, pencernaan dan mineral lemak tak tepu (PUFA). Walaupun logam dalam tubuh manusia membolehkan fungsi otak yang sihat, logam berat, sebaliknya, dianggap sebagai logam toksik yang merugikan kesihatan manusia. Tanah yang tercemar dengan logam berat akan memberi impak negatif kepada ekosistem apabila logam berat diambil oleh tumbuh-tumbuhan dan haiwan dan mengakibatkan mengancam ekosistem disebabkan pemindahan dan pengumpulan bahan-bahan racun makhluk perosak atau bahan kimia lain dalam organisma. Penggunaan ikan yang tercemar dengan logam berat oleh manusia menyebabkan kerosakan pada kesihatan manusia dan logam berat boleh menjadi toksik kepada tubuh manusia apabila melebihi had tertentu. Oleh itu, kajian ini bertujuan untuk mengkaji kepekatan logam berat dalam ikan dan menilai risiko kesihatan pemakanan di kalangan penduduk di Sabak Bernam.

3. APAKAH YANG PERLU ANDA LAKUKAN?

Kajian ini akan melibatkan satu set soal selidik dan ikan dari sawah di Sabak Bernam. Anda boleh memilih sama ada dalam kajian ini atau tidak. Jika anda sukarela dalam kajian ini, anda boleh menarik diri pada bila-bila masa tanpa sebarang akibat / penalti dalam apa jua bentuk. Sebelum menjawab soalan, anda dinasihatkan untuk membaca, memahami dan menjawab semua soalan dalam borang soal selidik. Selepas menjawab semua soalan, borang soal selidik harus dikembalikan kepada penyelidik. Berikutan itu, penyelidik akan mengumpul ikan dari sawah padi.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

Responden yang mempunyai syarat berikut dikecualikan daripada menyertai kajian ini:
i. Responden yang tidak bersetuju untuk mengambil bahagian dalam kajian ini.

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?

Kajian ini akan memberikan pandangan kepada responden mengenai kehadiran logam berat dalam ikan dari sawah padi. Logam berat berasal daripada racun serangga sawah.

b) KEPADA PENYELIDIK?

Kajian ini akan menjadi kajian perintis tentang potensi risiko kesihatan manusia mengenai penggunaan ikan dari sawah padi.

6. ADAKAH IA BERISIKO?

Tidak ada risiko yang boleh dijangkakan untuk mengambil bahagian dalam kajian ini selain daripada yang ditemui dalam kehidupan seharian.

7. ADAKAH MAKLUMAT DAN IDENTITI SAYA KEKAL RAHSIA?

Semua maklumat yang diperolehi melalui soal selidik dan borang maklumat dan borang kebenaran responden ini adalah sulit dan tidak akan didedahkan kepada mana-mana pihak ketiga. Soal selidik yang lengkap akan dikodkan menggunakan kod pengenalan unik. Butiran individu tidak akan dimasukkan ke dalam mana-mana bahagian notis penyelidikan dan penerbitan. Jika anda diminta untuk mengetahui hasil penyelidikan ini, kami senang memberikan hasilnya.

8. SIAPA YANG SAYA PERLU HUBUNGI SEKIRANYA SAYA MEMPUNYAI SOALAN TAMBAHAN SEMASA MENGIKUTI PENYELIDIKAN INI?

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Sila tandatangan di sini sekiranya anda telah membaca dan memahami kandungan halaman ini _____

9. PERSETUJUAN

Saya..... No Kad . Pengenalan.
beralamat.....
.....dengan ini bersetuju untuk mengambil bahagian secara sukarela dalam penyelidikan yang tersebut di atas *(kajian klinikal/percubaan ubat-ubatan/rakaman video/kumpulan sasaran/temuduga/ soal selidik).

Saya telah diberi penjelasan secara menyeluruh mengenai penyelidikan ini dari segi metodologi, risiko dan komplikasi (seperti tertulis pada Helaian Penerangan Responden). Saya memahami bahawa saya berhak menarik diri dari penyelidikan ini pada bila-bila masa tanpa memberi sebarang alasan.Saya juga memahami bahawa sebarang maklumat yang berkaitan identiti saya akan dirahsiakan.

Saya* berminat / tidak berminat untuk mengetahui keputusan kajian yang melibatkan saya.

I setuju/tidak bersetuju untuk imei/gambar/rakaman video/ rakaman suara digunakan dalam apa jua bentuk penerbitan atau pembentangan. (sekiranya berkaitan).

*potong yang tidak berkenaan

Tandatangan Tandatangan
(Responden) (Saksi)

Tarikh :..... Nama :.....
No. K/P:

Saya mengesahkan bahawa saya telah menerangkan kepada responden ini sifat dan tujuan penyelidikan yang tersebut di atas.

Tarikh Tandatangan
(Penyelidik)

ID RESPONDEN

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BERILMU BERBAKTI

PROJEK PENYELIDIKAN TAHUN AKHIR

B.S. KESIHATAN PERSEKITARAN DAN PEKERJAAN
FAKULTI PERUBATAN DAN SAINS KESIHATAN
UNIVERSITI PUTRA MALAYSIA
SERDANG, SELANGOR

**BORANG SOAL SELIDIK KAJIAN PENILAIAN RISIKO KESIHATAN
LOGAM BERAT MELALUI PENGAMBILAN PEMAKANAN IKAN PUYU DI
SAWAH PADI, SABAK BERNAM.**

Nama responden	
Alamat responden	
Nombor telefon	

BAHAGIAN - A (MAKLUMAT PENDUDUK KAMPUNG)

Sila pilih pilihan yang menunjukkan pilihan anda. Responden perlu menandai atau menulis dalam kotak yang disediakan.

1. Kaum:

- Melayu Cina India
 Lain-lain

2. Jantina:

- Lelaki Perempuan

3. Umur: _____

4. Tahap pendidikan

- Sekolah rendah Sekolah menengah Diploma
 Ijazah Lain-lain

5. Apakah keadaan anda ?

- Bekerja Tidak bekerja Pelajar
 Pesara

6. Pekerjaan

- Petani Bukan petani

7. Status pendapatan:

- <RM 1000 RM 1000-RM 3000 > RM 3000

8. Weight: _____ kg

9. Height: _____ cm

BAHAGIAN - B (GAYA HIDUP)

Sila pilih pilihan yang menunjukkan pilihan anda. Responden perlu menandai atau menulis dalam kotak yang disediakan.

1. Adakah anda seorang perokok rokok?

- Ya tidak

2. Jika ya, berapa kali anda merokok setiap hari?

_____ Rokok

3. Berapa umur anda mula merokok secara teratur?

_____ tahun

4. Jika anda telah berhenti merokok, berapa umur anda berhenti merokok?

_____ tahun

5. Adakah anda mengambil minuman keras (alkohol)?

Ya Tidak

6. Berapa banyak botol untuk diminum dalam sehari?

BAHAGIAN - C (PENGGUNAAN RACUN PEROSAK)

Sila pilih pilihan yang menunjukkan pilihan anda. Responden perlu menandai atau menulis dalam kotak yang disediakan. (Bahagian ini akan memasukkan soalan mengenai penggunaan racun perosak di sawah anda)

1. Adakah anda menggunakan sebarang racun makhluk perosak di sawah padi anda ?

Ya Tidak

2 Adakah anda membeli racun perosak dari satu atau lebih pembekal lain ?

Ya Tidak

3. Adakah anda membeli jenama racun serangga yang sama untuk digunakan di ladang anda, atau anda sering membeli jenama yang berbeza ?

Ya Tidak

4. Berapa kerap anda menggunakan racun perosak untuk penanaman padi setiap tahun?

_____ setahun

5. Senaraikan jenis racun perosak yang digunakan untuk penanaman padi.

Bil.	Jenis tumbuhan	Keadaan padi	Jenis racun perosak	Kuantiti penggunaan racun perosak makhluk

6. Adakah anda kesan racun perosak ke medan padi air?

Ya Tidak

7. Pernahkah anda menyaksikan kematian ikan secara besar-besaran di sawah padi anda?

Ya Tidak

8. Adakah ikan menunjukkan apa-apa tanda yang tidak normal sebelum kematian?

Ya Tidak

9. Adakah anda bimbang tentang kesan racun perosak kepada kehidupan akuatik? Terangkan.

Ya Tidak

BAHAGIAN - D (KONSUMSI IKAN)

Sila pilih pilihan yang menunjukkan pilihan anda. Responden perlu menandai atau menulis dalam kotak yang disediakan.

1. Adakah anda atau rumah anda menangkap dan memakan ikan dari 12 bulan yang lalu?

Ya Tidak

2. Berapa kerap dalam tempoh 12 bulan yang lalu adakah anda makan ikan haruan dan puyu apabila ikan berada pada musim?

- 1-6 kali setahun
- 7-11 kali setahun
- 1 kali sebulan
- 2-3 kali sebulan
- 1 kali seminggu
- 2 kali seminggu
- 3-4 kali seminggu
- 5-6 kali seminggu
- Setiap hari
- 2 atau lebih kali sehari

_____ (Hari, minggu, bulan, tahun)

3. Bagaimanakah anda memasak ikan puyu dan haruan?

- Wap (Steam)
- Panggang
- Menggoreng
- Merebus

Cara memasak lain: _____

4 .Apakah faktor yang mempengaruhi anda untuk menangkap ikan?

5 .Bagaimanakah anda biasanya memberitahu sama ada ikan segar yang anda jangkakan adalah kualiti makanan atau tidak.

- Warna kulit Warna Gill Bau Mata
 Keteguhan daging Tidak tahu

6. Berapa banyak ikan yang anda makan dalam satu hidangan?

_____ Setiap hidangan

BAHAGIAN - E (STATUS PERUBATAN)

Sila pilih pilihan yang menunjukkan pilihan anda. Responden perlu menandai atau menulis dalam kotak yang disediakan.

1. Adakah anda mempunyai apa-apa rekod perubatan kesihatan anda?

- Ya Tidak

2. Adakah anda mempunyai pengalaman satu isu kesihatan selepas makan Ikan Puyu dan Channa striatus?

- Ya Tidak

3. Adakah anda mempunyai sekatan yang diperintahkan oleh doktor mengenai kebiasaan makan? Terangkan.

- Ya Tidak

4. Adakah anda mempunyai keadaan jantung seperti kegagalan jantung kongestif atau penyakit jantung koronari?

- Ya Tidak

5. Adakah anda mengalami gangguan paru-paru-seperti asma atau COPD?

- Ya Tidak

6. Adakah anda dirawat oleh ahli psikiatri atau ahli psikologi?

- Ya Tidak

7. Adakah anda mempunyai diabetes?

- Ya Tidak

8. Tekanan darah tinggi?

- Ya Tidak

9. Adakah anda mempunyai penyakit ginjal atau adakah dialisis?

- Ya Tidak

10. Adakah anda mempunyai kanser?

Ya Tidak

11. Adakah anda mempunyai penyakit darah, seperti anemia sel sabit atau Hepatitis?

Ya Tidak

12. Nyatakan keadaan kesihatan yang lain: _____



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