



UNIVERSITI PUTRA MALAYSIA

***HEALTH RISK ASSESSMENT OF HEAVY METAL VIA DIETARY
INTAKES OF CHANNA STRIATUS FISH BODY FROM PADDY FIELD
SABAK BERNAM.***

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BY

ERNNEY SHUHEIDA BINTI KHAMARUDIN

**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

HEALTH RISK ASSESSMENT OF HEAVY METAL VIA DIETARY INTAKES *CHANNA STRIATUS* FISH BODY FROM PADDY FIELD, SABAK BERNAM.

ERNNEY SHUHEIDA KHAMARUDIN

Introduction: *Sabak Bernam* is known as the rice producing area. For decades, the government introduced industrialized agriculture to increase the food crop yields in order to feed the growing population in the country. Synthetic pesticides are among the chemicals widely used throughout the industrial agriculture development. Nevertheless, the pesticide residues have gradually polluted and harmed the aquatic life, especially fish as it lives and feeds in the contaminated water. Therefore, the consumption of contaminated fish could affect the health of the consumer. **Objective:** The purpose of this study was to determine the concentration of different heavy metals that accumulate in the fish from paddy river and its dietary human health risk to the villager. **Methodology:** The study was conducted at Sungai Besar, Sabak Bernam where 120 Malay villagers were randomly selected to answer the questionnaire related to dietary health intake of *C. striatus* fish. The data from the questionnaire was analysed using Statistical Package for the Social Sciences (SPSS 20.0) and data for the level of heavy metal that accumulates in the fish collected from the paddy river were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) while for the estimation of dietary health. **Result and discussion:** The results will subsequently be used to estimate non-carcinogenic dietary health risks among fish consumer by using the Hazard Quotient (HQ) formula and Lifetime Cancer Risk (LCR) formula. Low concentrations of heavy metals for lead and arsenic are detected in fish muscles. Results showed that heavy metals detected in the fish muscles ranged as follows: lead (0.00828 mg/kg) > arsenic (0.00589 mg/kg) and cadmium was found undetected. As compared to the Food Act 1983, the concentration did not exceed the permitted level of arsenic and cadmium. The calculated non-carcinogenic health risk due to consumption of selected *C. striatus* fish showed that HQ not exceeds to one and the result for LCR was within 10^{-4} and 10^{-6} . **Conclusion:** The consumption of *C. striatus* fish in the *Simpang Lima* trench can be considered as safe because the HQ value was below one and LCR was in range. Consumption of fish would be considered dangerous if the HQ value is greater than one while for LCR more than 10^{-4} . Although the heavy metals residuals found in fish were considered low, the low level chronic exposure via ingestion of contaminated fish is of particular concern. Villagers need to limit the consumption of contaminated *C. striatus* fish, especially directly caught from paddy river. At the same time, local farmers should reduce the pesticide used, as well as to minimize the pesticide drift that would increase the chances of cross-contamination in the water and soil near to the village and paddy field.

Keyword: Fish body, pesticide, paddy field, arsenic, lead, cadmium, hazard quotient

ABSTRAK

PENILAIAN RISIKO KESIHATAN LOGAM BERAT MELALUI PENGAMBILAN PEMAKANAN IKAN *CHANNA STRIATUS* DI SAWAH PADI, *SABAK BERNAM*.

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Pengenalan: *Sabak Bernam* dikenali sebagai kawasan pengeluaran beras. Selama beberapa dekad, kerajaan memperkenalkan system pertanian perindustrian untuk meningkatkan hasil tanaman makanan untuk menampung makanan disebabkan peningkatan populasi penduduk di negara ini. Racun perosak sintetik adalah antara bahan kimia yang digunakan secara meluas dalam pembangunan pertanian perindustrian. Walaubagaimanapun, sisa-sisa racun makhluk perosak secara perlahan-lahan mencemarkan dan merosakkan kehidupan akuatik terutama ikan kerana ia hidup dan makan makanan dalam air yang tercemar. Oleh itu, penggunaan ikan yang tercemar dapat menjejaskan kesihatan pengguna. **Objektif:** Kajian ini adalah untuk menentukan kepekatan logam berat yang berbeza yang terkumpul di dalam ikan dari parit dan risiko kesihatan manusia kepada penduduk kampung. **Kaedah:** Kajian ini dijalankan di kampung *Simpang Lima* di *Sungai Besar, Sabak Bernam* di mana 120 penduduk Melayu dipilih secara rawak untuk menjawab soal selidik yang berkaitan dengan pengambilan makanan kesihatan *Channa striatus*. Data dari soal selidik dianalisa menggunakan Pakej Statistik untuk Sains Sosial (SPSS 20.0) dan data untuk tahap logam berat yang terkumpul di dalam ikan yang dikumpulkan dari sungai padi dianalisis menggunakan Spektrometri Massa Plasma yang Digabungkan secara Induktif (ICP-MS) untuk mengira risiko kesihatan pemakanan. Hasilnya digunakan untuk menganggarkan risiko kesihatan diet bukan karsinogen di kalangan pengguna ikan dengan menggunakan formula Darjah bahaya (HQ) dan risiko kanser seumur hidup (LCR)) formula. **Hasil dan perbincangan:** Kepekatan logam berat yang rendah untuk plumbum, dan arsenik dikesan dalam otot ikan. Keputusan menunjukkan bahawa logam berat dikesan dalam pelbagai otot ikan seperti berikut: plumbum (0.00828 mg/kg) > arsenik (0.00589 mg/kg) dan kadmium didapati tidak dapat dikesan. Berbanding dengan Akta Makanan 1983, kepekatan itu tidak melebihi tahap arsenik dan kadmium yang dibenarkan. Risiko kesihatan bukan karsinogenik yang dikira kerana penggunaan ikan *C. striatus* yang terpilih menunjukkan bahawa HQ diperoleh di bawah satu dan Keputusan LCR antara 10^{-4} sehingga 10^{-6} . **Kesimpulan:** Ikan *C. striatus* yang ditemui di parit *Simpang Lima* boleh dianggap sebagai selamat untuk pengambilan makanan kerana nilai HQ berada di bawah nilai satu dan nilai LCR di dalam julatnya. Penggunaan ikan akan dianggap berbahaya jika nilai nilai HQ lebih besar daripada satu manakala untuk LCR lebih daripada 10^{-4} . Walaupun sisa logam berat yang didapati dalam ikan dianggap rendah, pendedahan kronik tahap rendah melalui pengambilan ikan tercemar adalah kebimbangan khusus. Penduduk perlu mengindahkan pemakanan ikan *C. striatus* yang tercemar, terutamanya secara langsung ditangkap dari sungai padi. Pada masa yang sama, petani tempatan harus mengurangkan racun perosak yang digunakan, serta meminimumkan racun perosak yang akan meningkatkan peluang pencemaran silang di dalam air dan tanah berhampiran kampung dan sawah padi.

Kata kunci: Isi ikan, Sawah padi, Darjah bahaya (HQ).

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LIST OF ABBREVIATIONS

ICP-MS	Inductively coupled plasma mass spectrometry
HQ	Hazard Quotient
NPIC	National Pesticide Information Center
ATSDR	Agency for toxic substance and disease registry
GDP	Gross domestic product
WHO	World Health Organization
USEPA	United States Environmental Protection Agency
USAID	United States Agency for International Development
DHSS	Department Of Health and Social Security

CHAPTER 1

INTRODUCTION

1.1 Background

For decades, increases of population growth and economic development have moved Malaysia into a fast developing country in the Association of Southeast Asian Nations (ASEAN) region. Since rice is the most important food crop of the developing nation, Malaysia has initiated the effort to provide sufficient rice for the nation through industrialising the agricultural sector (Mohd-Taib et al., 2018). The prevailing agricultural system has vast health and environmental impacts, especially with the use of synthetic pesticides. Agricultural contaminants not only increase the myriad of health risks to farmers, they also impact ground and surface water, as well as depleted soil health. *Sabak Bernam* is one of the districts in Selangor that adapted the use of industrialized farming to enhance the rice production yield in large quantities while combating pests, weed, fungal and climate change. There were little types of pesticides such as herbicide, insecticide, fungicide, and bactericides being used in the paddy field in order to protect their crop and also to fasten the process. (Aktar, Sengupta and Chowdhury, 2009). Figure 1.1 show the location of the paddy field for the study location.

Synthetic chemical pesticides can easily be dispersed and dissolved into the environment and pollute the air, water and soil by spraying activity, soil seepage and water pollution (Pesticides reduce biodiversity, 2010). This problem did not only causing farmer's health who works directly in the farmland, but also causing danger the farm villagers' health from contaminated fish, soil and water. The use of pesticide without proper control in agricultural activity cause adverse health and environment effects to a large extent, include the aquatic lives. The use of synthetical chemicals in the farmland for a long duration of time would change the healthy ecosystem which later disrupts the harmony ecologically.

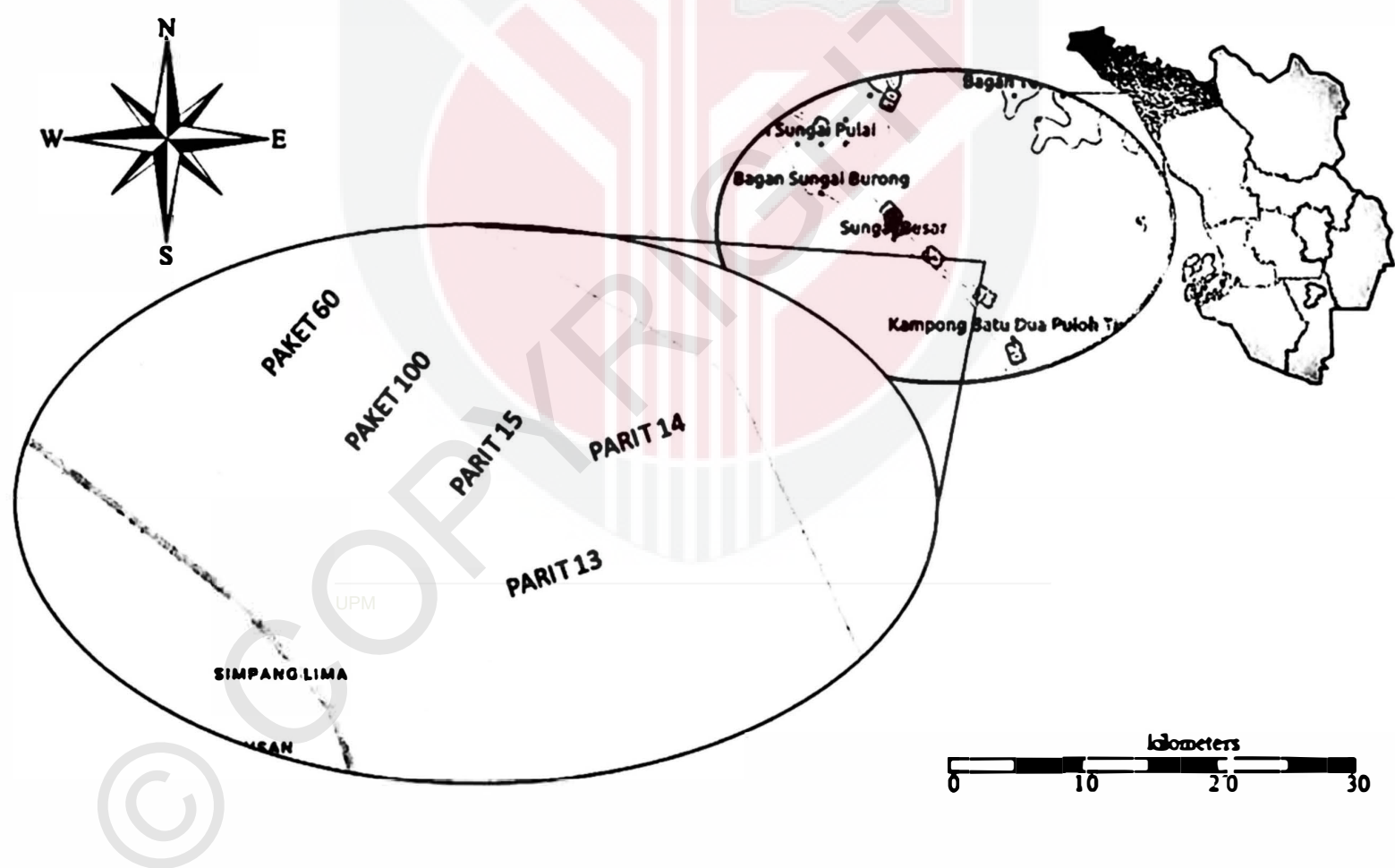


Figure 1.1: *Simpang Lima* paddy field in *Sungai Besar, Sabak Bernam, Selangor*.

Sabak Bernam is one of the districts in Selangor that had a total area of about 99,710 hectares consisting of the following village *Sabak, Bagan Nakhoda Omar, Panchang Bedena, Sungai Panjang, and Pasir Panjang*. 43.54% of the *Sabak Bernam* area was used to do an agricultural activity such as paddy plant, palm oil plant, coconut plant, and others. One of the significant agrarian activities was paddy cultivation, which uses around 13,375 hectares out of the *Sabak Bernam* area in 2013. Recently, this district has produced a new economy called 'Homestay' and chalet business which give the local population opportunities of new economic activities promoted by the Ministry of Tourism Development and the state government (Ngah, 2014). The business offers extra income to the villagers due to the attractions, beautiful scenery of the paddy field which provides calmness and visitors can experience rice cultivation activities during the stay as well as local culture. The significant impact on the potential health risks from synthetic chemical used at farmland will not only affecting the villager's health, the tourists would also experience the relatively health impact while visiting the pesticide-treated farmland.

The most famous nutrient sources in *Sabak Bernam* came from the paddy field was a freshwater fish such as *Channa striatus*. In Malaysia, *C. striatus* also known as *Haruan*, and this fish was very famous among other types of freshwater fish that live in the slow-moving water such as paddy field irrigation and trenches. This fish is a type of hardiness fish which capable live muddy area that has low level of oxygen and able to live without water for a long period. *C. striatus* is known as carnivore as their primary diet where it can eating small insect, tadpole and small fish and in some condition it also will become cannibalism which eating its own species.

C. striatus fish is famous in the Southeast Asian region as it contains a lot of health benefits and traditional remedy. Studies by Zubaidah et al., (2015) have shown that *C. striatus* provides an excellent source of health benefits because it has a high level of amino acid and is commonly used by postnatal women as a traditional remedy to promote wound healing to reduce pain after surgery. Also, it helps with the beneficial aspects of anti-inflammatory, antimicrobial, anti-nociceptive, and anti-cancer properties (Shafri and Manan, 2012; Michelle, Shanthi and Loqman, 2004). Besides, *Haruan* had to produce polyunsaturated fatty acid which helped to regulate prostaglandin synthesis inducing wound healing.

Other than that, *C. striatus* could be an antimicrobial agent that acts as an agent which kills microorganisms and stops their growth. Study had found that the antimicrobial from the animal extract was better for human consumption to avoid unnecessary repercussions. Anti-nociceptive properties in the *C. striatus* could be used to treat skin diseases like eczema because it was reported to help the patient relief from pain and irritation (Dambisya et al., 1999; Ganabadi, 2009). Mat Jais et al. (1997) state that, fillet and the mucus extracts was found to exhibit a concentration-dependent anti-nociceptive activity. Another study also shows that *C. striatus* extracts proved to be better in healing medicine than other traditional fish because it contains the density of protein gene product (PGP)-immunoreactivity nerve fibres in the synovial membrane in the rat model. *C. striatus* was the dominant freshwater fishes to have antioxidant activity. Lastly, *C. striatus* extract influence the serotonergic receptor system, thus it could be functioning as an anti-depression.

Due to *C. striatus*'s benefit, it is essential to ensure the fish muscle was safe for consumption. Since *C. striatus* fish is highly demanded and consumed by villager, this study was to emphasize the dietary health risk among fish consumer from rice farming villages at *Sabak Bernam*. The benefits of the fish should be protected, so the remedy would not give harmful to the user. Furthermore, the health risk of diet among fish consumers from the paddy fields in *Sabak Bernam* is more assured.

1.2 Problem Statement

Rice cultivation required a series of complex processes to achieve the finished product. In the process of paddy cultivation, the primary stages of cultivation include seed selection, land preparation, crop establishment, water management, nutrient management, crop health management, harvesting and post-harvest. In 1960's, Malaysia had change their paddy cultivation technique by introduce the used of irrigation system and used of synthetic chemical which help conventional paddy cultivation can be done twice in a year. By having this two innovation, it helped the farmer to combat againt pest and also increased the rice production. Unfortunately, this technique requires higher levels of pesticide application due to the resistance problem among the pest after a particular time. For example, herbicides were used to destroy weeds and harmful vegetation, insecticides to control a wide variety of insects, fungicides prevent the growth of mould and mildew, disinfectants prevent

the spread of bacteria, and compounds used to control mice and rats (Agrawal et al. 2010).

Inevitably, applying mixtures of pesticides to the field twice a year increased the pesticide exposure level to the environment and harm the human health (Kamarudin et al., 2016). The duration of paddy to complete the cultivation process took around one hundred days in average which mean the pesticide can stay in paddy water almost two hundred days in one year after the build of irrigation. In 1994, Ismail discovered that heavy metal lead and cadmium were present in the paddy field water due to pesticide and fertilizer used. In some factors, the pesticide can be spurted to the environment and transferred thousands of miles away and can be infiltrated into a plant and also animal especially fish. In fact, pesticide was very dangerous as it could enter into the fish's body by two ways which were by direct consumption of water and food through the digestive tract and non-dietary routes across porous membranes such as gill surface to higher levels than the environmental concentration that potentially pollute by the pesticide (Annabi, Said and Messaoudi, 2013; Ribeiro et al., 2005). This was because it could accumulate heavy metals in their tissues by absorption. Therefore, massive heavy metal levels in fish usually reflect levels found in sediment and water on the particular aquatic environment from which they sourced.

The other hand, non-essential heavy metals such as cadmium, arsenic, and lead have an unknown essential role in living organisms could exhibit extreme toxicity even at shallow exposure levels and have been regarded as the main threats

to all forms of life especially human health. Once human are exposed to the heavy metal by consuming the fish, it could be distributed to the brain, liver, kidney, and bones for the lead while for the Cadmium exerts toxic effects on the kidney, the skeletal and the respiratory systems (Soisungwan, 2018). As the fish was an essential part of the human diet, the content of toxic heavy metals exposed from pesticides in fish can counteract their beneficial effects. Several adverse effects of heavy metals on human health have been known for a long time. Hence, fish considered as one of the best indicators of heavy metal pollution in the coastal environment. For the extreme condition, these heavy metals could bring another type of health problem because there will be interaction between two or more agents among the heavy metal which called synergism.

1.3 Conceptual Framework

The aim was to determine the concentration of heavy metal (lead, arsenic, and cadmium) that accumulate in the muscle of *Channa striatus* fish. The objective was to determine whether the consumption of fish can affect human health by using hazard quotient formula (HQ) and lifetime cancer risk formula (LCR). Figure 1.2 shows the synopsis of the study. Since the farmer applied mixture of pesticide during paddy cultivating in the wetland paddy field, it is likely to presume that fish is possibly being contaminated with heavy metals that are presents in the farm water. Therefore, this study was to examine the level of heavy metals in the fish based on the recommended dietary intake as suggested by Food Act 1983 to prevent potential

health risks arising from the consumption of fish. Farmers were selected randomly as respondents to participate in this study. A set of self-administered questionnaires were used to examine the dietary intakes and behaviours of *C. striatus* fish from the paddy field. The dietary health risk was assessed based on the accumulated heavy metals found in the muscle of *C. striatus*. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to measure the level of heavy metal accumulation in the fish body that leads to adverse health effects toward the consumer. By having the data, we can obtain the non-carcinogenic health risks through dietary *C. striatus* fish intake among the study respondents.

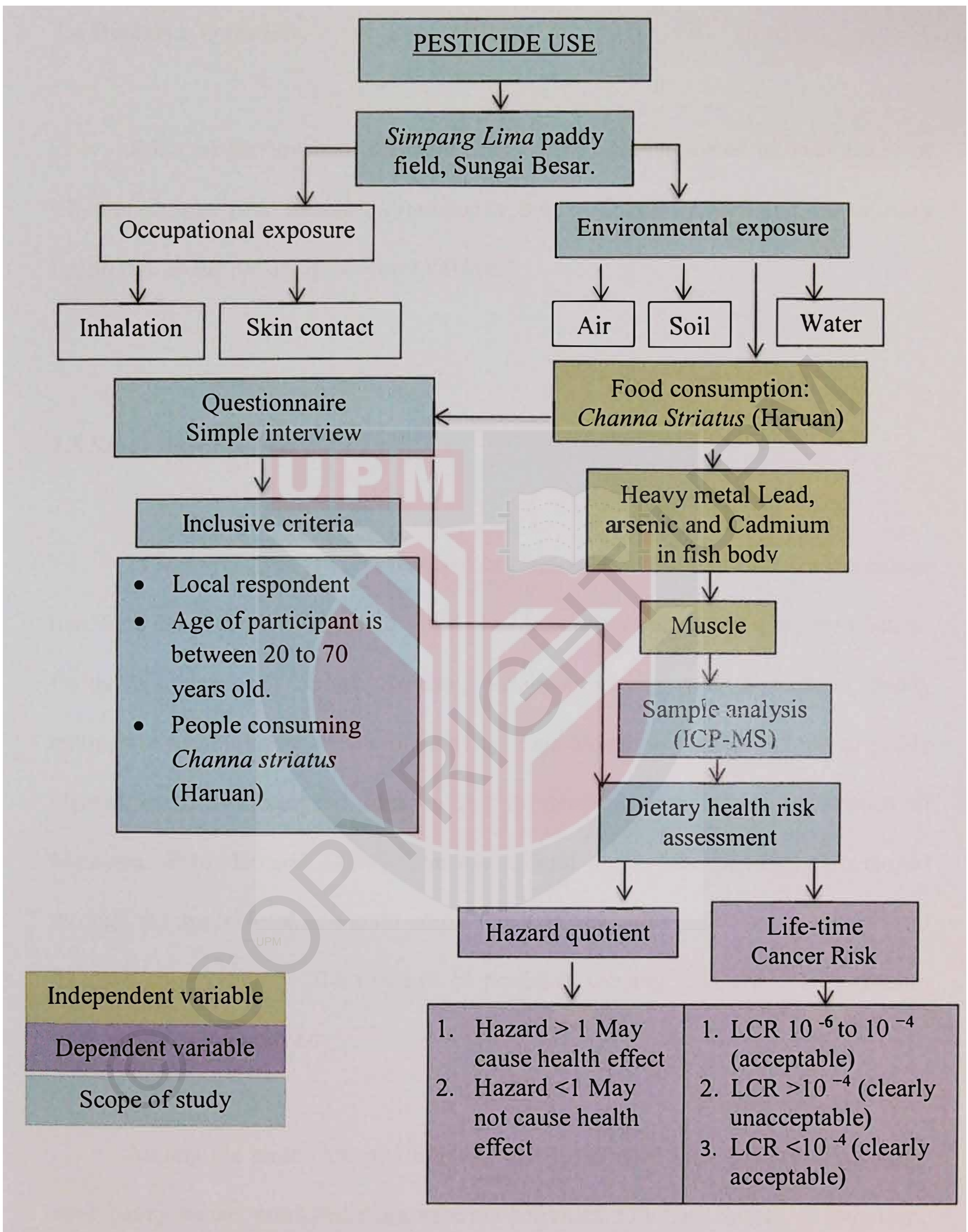


Figure 1.2: Conceptual Framework

1.4 Research Question

What are the levels of different heavy metals contaminated in body fishes of *Channa striatus* from *Simpang Lima* paddy field at *Sabak Bernam* and their dietary health risk to the paddy agricultural villager?

1.5 Study Justification

In Malaysia, the government had implemented a different initiative to ensure that food crop production could fulfil the demands of the increasing population. Paddy cultivation in *Sabak Bernam*, Selangor mostly was a wetland paddy cultivation technique for their crop activity. This technique can be defined as paddy planted in the waterlogged field during its growing stage (Paddy Statistics of Malaysia, 2015; Donald, 2004). The agricultural sector has massively developed through the use of fertilizers and various types of pesticides to accelerate growth and increase crop yields — the overuse of pesticide causing environmental pollution especially to aquatic lives.

Among the main elements found in fertilizers used for agricultural purposes were heavy metals produced from agrarian activities. Othman, Samat, & Sulaiman, (2006) reported that, there was a presence of heavy metal lead (Pb) and cadmium (Cd) in the paddy field water due to the use of synthetic pesticide during cultivation activities in *Sungai Burung, Tanjung Karang* which had share the similar paddy

cultivation as *Sabak Bernam*. Since the whole paddy cultivation process took 90 -120 days to complete, it is no wonder that the water and soil will be greatly polluted by continuous introducing the mixture of pesticides into the paddy farmland (Kamarudin et al., 2016).

The problem arise when agricultural authority subsidize the use of synthetic chemicals in the farm, encouraging, intensive farming system and targeted continuous land used for rice production within short interval of time. Synthetic pesticide will cause hazardous to all biotic and abiotic in the earth if the paddy field agrarians in the study area tended to continue with the farming method (Aktar, Sengupta and Chowdhury, 2009). Due to the activity, all the river water became contaminated with mixture of pesticides along with the paddy cultivation process. Moreover, killing insects or weeds using pesticides could cause toxic to a host of other organisms, including birds, fish, beneficial insects, and non-target plants. Both insecticides and herbicides can kill not only the targeted pest/weed, but also the non-target organisms due to its systemic effects.

Study shows that fish was the best sample that can be used to identify the level of heavy metal that the consumer was possibly exposed to. Fish could be an excellent indicator for the heavy metal contamination level in the aquatic system because fish occupies different food chain levels (Karadede-Akin and Unlu, 2007; Zhao et al., 2012). Fish can accumulate heavy metal in their tissue by absorption along the gill surface and kidney, liver and gut tract wall to a higher level than the environmental concentration (Annabi, Said and Messaoudi, 2013). In other words,

level of contaminants in the fish usually uses to reflect the level of contaminants found in the water from which they are sourced. This highlight of particular concerned, especially fish consumption has increased demand with the growing concern of its nutritional values. Therefore, it is very important to identify the levels of non- essential element in fish such as heavy metal are to ensure that it does not counter the nutritional benefits of fish.

1.6 Objective

To determine the concentration of different heavy metals (lead, arsenic, and cadmium) that accumulate in the *Channa striatus* fish from *Simpang Lima* paddy field water and its dietary human health risk to paddy agricultural villager.

1.6.1 Specific Objective

1. To determine the socio-demographic background, occupational information and self-reported health symptoms among the paddy farmers and villagers.
2. To examine the fish dietary habits among paddy agricultural villagers.
3. To determine the contamination level of heavy metals (lead, arsenic and cadmium) that accumulate in body fish of *Channa striatus*.
4. To compare the level of heavy metals contaminated in on *Channa striatus* fish's body with Food Act 1983.

5. To assess the dietary health risk based on the levels of heavy metal lead, arsenic and cadmium accumulated from up taking *Channa striatus* of fish by paddy agricultural villagers using hazard quotient (HQ) and lifetime cancer risk (LCR) formula.

1.7 Hypothesis

1. There is a significantly different level of heavy metal contamination in *Channa striatus* fish's body compared to the guidelines in Food Regulation 1983.

1.8 Terminology

1.8.1 Conceptual definition

I. Pesticides

The pesticide is a chemical compound that is used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). Pesticides are used by the human to kill vectors that causing disease, such as mosquitoes, and in agriculture, the pesticides are used as pest killers. By their nature, pesticides are potentially toxic to biotic and abiotic, which need to be used safely and disposed of properly (WHO, 2018).

II. Heavy metal

Heavy metals defined as metallic elements that have a relatively high density compared to water (Fergusson, 1990).

- **Lead**

The lead is a naturally occurring element, a member of Group 14 of the periodic table and has an atomic weight of 207.2. It also exists in three states which were Pb (0), Pb (II) and Pb (IV). The lead was a bluish-grey heavy metal, and it's usually found combined with two or more other elements to form lead compounds (Agency for Toxic Substance and Disease Registry, 2007). Most of them came from human activities. The lead concentration was

found high in the muscles and organs of fish. When accumulates in the human body, it replaces calcium in bones (DHSS, 1980).

- **Arsenic**

Arsenic is an element which categorise as a toxic in its inorganic form. It is naturally present at high level in the groundwater in number countries. Consumption of contaminated water or food with arsenic will cause chronic diseases. It also associated with cardiovascular and diabetes disease (WHO, 2018).

- **Cadmium**

Tobacco smoking, mining and manufacture of phosphate fertilizers are one of the cadmium sources that can be found naturally from volcanic activity and also human activity. Some crop such as rice highly accumulates with cadmium is daily consumed by the large population. Long-term exposure to the cadmium leads to the cancer and kidney disease. (WHO, 2019)

III. Oven dry

The drying oven method is a thermogravimetric method (loss on drying) in which the sample is dried for a defined period of time at constant temperature. A precise and rapid technique that allows the sample heated under specified conditions. (Foodtechsource, 2019)

IV. Closed vessel microwave digestion

Closed systems offer the advantage that the operation show the image of the food digestion similar to human process in short duration and detail result. It is essentially isolated from the laboratory atmosphere, thereby minimizing contamination. Digestion of the sample is essentially ensured by a common wet digestion procedure, which is performed under the synergistic effects of elevated temperature and pressure; digestion occurs at relatively high temperature due to boiling-point elevation (Henryk, 1999)

V. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was an analytical technique used for elemental determinations. The method was commercially introduced in 1983 and has gained general acceptance in many types of laboratories. Geochemical analysis labs were early adopters of ICP-MS technology because of its superior detection capabilities, particularly for the rare-earth elements (REEs) (Wolf, 2005).

VI. Body mass index

BMI is a weight (in kilograms) divided by height (in metres) squared. As an individual's height and weight can be readily and inexpensively measured, BMI has become a popular heuristic approximation for body fatness in epidemiology and clinical practice (Sperrin et al., 2015).

1.8.2 Operational definition

I. Pesticides

The pesticide was known as a hazardous chemical used in paddy cultivation activity for ensuring crop protected. The level of pesticide concentration can be measured using aquatic life such as fish that live in the paddy field where exposure takes place.

II. Heavy metal

Heavy metal was tiny particles that came from the pesticide sprayed during paddy cultivation. These activities cause health impact on a human because heavy metal was categories as a carcinogen that will cause carcinogenic. The level of heavy metal includes lead, arsenic, and cadmium contained in the pesticide measured by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to assess the presence of the heavy metal.

- **Lead**

Naturally, the lead was found in small amount in the earth's crust and also in all parts of our environment, but lead also can be found in human activity such as spraying pesticide. The lead that present in the water due to pesticide exposure could be accumulated in the bodies of fish since the fish live and feed in that contaminated water. Therefore, the consumption of fish can give a health problem to a consumer. The hazard quotient used as a tool to measure the level of lead which could give hazardous to health to prevent consumers from exceeding the recommended dietary intake of the lead.

- **Arsenic**

Heavy metal arsenic most present in the pesticide for industrial agriculture. Arsenic is used as a pesticide primarily to preserve wood from rot and decay. In the past, arsenic was also used in rat poisons, ant poisons and weed killers. Exposed to the heavy metals cause long term effect such as cancer. Precaution need to prevent from exposed too much arsenic by ensuring ingestion to food contaminated with arsenic does not exceed the limit.

- **Cadmium**

Cadmium cause human health such as pulmonary, cardiovascular, and musculoskeletal systems. Because of increase used of fertilization especially in industrial agriculture, it increases the risk of cadmium when it transfers to the food chain.

III. Oven dry

Oven dry is equipment used to dry the fish sample until the sample could be form a solid. Each of equipment had different types of procedure based on their brand. Major important for the sample to dry is the temperature and the duration taken.

IV. Closed vessel microwave digestion

Closed vessel microwave is an instrument that completing the digestion process in short duration. The processes take different duration and temperature based on the samples that need to be digesting.

V. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is an instrument for analysis of multiple particles like Pb, As, and Cd. The extraction of fish can give data of the concentration of heavy metal that accumulated in the fish. By having this data, we can estimate the amount that could be safe to consume.

VI. Body mass index

This measurement was collected by using body weight and height instrument machine. Two type of instruments need during the process which are Body Composition and measuring tape. All the measurement is inserting into BMI formula to get the result.

$$\text{BMI: weight (kg) / [height (m)]}^2$$

CHAPTER 2

LITERATURE REVIEW

2.1 Characterisation of *Channa striatus*

Channa striata is native to east and Southeast Asia. In Malaysia, *Channa striatus* is called Haruan fish. This fish lives in most types of slow-moving freshwater habitat, including rivers, swamps, ponds, canals, lakes, and paddy fields. Their food habit is known as carnivore as their primary diet. It is generally type of fish that hunt their food such as small insect, tadpoles and small fish. (Munshi and Hughes, 1992). *C. striata* are capable to live in muddy areas that lack of oxygen (Singh et al., 1986). All *Channidae* family members have acquired the ability to breathe using their gills and skins, as well as through their suprabranchial space (Chandra and Banerjee, 2004). This fish can withstand poor environment due to its excellent air rigidity and respiratory organs. This allows these species to survive during their burial grounds. Compared to most freshwater fish, it is quite tolerant of turbid conditions and also able to survive lived despite being isolated from the water for a long time. However, the fish remains alive. Environmental pollution has physically changed the fish in response to contaminated aquatic habitats. Although *C. striata* can survive in a harsh environment, this species conservation planning is required because of its very high commercial value. *C. striatus* suggests that

environmental factors have a significant effect on the evolution of specific characteristics. Li (2013) shows that, a molecular diffraction patterns have changed with morphological change patterns because environmental conditions play an essential role in the differences in population morphology. Though the fish can last for a couple of hours without water due to the prolongation of air-breathing through their accessory respiratory organs, it will also die as its breathing fails to function further. Dead fish is not only due to respiratory failure but also due to the retention of toxic metabolites (heavy metals), including nitrogen residues (ammonium) products (Chandra and Banerjee, 2004).

2.2 Synergism

synergy is a word from the Greek, which is "synergos" that means working together. It is the interaction between two or more agents, entities, factors, or substances that produce a more significant impact than the number of individual effects. The effect can give either beneficial or dangerous because the combined effect is more significant than if it added to other harmful substances. (Baker and Sinkula, 1999). Synergism usually occurs if at least one of the components is present to affect the biological system. It occurs when the combined effect is more significant than is predicted by the activity that each part individually concludes at the same level of exposure that occurs in the mixture. Toxic kinetic interactions arise when one substance changes the metabolism of other potentially more toxic substances to increase the internal dose or systemic exposure for the active form of the poisonous

component (parent compound or metabolite). Such interactions can increase the activity of toxic substances that increase the activity of pesticides in the formulation of target organisms. (Singh, 2017).

2.3 High Demand in Staple Food

The agriculture sector was one of the significant pollutant factors in Malaysia. GDP had declined this caused a decrease in the agricultural industry from 30% in 1970 to only 7% in 2013. However, people are still concerned about this industry due to the high use of pesticide which could lead health hazards. Through the calculated statistics it cannot reflect the real significance of Malaysia's industrial economy (Jamal, 2014). One-fifth of the world's population or more than a billion household in Asia, Africa, and America rely on rice systems for their primary sources of employment and livelihoods. It is also on the frontline in the fight against world hunger and poverty (Nguyen and Ferrero, 2006). Global rice production has had to meet the demands of the growing populations. To overcome this problem, the Malaysia government has already made plans to improve the paddy cultivation method. In the 1960s, the green revolution program was developed by the government to introduce the double-cropping system. This system included the packed schedule of new high-yield variety seeds, an input of chemical fertilizer and use of agricultural machinery. These post-independent programs (with subsidy), enabled farmers to produce more rice for sale, and thus increased their income. Besides, In the 1970s the Malaysian government introduced a newly initiated irrigation scheme to help control the water movement, as the farmers used wetland

paddy cultivation style. (Drakakis-Smith, 1992). During the 1970s, there were 131,700 hectares of paddy land in Peninsular Malaysia which improved through irrigation facilities, of which 110,563 were provided in double-cropping areas (Tenth Malaysia Plan p. 286).

2.4 Pesticide Usage.

Pesticides are substances that can control the shape of individual plants or animals considered pests. Pesticides such as herbicides are used to destroy weeds and other undesirable plants while insecticides are used to manage various types of insects. Also, a fungicide is used to prevent the growth of mould, disinfection to avoid from the spreading of bacteria, and compounds used to control rats and mice (National Institute of Environmental Health Sciences, 2018). The use of many insecticides and chemicals in the agricultural sector has led to widespread dangers in food production — the result shows that many people could be exposed to low residual pesticides through their diet.

The use of pesticides in an excessive way or poor control would result in environmental pollution, especially for aquatic systems. Among the significant elements found in fertilizers used for agricultural purposes were heavy metals. Heavy metals were those metallic elements with relative atomic masses higher than iron (Duffus, 2002). Lead is an element that causes harm when exposed to humans especially to pregnant women. The lead quickly passes the placental barrier and

causes the conceived baby to be exposed to lead which could result in damage of the developing nervous system of the baby because low-level lead exposure in a developing baby has been found to affect intelligence. Previous exposure can cause miscarriage, dying, and infertility among men and women (The National Institute for Occupational Safety and Health (NIOSH)). While when eaten, large amounts of cadmium could severely irritate the stomach and cause vomiting and diarrhea. Exposure to cadmium could damage the lungs of people and prove fatal if sniffed at in high amounts. Exposure to low levels, such as cadmium in the air, food, water, and especially in tobacco smoke over time could build up cadmium in the kidneys and cause kidney disease and brittle bones. Cadmium is also considered a cancer-causing agent (Center for Disease Control and Prevention (CDC)). Exposure to cadmium in the various routes can cause excessive amount of heavy metal resulting in health problems.

When the hazardous chemicals are used without monitoring during paddy cultivation, this could cause soil, air and water pollution. These problems have a significant impact on aquatic life, especially on fish living in such waters. Pesticides like insecticides could accumulate in the tissues of both flora and fauna in the ecosystem (USAID, 2009). In the bioaccumulation process, insecticides were transported, and it would increase the concentration of a pollutant in an organism, especially when an organism ingests a particular substance at a faster rate, and magnified along the food chain. Pesticides could also accumulate in soil and sediments and were potentially carried along the paddy field trench by water and air. Many pesticide products in the market help the rice farmer to keep pests away from

paddy field. Used prudently, fertilizers and pesticides increase yield, and this was crucial for ensuring food security in Malaysia where rice is the staple food. However, applied in excess or an uncontrolled manner, pesticides will taint the water surface. Various studies have already demonstrated that rice cultivation was one of the critical sources of environmental pollution (Ramachandran et al., 2006; Hod, 2011).

In fact, it could also show the farmers themselves are exposed and gradually poisoned when they work in pesticide-sprayed fields. The use of a machine with high pressure causes the concentrations of heavy metal to disperse far which lead to air pollution. Heavy metals have also been discovered in paddy field waters, and these have contributed to the excessive use of agrochemicals which are known to contain cadmium, copper, lead and zinc (Ismail, 1994). For example, in the Sabak Bernam paddy field, the most famous freshwater animal that lives in it was *Channa Striatus* fish. This fish is easy to be found and is one of the nutrient sources for the population. So, because of the contaminations of water in paddy field due to pesticides use, it might give significant impact to both of the fish.

Heavy metals were giving significant impact to the ecosystem. Water in the paddy field was contaminated with lead, arsenic, and cadmium affecting the aquatic life, especially fish that live in the paddy field irrigation system. The heavy metals will transport into fish bodies by three possible ways which are gills, digestive tract, and body surface. The gills are considered as the essential site for direct uptake of metals from the water (Romeo, 1999; Padmaja and Rao, 2000), though the body surface usually is estimated to take a minor part in the uptake of heavy metals in fish

(Selda and Nursah, 2012). Heavy metal accumulation can also be caused by the food source, possibly leading to bio-magnification, the augmentation of toxins up the food chain (Per-Arne, 1997). As human food, Fish considered as an excellent source of polyunsaturated fatty acids (predominantly omega-3 fatty acids), protein, zinc, iron, and calcium (Toth and Brown, 1997). Seafood will be an even more critical and safe source of food in the future for protein and fatty acids for human intake and products made from aquaculture (WHO, 1999).

2.5 Paddy Field Location

Sabak Bernam is a district in the northern part of Selangor, covering an area of 992 square km or about 11% of the Selangor land area. It has a population density of 106 respondents per sq km and an urbanization rate of 31 per cent (compared to 91.4 per cent for Selangor in 2010). The composition of the population consists of a majority of Malay (74%), followed by Chinese (19%) and Indian (3.9%). About 47 per cent of the total area of Sabak Bernam is under agriculture, 40.18 under forest and less than 5 percent occupied by urban settlements. Paddy, coconut and palm oil have become main crops in Sabak Bernam. However, paddy considered as an essential food crop, and naturally, Sabak Bernam as the rice bowl of the nation among the primary producer of rice in the country. The area of study chosen was in the agricultural area (rice field) in Sungai Besar, Sabak Bernam. The primary source of water used for paddy cultivation here is the Sungai Tengi, which streamed through the Great Canal, which built before being flushed into a small canal through the

Watergate. The drainage system consists of canals, entrances, floods, paddy fields, and drainage drains. In the paddy season, especially during the growing season, inland drainage, floodplain, paddy fields, and drainage will be filled with water, while in the harvesting season it is dry. Sampling has been done in the trenches where it was the last location of contaminated water that fish live in.

2.6 Consumption of Fish Contaminated With Cadmium

Cadmium was non-essential and also a very toxic heavy metal that widely distributed in the aquatic environment and the earth's crust. In the list of heavy metals, cadmium is considered to cause a public health hazard (Sastry and Gupta, 1979). As fertilizer was one of the chemicals used by the farmer, it contained some cadmium. So, fish in the paddy fields became contaminate if they consumed water which was contaminated with cadmium. Fish are significant sources of protein for human beings. Fishes were the significant part of the human diet, and it was therefore not surprising that many studies have been carried out on metal pollution in different species of edible fish (Andersen, 1997; Prudente, 1997; Erdoayrul, 2006). Unfortunately, the chemical contaminants stored within the lipid component of the fish (OSTOW, 2003), so they would be protected when entering the human body. The human being exposed to Cd mainly through food consumption. Food materials containing higher Cd can significantly increase the Cd concentration in human bodies. The food material that contains higher Cd was fish liver. In results, this causes the health effects to a consumer such as fractures of bone, failure in

reproduction and fertility, cause damage to the nervous system and the immune system. Sometimes, it also would cause cancer if exposed to high concentration of cadmium.

2.7 Consumption of Fish Contaminated With Lead

When the accumulation of heavy metal lead reaches a substantially higher 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). Fish were usually among the top consumers (Bunton, 1987). Fish and people were 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 susceptible to even low levels of lead (Elder and Collins, 1991). Consumption of lead can affect organs such as brain, which would cause memory problem, hearts, like high blood pressure and other organs.

2.8 Consumption of Fish Contaminated With Arsenic

Arsenic is a solid substance that naturally exists in the Earth's crust and crushed rock. The exposure was natural, but can be aggravated by human activities. Arsenic in the pesticide comes in the inorganic form, and it was found in the paddy field due to the excessive use of pesticide. Water was an essential element that had

been used lots during the paddy cultivation process, and this water was flowing to the trenches through the irrigation system. This pollutant was because the farmers were using the pesticide during the paddy cultivation. There were many exposure routes for the arsenic to enter the body, such as drinking water, inhalation and also food consumption. As the primary food that easily exposed to the arsenic was like a fish, seafood, algae, and rice, most of the aquatic life had exposed to the arsenic by consuming the contaminated water and food in sediment. Fish such as Haruan was the one that potentially being contaminated with the arsenic. The consumption of this fish causes health effect and for long-term exposure, arsenic could cause cancer and skin lesions. It also associates with cardiovascular disease and diabetes. For early childhood, disclosure has linked to negative impacts on cognitive development and increased deaths in young adults. The effect of the arsenic exposure also causes heart and blood vessels (cardiovascular), Stomach and intestines (gastrointestinal), kidney effects, liver, nerves and nervous system (neurological), lungs (pulmonary), childbirth (reproductive), respiratory blood and blood-forming organs (hematology).

2.9 Health impact assessment (HIA)

HIA is a process of estimating the potential impact of a chemical, biological, physical or social agent on a specified human population system under a specific set of conditions and for a certain timeframe (Health Council, 2001). Two formula had been used from HIA which are hazard quotient (HQ) and lifetime cancer risk (LCR). The hazard quotient (HQ) was a ratio of the consumed dose of a toxic metal

via an oral reference dose (RfD) proposed by the USEPA. HQ values above 1 means that the contaminated food intake has likely some noticeable harmful effects on the exposed population. The higher the HQ value is, the higher the probability of the hazard risk of the human body will be. Based on HQ values, studies have performed on the potential risk assessment of dietary intake of heavy metals via the consumption of seafood (Li, 2013; Storelli, 2008). The lifetime cancer risk (LCR) is estimated by the product of the lifetime average daily dose (LADD) and the cancer slope factor (CSF).



CHAPTER 3

METHODOLOGY

3.1 Study Design

This is a cross-sectional study with the aims to determine if the fish accumulate with heavy metals (lead, arsenic, and cadmium) that contaminated in the water from *Simpang Lima* trenches.

3.2 Study Location

Location of the study had been chosen using purposive sampling based on a few criteria which is the location must have paddy field area. In Selangor, there were two districts that have paddy field area which is *Sabak Bernam* and *Kuala Selangor*. By using simple random sampling, *Sabak Bernam* had been chosen. From the districts, *Sungai Besar* had been selected from few 'Mukim' that having paddy field area. Lastly, one out of eleven villages in the *Sungai Besar* had been selected randomly as study location. Five trenches in the *Simpang Lima* villager have been selected randomly for sample location and respondent location. The locations were *Parit 13, Parit 14, Parit 15, Paket 60* and *Paket 100*.



Figure 3.1: *Simpang Lima* trench

(*Parit 13, Parit 14, Parit 15, Paket 60 and Paket 100*).

3.3 Study Population

3.3.1 Target population

The target population was the resident who lives in *Sabak Bernam*. Sungai Besar is a town in Sabak Bernam that being chosen randomly in the study to select villages as a responden to representing the location. For, the sample population, the participant must refrain from the Sungai Besar to representing the location.

3.3.2 Population sampling

The sample of the study population was chosen based on a few criteria. This criterion divided into an inclusive and an exclusive criterion.

The requirements for the community was the study group, should be a resident or a local paddy farmer who had been working and living in the *Sabak Benam* for 20 years. The participants in the study should have experienced eating the *Channa striatus* fish for at least seven years. The age range of sample population must be 20 to 70 –year –olds. The participants also needed to have a medical record about their health as evidence for answering the questionnaire. The criteria requirements can be seen on the sampling flow chart below:

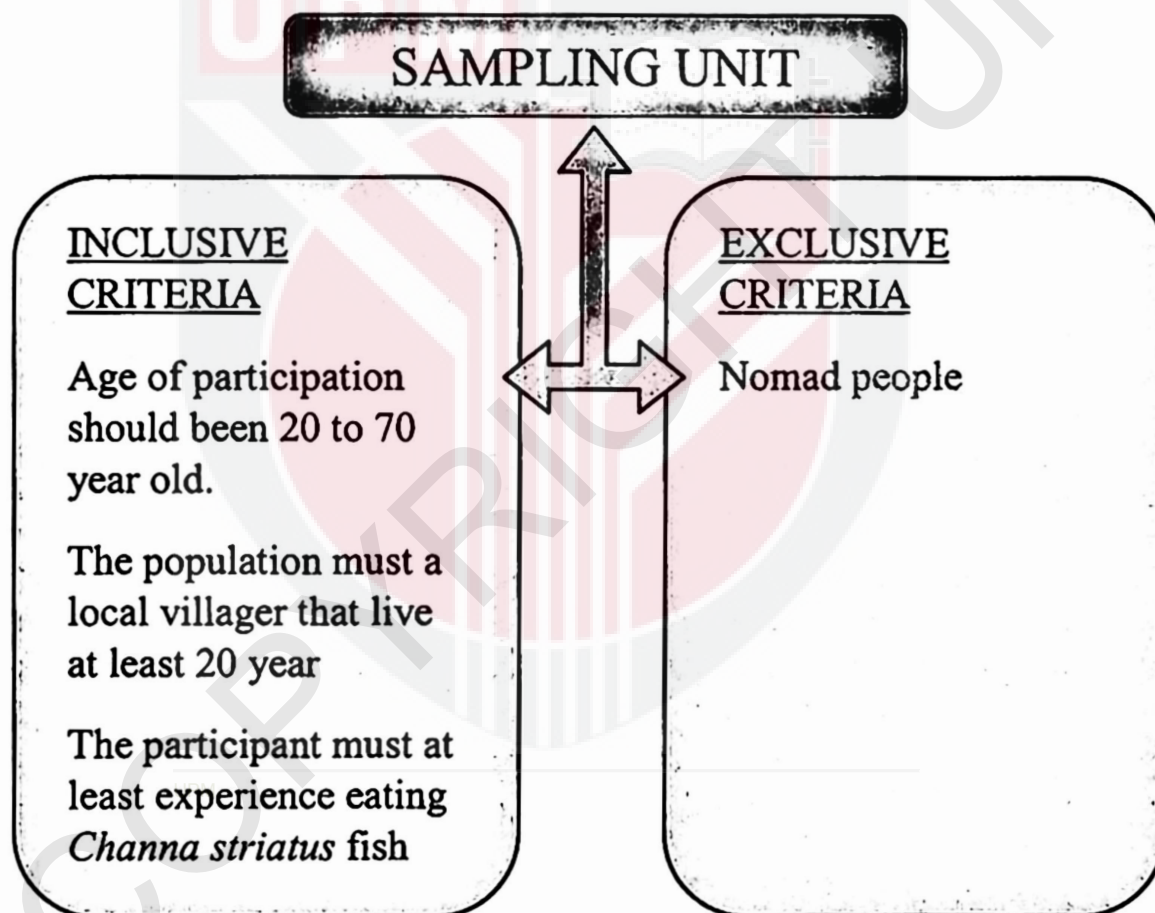


Figure 3.2: sampling unit

3.3.3 Sample size calculation

The sample size was calculated to determine the minimal number of samples to be collected to represent the farming villages in this study location. In this context, the proportion of a paddy agricultural villager who consumes fish was therefore estimated by using the sample size determination formula: Fischer et al. (1991).

$$N = \frac{Z^2 pq}{P^2} = \frac{(1.96)^2 (0.5) (0.5)}{(0.1)^2} \times \frac{20}{100} \times 97 = 20$$

$$97 + 20 \text{ (20\% strength of analysis of the study)} = 117$$

N – Desired sample size

P – Proportion of the large population estimated to contain pesticide and heavy metal residues

q – Expected contaminated proportion

z – The standard normal deviation is set at 1.96

D – The degree of accuracy desire sets at 0.1 significant

Based on this sample size calculation, a minimum number of 97 paddy agricultural villagers who fulfils the inclusion criteria will be recruited from villages located at *Sungai Besar, Sabak Bernam*.

3.4 Instrumentation

3.4.1 Questionnaire

A questionnaire was used to identify the general information of villagers; a pesticide used during paddy cultivation that may be exposed to the fish, fish consumption and also medical status. Data from the questionnaire would be used to identify their daily intake of contaminated fish during the past seven years, whether it can potentially affect the human health. This study applies a simple random sampling method to recruit study population in 4 selected locations in Sungai Besar to answer the questionnaire. The question items gathered from the differently standardized survey. Face-to-face interviews also were conducted among respondents by the researcher to ensure that all information needed is not missing.

3.4.2 Anthropometric Measurement

Body mass index was measure using two type of equipment. Body scale form Omron body composition brand, model: BF508 had been used to measure the respondent current weight and measuring tape brand: Stanley Hardware, model: ST30696 had been using as equipment to measure height of the respondents.

3.4.3 Fish sampling

Two kilograms of fish were collected randomly during the harvest season in December 2018 at five different locations to represent the *Simpang Lima* trenches that linked to paddy irrigation. The selected locations for study site were *Paket 60, Paket 100, Parit 13, Parit 14* and *Parit 15*. To catch the fish, landing net and also fishing rods were used to lift the fish out of the water. All the fish was selected by measuring the size to get the same exposure duration. The landing net was a large handheld net used to lift caught fish out of the water, the most common in anglers and fishers. The landing net was usually used for large fish *Channa striatus*. Then, the fish was being washed immediately with aqueous water, stored in a clean polyethylene bag and kept cool in the icebox. The fish was immediately sent to the IBS laboratory where the item was frozen at -20°c so that, they could be prepared for extraction. The reason for fish collected in the trench was because the primary sources of pollutants were pesticides from paddy farming where farmers use pesticides such as pesticides and fertilizers during activities.

3.4.4 Heavy metal analysis (oven-dry, closed vessel microwave digestion, Inductively Coupled Plasma Mass Spectrometry (ICP-MS))

Three steps had been conducted to get the level of heavy metal concentration that accumulated the *Haruan* fish. The first step that had been conducted in FPSK laboratory was extraction process. During the preparation of material and equipment for extraction, the fish were thawed to room temperature. The muscles were removed with stainless steel knives, homogenized and weighed. After completing the process, samples were then oven-dried to constant weight at 80°C for 48 hours in acid-washed petri dishes. Then the sample was allowed to cool in the desiccators and was ground to a fine powder using porcelain pestle and mortar.

Five grams of the sample from the previous process have been triplicated and sent to an IBS laboratory for digesting using closed-vessel microwave digestion with 3 ml of an ultra-pure nitric acid (64%). The process took about 10 minutes to complete and the entire sample was diluted using deionised water, filtered through 0.45µm *Whatman* filter paper and stored in a 15 ml centrifuge tube for 10ml. For the last step, the sample was continued analysis using inductively-coupled plasma mass spectrometry (ICP-MS) (model ELAN 9000 Perkin Elmer ICP-MS, USA) at environment faculty laboratory, UPM. Element standard solutions used for calibration were prepared by diluting stock solutions of 30 ug/kg, 50 ug/kg, 100 ug/kg, 300

ug/kg and 500 ug/kg were Element standard solutions: 5% HNO₃ multi-element calibration standard of each element. Three types of heavy metals: Lead (Pb), cadmium (Cd) and Arsenic (As) was chosen to be detected the fish's body for this study.

Data collection procedure

There were two sections of data collection in this study, which was the analysis of *Channa striatus* fish that contaminated with heavy metal in part one while in part two; we want to collect data on the level of contaminated fish consumption by villagers using a questionnaire. In part one; we will catch the *Channa striatus* fish in five different trenches from *Simpang Lima* villages, Sungai Besar. All the sample collection will be sent to a laboratory at Environmental faculty (get the correct faculty name), UPM to measure the level of contamination with heavy metal such as lead, arsenic, and cadmium in the fish muscle by using the ICP-MS. In part two, we distribute the questionnaire randomly to 120 local villagers in Sabak Bernam to determine the daily intake of *Haruan* fish.

The process of data collection and environmental samples were as follows:

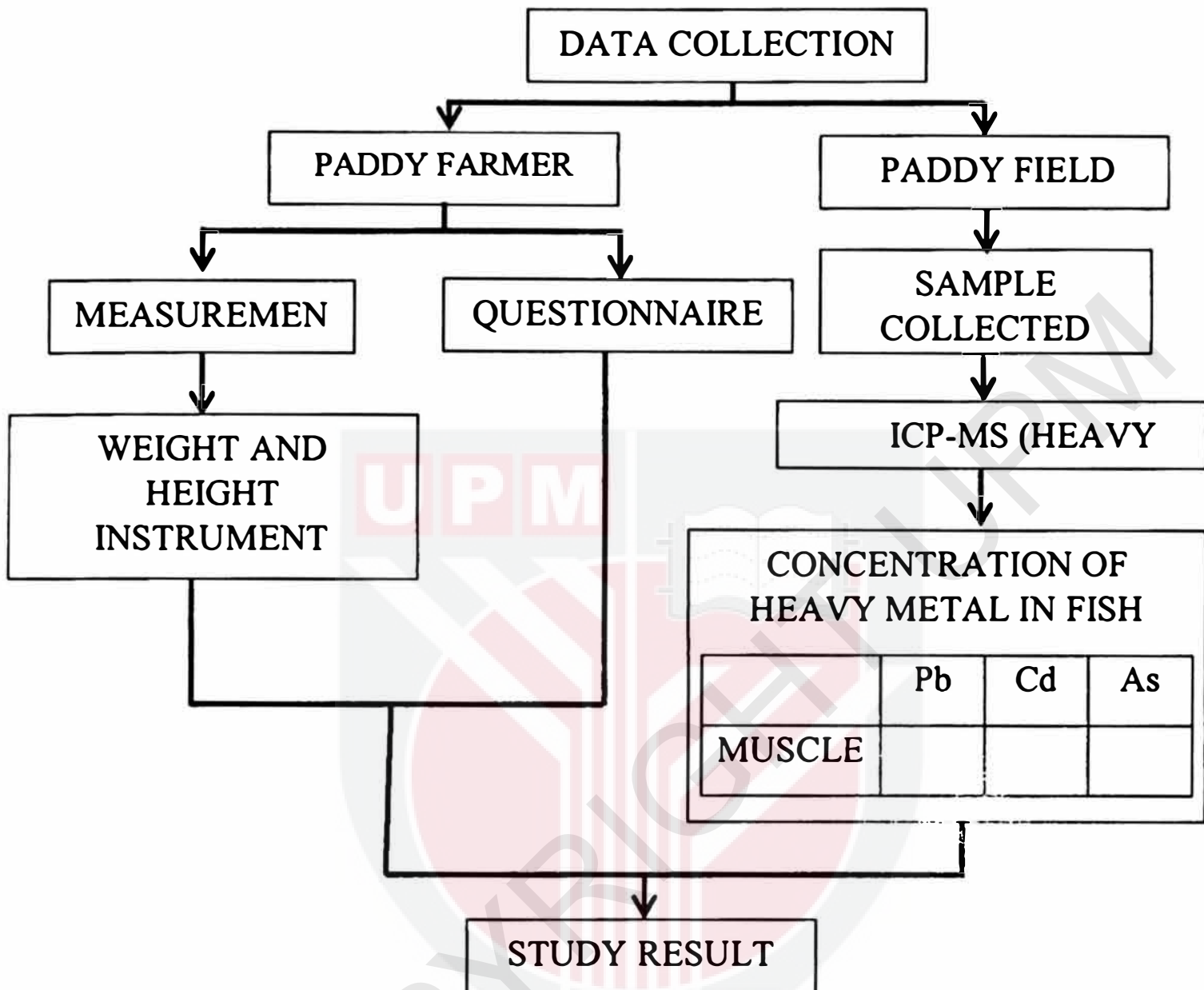


Figure 3.3: Data Collection Procedure

3.4.5 Hazard Quotient and Lifetime Cancer Risk Formula

Hazard quotient formula was used to determine ratio of the consumed dose of a toxic metal via an oral reference dose:

$$\text{For ingestion: } \text{HQ} = \text{ADD} / \text{RfD}$$

$$\text{ADD} = \frac{(\text{Cf} \cdot \text{Ri} \cdot \text{Fi} \cdot \text{EF} \cdot \text{ED})}{(\text{BW} \cdot \text{AT})}$$

HQ = Hazard quotient (unitless)

ADD = Average daily dose (mg/kg-day)

RfD = Reference dose (mg/kg-day)

Cf (lead) = 0.00828 mg/kg

Cf (arsenic) = 0.00589 mg/kg

Ri (fish ingestion rate) = Ri = 0.28 kg/meal

Fi (fraction ingestion contamination) = 0.1

EF (exposure frequency) = total consumption

AT (average time)/ED (exposure duration) = age of respondent

BW = weight individual

RfD (lead) = 0.002

RfD (arsenic) = 0.0003

The lifetime cancer risk (LCR) is estimated by the product of the lifetime average daily dose (LADD) and the cancer slope factor (CSF).

$$\text{For ingestion: } \text{LCR} = \text{LADD} \times \text{CSF}$$

$$\text{LADD} = \frac{(\text{Cf} \cdot \text{Ri} \cdot \text{Fi} \cdot \text{EF} \cdot \text{ED})}{(\text{BW} \cdot \text{AT})}$$

LCR = Lifetime cancer risk

LADD = Lifetime average daily dose

CSF = Cancer slope factor (1.5)

3.5 Quality Control

Sampling protocols, equipment supplies, sampling containers, and documentation were designed with extra precautionary measures to ensure that quality of the data being secured throughout the data collection procedures.

3.5.1 During sampling in the field

To ensure sample quality during sampling, equipment should be cleaned by absorbing into nitric acid before going to the field. Fishing equipment such as a fishing rod or fishing net should ensure that there was no pollutant such as heavy metals when collecting fish. The fish should be placed in a separate ice box labelled after being wrapped with a polyethylene bag to prevent the sample from being damaged. Researchers should keep their hands clean from any chemicals to prevent unwanted pollution by wearing gloves or wiping with alcohol. Finished arrest, the sample should be taken to the laboratory as soon as possible and frozen in 0°C in the icebox to ensure the level of pollution that represents the rice field area. During transplantation, the refrigerator has been checked every one hour to ensure that the temperature is maintained.

3.5.2 During laboratory analysis

To ensure that the experiment works correctly, the QA / QC in the laboratory needs to be more focused on the laboratory's tools, equipment, and cleanliness. When handling samples, researchers need to keep themselves clean to avoid samples from anything contaminated with other types of heavy metals. Triplication of *Channa striatus* collected from the muscles must be done, $n = 3$ to get the best values at the end of the analysis. Before sample analysis, all glasses and plastics must be soaked throughout the night in 10% (V / V) of nitric acid, rinse with distilled and deionized water and dry. The machine also needs to be calibrated because the data presented will be inaccurate and cannot represent the contaminated at the actual location.

3.5.3 During survey

The questionnaires were given to the respondent randomly based on name list given by the head of villagers. All the respondent also being chose based on the criteria needed. Instruction had been provided to guide the respondent how to filling up the form. For the respondent that cannot write, the researcher was help to filling up the form.

This measurement was collected by using body weight and height instrument machine. During measure activity, a few precautions were taken care of by following the correct step during

measure the height of a participant. Firstly, we need to ask the participant to remove their footwear (shoes, slippers, sandals, and other) and also headgear (hat, cap, hair bows, comb, ribbons, and other). The measurements could be taken on the light cloth to ensure the accuracy of the size if this would be insensitive to seek the removal of a scarf or veil. Next, the participant needs to stand on the board and make sure the participant holds with Feet together, Heels against the backboard and knees straight and also look straight ahead and not look up. After that, the arm would be measured gently down onto the head of the participant, and the participant needs to breathe in and stand tall, so the height in centimeters could be measured at the exact point. All participants ID were recorded to prevent data missing. To measure the respondent body weight, they also need to follow a few steps which were; asking the participant to remove their footwear and socks, step onto scale with one foot on each side of the range, ask the participant to stand still, face forward, place the arms on the team and wait until asked to step off and finally, record the weight in kilograms on the participant's Instrument.

3.6 Statistical Analysis

One sample t-test was performed in the experiment to determine the mean value of the heavy metal concentration and also to compare with the permissible value in the food regulation 1998 with SPSS for window (version 20) software. The significant level was $p < 0.05$.



CHAPTER 4

RESULT

4.1 Determination of socio-demographic background of the respondent, occupational information and health status among the paddy agricultural and the villagers.

Simpang Lima was randomly selected among the area in *Sungai Besar* where 5 different trenches; *Parit 13*, *Parit 14*, *Parit 15*, *Paket 60* and *Paket 100* been chose for sample location. Two kilograms of *C. striatus*'s fish had been collected in the trenches to ensure the concentration of heavy metal which accumulates in fish muscles could representing the level of pesticide concentration in *Sungai Besar* paddy field. The selected sampling locations were the main trenches that act as water disposal site from paddy irrigation during the cultivation process and throughout the harvesting season. The location had been checked as the *C. striatus*'s habitat as it was a freshwater fish.

120 respondents from the *Sungai Besar* area were selected randomly based on the name list of households located around provided by the head of the five identified sampling location to examine the fish consumption habit among villager at *Sungai Besar* paddy field. Table 4.1 reveals the socio-demographic characteristics of the respondent where there the numbers of participants were almost balance between

male and female respondent. 120 sample size population needed in this study, 52.5% were a male, while the female was 47.5% with the mean age of 46.63 and 44.51 that volunteered to participate.

Based on 52.5% of male respondent and 47.5% of female respondents, 9.5% male and 26.3% female was between 20-30 year old, followed by 27% male and 8.8% female (31-40 years old), 15.9% male and 28.1% female (41-50 years old) and 27% male together with 17.5% female (51-60 years old). Lastly, 20.6% of male and 19.3% of the female was between 61-70 years old. Data showed that 27 people participants from 51-60 year old were mostly participants who willing to interview followed by 41-50 year old 19 respondents from age 31-40.

Educational level was divided into five categories which were primary school, secondary school, diploma, degree and other type of educational level. All the respondent that participate had a chance to go to school. About 20.6% of the male and 14% of female got an education in the primary school while 44.4% of the male and 35.1% of female got to enter into the secondary school. 6.3% of male respondents and 14.0% from female respondents were joined other type of education program. The rest of respondent got chance to study further where for the diploma education, 20.6% of the male and 21.1% of female while 7.9% of male and 15.8% of female got degree education.

The respondents were asked about their weight and height to identify if body composition cause health problem among villager. Comparing the BMI categories, data for male respondent BMI shows that majority of them were in healthy body

weight, but for a female respondent, most of them were overweight due to their body composition like storage of fat and also their metabolism. The results could be categories as follows: normal > overweight > obese > underweight for male respondents while for female respondent; overweight > normal > obese = underweight.

Table 4.1: Socio-demographic background of village (N=120)

Socio-demographical		Percentage (%)	
		Male(n=63)	Female(n=57)
Age (Mean ± SD)		46.63(14.41)	44.51(14.40)
Education	Primary School	20.6	14
	Secondary School	44.4	35.1
	Diploma	20.6	21.1
	Degree	7.9	15.8
	Others	6.3	14.0
Income	RM 3,000	33.3	14.0
	< RM 3,000	66.7	86.0
BMI	Underweight	0	1.80
	Normal	63.5	45.6
	Overweight	33.3	50.9
	Obese	3.2	1.8

Table 4.2 showed the occupational background of the respondent. Majority of the respondents were self-employed where 77.8% of the participants were among male, and 42.1% among female. they admits that they would work on owed farmland such as paddy cultivation activity, planting a vegetable or rearing animals as one of their sources of income. Most of the unemployed participants were a female with a percentage of 42.1%, while the unemployed male was 3.2%. There also has a student

who joins the study were 7.9% of male student and 12.3% from a female student. Data showed that 11.1% of male and 3.5% from a female was retired due to specific reasons.

The respondents were asked about their job status, whether they work as a paddy farmer or not. Data from the SPSS analysis shows that both of the genders have worked as a farmer. From the 52.5% (n=63) of the male, 34.9% were a paddy farmer while the 65.1% was a villager that work other than paddy farmer and for the female 5.3% out of 47.5% (n=57) working as a farmer but the rest mostly was a housewife. During face to face interview season, we asked whether pesticide were applied in paddy field and all the farmer admit that their farmland were treated with a bunch of pesticide to combat against pests, weeds, and rodents.

Following the interview session, pesticide usages were further examined. Among the self- employed from male and female respondents, only 34.9% of the male farmer and about 8.8% female farmer admits that they had used the pesticide during planting activity. The percentage of pesticide used by the male farmer was significant with their occupation as a farmer, but the pesticide usage the female showed it was slightly higher than the occupational status. Most of the 25.4% male respondents and 5.3% female respondents use the source of pesticide more than one seller, whereas 20.6% of the male respondents and 5.3% of female respondents chose to depend on more than one type of brands as the entire pest quickly immune if they used the same kind of pesticide.

Table 4.2: Occupational Information of Villagers (N= 120)

Occupational Information		Percentage (%)	
		Male (n=63)	Female (n=57)
Job Status	Self-employed	77.8	42.1
	Unemployed	3.2	42.1
	Student	7.9	12.3
	Retired	11.1	3.5
Occupation	Farmer	34.9	5.3
	Non-farmer	65.1	94.7
Pesticide usage	Yes	34.9	8.8
	No	65.1	91.2
Source of Pesticides	Not using	65.1	93.0
	From one source of seller	9.5	1.70
	From more than one seller	25.4	5.3
Brand of Pesticides usage	Not using	65.1	93.0
	Depends on one single brand	14.3	1.70
	Depends on more than one brand	20.6	5.3

Table 4.3 shows the health status among the respondents who participated in this study. There were six types of the health problem were examined in this study; heart disease, asthma, diabetes, hypertension, kidney disease, and cancer. Among all the health problem, only four disease was confirmed to have by respondents, 9.5% of male and 7.0% of female respondents reported to have heart disease, 7.9% of male were claimed to have asthma. Lastly, 14% of female respondents reported having diabetes while 27.0% of male respondents were having diabetes and hypertension.

19.3% of female respondents were reported having hypertension. There was none of respondents had developed kidney disease or got cancer in both gender.

Table 4.3: Health status of villagers

Health Status		Percentage (%)	
		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 Fish dietary habits among paddy agricultural villagers

Data revealed in the table 4.4 that most of the respondents like to go fishing, especially during the harvesting season. Most of them would use fish rods to catch the fishes because it's more convenient due to some reason and also because of the water level in trench was low. About 58.3% of total respondents' answer fishing was the best way for them use to get the *C. striatus* fish. About 48.3% from the total fisher respondent preferred fishing in the trench rather than in irrigation because,

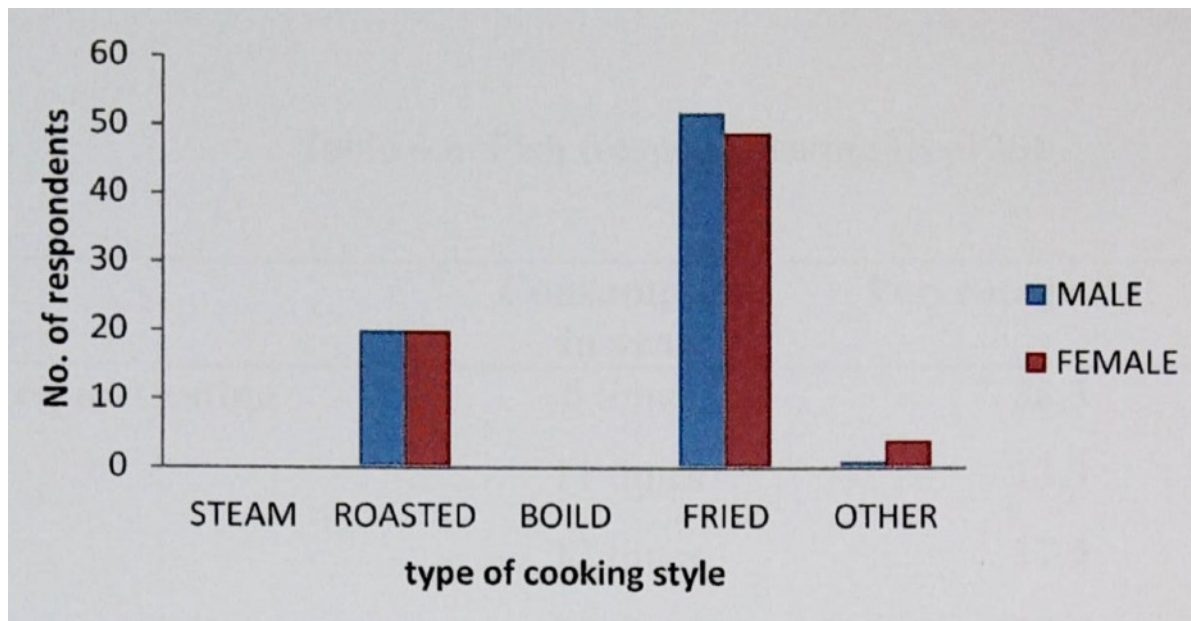
during the harvesting season, the water level in irrigation was low. Another 41.7% respondent chose to buy in the market, although the price was a bit high.

Table 4.4: Fishing status among the villagers (N=120)

Fishery status		Percentage (%)
Fish catches	Yes	58.3
	No	41.7
Fishing location	Yes	<ul style="list-style-type: none"> • Trench 48.3 • Paddy Irrigation 10.0
	No	Buy at market 41.7

In table 4.5, it was tabulated to examine how the respondent chose to cook the fish. Most of the respondents were preferred eating the *C. striatus* fish if it was cooking roasted, fried and also Malaysian food styles such as fish cooked in thick and thin chili gravy, fish curry, and fish cooked with coconut milk mixed with other spices and flavourings. Even though they prefer these three types of fish cooked style, but the majority chose fried as their dish. The mean value of each sized in one serving while eating the fish was equal to two fish for every respondent due to the size of *C. striatus* which was big enough to eat per person.

Figure 4.5: Fish cooking style reported among study population (N=120)



The frequency of eating fish was being calculated to determine the dietary habit of the respondent during from 12 months before. 38.3% of the respondent were eating about 6 times in a year, 13.3% eating 11 times in a year, 17.5% 12 times in year, 25% for 36 times in years, 3.3% eating 48 times in year, 1.7% eating 96 times while only 0.8% eating about 288 times in years. This data was collected during the interview where most of the respondents state that the fish was hard to find now a day.

Table 4.6: Fish frequency eating (N=120)

	Consumption in year	Percentage (%)
Frequent eating	6 times	38.3
	11 times	13.3
	12 times	17.5
	36 times	25.0
	48 times	3.3
	96 times	1.7
	288 times	0.8
Fish size per serving		1.90(0.627)
Fish (Mean ± SD)		

4.3 Contamination levels of heavy metal lead, arsenic and cadmium that accumulate in body fish of *Channa Striatus*.

All data for heavy metal concentrations were analyzed using the Inductive Cross-Plasma Mass Spectrometry (ICP-MS). A few studies on agricultural chemicals have been reported containing toxic trace elements in the Selangor paddy field. Therefore, this study aims to identify if lead, arsenic, and cadmium might present in the river. By replicating the fish sample, the result could show the accuracy of the heavy metal concentration in the fish muscle. Data from the ICP-MS showed the mean of the lead in the sample one was 9.147 ug/kg while Arsenic 6.042ug/kg. In the sample two, mean value of lead was 6.286ug/kg while arsenic was 5.960ug/kg. For the third sample mean value lead was 9.394ug/kg and arsenic was 5.725ug/kg.

Unfortunately, there was no detection level of cadmium in the fish filet. The presence of heavy metal in the paddy field and trench from excessive pesticide usage cause bioaccumulation to *Haruan* as fishes consume contaminated water. Based on analysis of heavy metal in fish sample collected, the trace element levels in the edible parts of these three fish were below the maximum permitted proportion of metal contaminants in food.

Table 4.7: Concentration levels of heavy metal accumulates in body fish (2kg) of *Channa striatus*

Heavy metal	Mean (SD)ug/kg		
	Sample 1 (Pb)	Sample 2 (Pb)	Sample 3 (Pb)
Lead (Pb)	9.147	6.286	9.394
Arsenic (As)	6.042	5.960	5.725
Cadmium (Cd)	ND	ND	ND

4.4 Compares the level of heavy metal contaminated in on *Channa striatus* fish's body (flesh muscle) with Food Act 1983.

Three types of heavy metal lead, cadmium, and arsenic were selected to identify the presence of any heavy metal that enters into the fish's body. Table 4.7 showed the mean level of heavy metal content in the pesticide that accumulates in the sample 0f *Channa striatus* body. Two out of three trace elements showed the average of mean concentration level of heavy metals inside the fish flesh where for

the lead was 0.00828mg/kg higher than arsenic 0.00589 mg/kg but the concentration of cadmium was not detected.

The result of the heavy metal in fish from table 4.8 was compared to the Food Act 1983 value and it showed that the Arsenic and lead concentration value in fish was a significant ($P < 0.01$). All the concentration showed the heavy metal in the fish filet was below than the permissible standard amount compared to the permissible level concentrations in food Act 1983. The values that maximum permitted proportion of metal contaminant in specified food for lead was 2.0 ppm while for the arsenic and cadmium was 1.0 ppm.

Table 4.8 Levels of heavy metal lead, arsenic and cadmium with permissible level concentrations in Food Act 1983

Heavy metal	Mean (SD) (ug/kg)	Standard (ug/kg)	Mean Difference (95% CI)	p-value
Lead (Pb)	8.28(1.73)	2000 ^a	-991.72 (-996.02,987.43)	<0.001**
Arsenic (As)	5.90(0.16)	1000 ^a	-994.09(-.994.49,-993.68)	<0.001**
Cadmium (Cd)	ND	1000 ^a	ND	ND

^a Food Act Malaysia

**p-value is significant at 0.05 levels

4.5 Dietary health risk assessment based on the levels of heavy metal lead, arsenic and cadmium accumulated from up taking *Channa Striatus* of fish by Paddy agricultural villagers.

Data was determined by using the Hazard Quotient to identify the estimated value of heavy metal that would expose to the body from consumption of the contaminated fish. Mean values from the tables indicate that the villager can have

safety consumption. From the table, mean value does not exceed the HQ limit either from respondent BMI or respondent's age.

The result showed that there was no factor from BMI and age categories that contributed to Hazard Quotient that exceeds the limit. By dividing those BMI and age factor into several categories, results showed that the higher value of HQ for lead and arsenic was among the older age, which was 17 male respondents from 61-70 years old while 10 respondents from 51-60 and 11 respondents from 61-70 years old female respondents. Among the BMI categories, the overweight body condition showed the highest value for male in arsenic concentration. For female respondent, the higher values were coming from normal and obese body condition. The result could be categories as follow: overweight > normal > obese > underweight for male in arsenic exposed and to the lead. For female that exposed to arsenic and lead the body condition were: obese > normal > overweight > underweight. This evidence could tell the villagers that consumption of contaminated *Haruan* does not cause health effect as long as the followed the guidance. For the female villager, they still need to control their dietary intake of fish because there was slightly higher in the estimation value from the HQ then a male villager although the average of fish consumption showed that male (51.23) was higher than female (41.91).

Due to the reference dose for each heavy metal could permissibly entering the body, by comparing the heavy metal concentration between table 4.9 (arsenic) and table 4.10 (lead), the HQ value for arsenic was higher than HQ for lead.

Table 4.9: Hazard Quotient value estimated from contaminated fish for daily consumption (arsenic).

Respondent Hazard Quotient Average	Mean ± SD			
	Frequency (n)	Male (n=63)	Frequency (n)	Female (n=57)
Age				
20-30	6	0.19(0.23)	15	0.43 (0.45)
31-40	17	0.30 (0.29)	5	0.38(0.24)
41-50	10	0.18 (0.19)	16	0.26 (0.25)
51-60	17	0.39 (0.47)	10	0.46 (0.33)
61-70	13	0.84 (2.36)	11	0.46 (0.43)
BMI				
Underweight	0	-	1	0.24 (0.00)
Normal	40	0.30 (0.36)	26	0.48 (0.40)
Overweight	21	0.64 (1.85)	29	0.30 (0.31)
Obese	2	0.05 (0.02)	1	0.65 (0.00)

Table 4.10: Hazard Quotient value estimated from contaminated fish for daily consumption (lead)

Hazard Quotient Average	Mean \pm SD			
	Frequency (n)	Male (n=63)	Frequency (n)	Female (n=57)
Age				
20-30	6	0.04 (0.49)	15	0.09 (0.09)
31-40	17	0.06 (0.06)	5	0.08 (0.05)
41-50	10	0.03 (0.04)	16	0.05 (0.05)
51-60	17	0.08 (0.10)	10	0.09 (0.07)
61-70	13	0.17 (0.4)	11	0.09(0.09)
BMI				
Underweight	0	-	1	0.05 (0.00)
Normal	40	0.06 (0.07)	26	0.10 (0.05)
Overweight	21	0.13 (0.39)	29	0.065 (0.06)
Obese	2	0.01(0.00)	1	0.13 (0.00)

Table 4.11 shows the hazard quotient for a person based on their frequency of eating, body weight and duration of eating. For the respondent that having less frequency of eating, they must not exceed the limit HQ which is 1. For those eating too frequent, they will exceed the limit.

Table 4.11: Individual Hazard Quotient

Frequency fish consumption	Individual HQ	
	Pb	As
6 times/year	0.030	0.147
11 times /year	0.036	0.342
12 times / year	0.073	0.347
36 times / year	0.186	0.549
48 times / year	0.203	0.965
96 times / year	0.353	1.675
288 times / year	1.829	8.675

Table 4.12 shows the mean value for lifetime cancer risk (LCR) based on age and body mass index. From the table, most of respondent were consider free from cancer as the value of LCR was not exceed the potential having cancer. Data shows that the value is in the range between 10^{-4} to 10^{-6} .

Table 4.12: Life-time Cancer Risk value estimated from contaminated fish for daily consumption

LCR average for As	Male	Female
Age		
20-30	3.2E-5(0.00004)	1.3E-4(0.00015)
31-40	7.42E-5(0.00007)	1.2E-4(0.00010)
41-50	5.3E-5 (0.000057)	8E-5(0.00008)
51-60	1.2E-4(0.0014)	1.2E-4(0.00008)
61-70	3.3E-4(0.00092)	1.4E-4(0.0001)
BMI		
underweight	-	-
Normal	9E-5(0.0001)	1.4E-4(0.00014)
Overweight	2.2E-4(0.0007)	8.6E-5(0.00009)
Obese	1E-5(0.000008)	-

CHAPTER 5

DISCUSSION

5.1 Determination of socio-demographic background of the respondent, occupational information and health status among the paddy agricultural and the villagers.

One hundred twenty respondents were selected randomly based on the name list of household located around provided by the head of the five identified sampling location. Most of the respondents from different age categories either they were male or female showed interest to join the study as it helped them to know whether the consumption of *Haruan* from the Simpang Lima trench was safe or would give adverse health effect. From the trend, the majority of the villagers were of the age range of 41 to 70 years old, which showed the most interest to participate in the study. Education levels of the villagers were asked to identify whether the respondent had any idea about the dangers and health effect of the use of pesticide while using these pesticides. Based on table 4.1, the people who were surveyed between the ages of 41 and 70 years were mostly educated to primary and secondary levels, and education was not of prime importance. One of the reasons for this was that the villagers could not afford to send their children for further studies. Even today, most of the villages were in the categories of lower income. From the results, it can be verified that lack of education could be a possible reason why the villagers were not aware of the

danger of pesticide usage. Possibly, very few villagers had a basic knowledge about the hazardous of pesticide toward the environment and human health.

Respondents were also asked about their smoking and alcohol consumption habits to determine if other factors contributed to their health problem. Unfortunately, some data showed the respondent from both genders smoked. Since in the study, we wanted to determine if there was any heavy metal Lead, Cadmium and Arsenic that could cause a health problem, we were concerned with smoking habit of the villagers as this could affect the result since it was known that cadmium and lead has been detected in cigarettes in several studies.

The majority of the 120 respondents were self-employed which meant that most of them were working as agrarian as most of Sabak Bernam location sector was paddy plant, palm oil plant, coconut plant and another type of plant activities. However, only 25 male and female respondents were working as paddy farmers. In the study of “Exploring Rural Transformation in Malaysia: A Case of Rice Cultivation Area in Sabak Bernam”, Ibrahim Ngah states that the area of rice cultivation continued to decrease until 2013 due to specific factors that contributed to the decrease amount of paddy farmer in Sungai Besar. Surprisingly, the table showed that the percentage of pesticide use was slightly higher. Still, we cannot assume the Sungai Besar trench was safe from heavy metal concentration because there were few activities involving the use of pesticide. Besides, respondents also admit that they would use various types of pesticide brand and sources to protect their crops. It was because, in the period, undesirable pests or plants were resistant to the same pesticide concentration that lead to farmers altering the type of pesticide or increasing concentration.

In table 4.3, high blood pressures become significant cause of health issue among the 120 respondents. Surprisingly, both male and female respondent faced the same health problems. High blood pressure was higher than diabetes followed by heart diseases. Many studies had being done to identify the association of heavy metal exposure with those three types of disease. Study of “Environmental toxic metal contaminants and risk of cardiovascular disease: systematic review and meta-analysis” claim that the concentration of heavy metal lead, arsenic, and cadmium associated with an increased risk of cardiovascular disease and coronary heart disease which linked to the increase of high blood pressure.

5.2 Fish dietary habits among paddy agricultural villagers

During harvesting season, the majority of the villagers chose to go fishing as they does not need to focus on handling the cultivation process anymore and also it was more convenient for them because during the season, the level of water was low and this helped them to catch the fish. Even though few villagers went fishing, but catching the fish during harvesting season was more like a tradition to them. Moreover, the study on the fish during harvesting season can show to the public that even in the rest cultivation period, the concentration of heavy metal still exists. Consumption of contaminated fish even in the low concentration level of heavy metal could pose the health hazard to them. In general, fish is widely consumed by both the low and high-income earners because of the easy accessibility through free fishing methods in open paddy irrigation or rivers.

From table 4.4, we can see the trend of fishing location that most of the respondents choose was in the trench rather than in the paddy irrigation. Based on response from the villagers, trenches were chosen as the best location because of the water level in a trench was high compared to irrigation that leads to the fish swimming and change their location to live. By referring to Mohd Rozali Othman et al. (year), based on value from Tanjung Karang paddy field, there were no substantial differences of heavy metal concentration between irrigation area and trench area. This evidence could tell us that, there was no difference on health problem regarding the consumption of *Haruan* in a different location. Even though there was some respondent who chose to buy fish from the market, the main habitat of *Haruan* was in the trench and irrigation that linked to the paddy field. So, there was a possibility that *Haruan* fish bought from the market was also exposed to the heavy metal.

Table 4.5 showed how the fish was cooked by the villagers. Data showed that the most favourable cooking method was deep-fried followed by roasted and another cooking style. Based on the journal by Nurul Izzah Ahmad et al. (2016), it showed that second most of the freshwater fish that was consumed by Malaysian was snakehead with the different cooking method. The Malaysian people mostly preferred to cook fish by the deep fry method, followed by grilled and then cooked with a curry flavour or coconut milk. Cooking style such as steam and boiled become moderately preferred by the study population. The consumption rate of *Haruan* fish was a primary factor that contributes to the health hazard calculation especially toward the contaminated fish. Increasing the quantity of fish consumption will increase the chances for villagers getting health problem. Based on the consuming frequency during a 12-month period, the

majority of the respondents were not entirely consuming the *Haruan* fish. This could be due to factors from table 4.4 where most of the villagers preferred to catch fish during harvesting season and also due to the high price of *Haruan*. Data also revealed that most of the respondents would take about two pieces of fish in one serving which means that in one serving the amount of fish consumption was equal to 280gram due to the size of *Haruan* that was big enough to eat per person.

5.3 Contamination levels of heavy metal lead, arsenic and cadmium that accumulates in body fish of *Channa Striatus*.

Different type of chemicals used had different purposes. There were several types of pesticide used to protect the crop. For example, an insecticide used for killing the insect and other arthropods, herbicide to kill the weed and other unwanted plants and also rodenticide to control mice and rat. That type of pesticide has used in controlling the farmer crops which contain a different type of active ingredient.

Arsenic is used as a pesticide primarily to preserve wood from rot and decay (NPIC,2015). In the past, arsenic was also used in rat poisons, ant poisons and weed killers. Due to the excessive use of rodenticide and herbicide, the concentration levels of arsenic continued to increase especially in the water and soil and this lead to people being exposed by various type of exposure routes. It became worse to the public if there was a synergism between arsenic and other heavy metals. For example, alteration in central monoaminogenic system Neurotoxicity and cytotoxicity could occur when arsenic combines with lead

(Mejia et al., 1997) or Change in relative liver weight MCV and PFC content of spleen when arsenic combines with dimethoate (Institóris et al., 2001b).

Cadmium was detected in the fertiliser also called plant food elements. This fertiliser was substance produced to supply these plant food elements in a readily available form for plant use and also maintain adequate plant food to sustain continuous healthy plant growth. In contrasts to lead and mercury, cadmium was readily absorbed by plants. Cadmium equally distributed over the plant. Cadmium was taken up through the roots of plants to edible leaves, fruits and seeds. During the growth of grains such as wheat and rice, cadmium is taken from the soil and was concentrated in the core of the kernel. Cadmium is generally considered the most toxic to humans and animals even at low concentration. Cadmium itself will cause cancer, congenital disabilities, and genetic mutations. Consider about synergistic exposure of Cadmium and Propoxur will cause to alter immune and neurotoxicologically function. (Institóris et al., 2002)

Lead has also becomes one of the heavy metal that gives cause for concern to many people due to the effect of the exposure. When accumulation reaches a substantially high level, accumulated heavy metals in the tissues of aquatic animals and may become toxic (Afshan et al, 2014). Marine organisms exposed to a higher concentration of heavy metals in water may take up substantial quantities of these metals. Bio-magnification of a pollutant may lead to toxic levels in species high up in the trophic chain and freshwater systems. Lead (Pb) is as a potent environmental pollutant. Lead toxicity has become very important due to its great concern for human health. Fish are usually among the top consumers. People and fish are mainly exposed to lead by breathing and ingesting it in food,

water, soil, or dust (Afshan et al, 2014). Lead accumulates in the blood, bones, muscles, and fat. Newborn babies and young children are especially at risk due to even low levels of lead.

Exposure to environment toxicant such as Lead, Arsenic and Cadmium in the fish was considered to be one of the risk factors in the development of dietary health risks among fish consumer. Use of agricultural chemicals has been indicated as the primary anthropogenic source of As and Cd pollution in aquatic environments.

Inductive Cross-Plasma Mass Spectrometry (ICP-MS) was used to analyse the concentration level of heavy metal in the sample. Three types of heavy metal had been analysed in the sample of fish and unfortunately, out of three heavy metals, lead, and arsenic concentration had been detected. Lead in the pesticides has found and caused soil levels to become a health concern among farmer (Mohd Rozali Othman et al., 2016) while Arsenic widely found in pesticide which has attracted much public attention since the last decade. Even though Cadmium is non-detectable in our sampled fish, the concerns started being highlighted when sources of paddy water found contaminated as shown in (P.A.C.T. Perera et al., 2016) study.

5.4 Comparison of heavy metals level in *Channa Striatus* fish body with Food Regulation 1998.

The data analysed using ICP-MS, showed that the amount of the heavy metal concentration found in the fish filet was considered to be safe for consumption. The results showed the significant value of $P < 0.001$ for lead and

arsenic which indicated that the level for the heavy metal was below the maximum permitted proportion of metal contaminant in fish and fish product. The mean for three different types of heavy metal lead, arsenic and cadmium from three samples showed that lead was 8.28ug/kg that was higher than the arsenic value 5.90ug/kg. However, there was no detection level of cadmium in the fish filet.

According to the Food Act 1983, the maximum permitted proportion of metal contaminant in specified food like fish for the lead was 2.0 mg/kg while for arsenic and cadmium were 1.0 mg/kg. Although the value of the heavy metal concentration was far from the levels that could cause the consumers a health hazard, the presence of heavy metal in the paddy field and trench should be the baseline for the farmer to begin to be concerned about their health.

There were a few factors that need to consider which could possibly cause the level of decline and elevation of heavy metal concentration in the trench. Malaysia is well known as Asian country with tropical rain forests, (S. Appanah & L. Ratnam, 1992). Even though, Selangor was located in the centre and having two inter-monsoons from April-May and October, in 2018 the average of rainfall was higher in months September until the end of January. Due to dilution effects of chemical compounds as an effect of high-intensity and extended rainfall.

This study could not say that the concentration of all the heavy metal might pose any health risk since the values were within the permissible limits. Another factor that might contribute to decreasing of heavy metal concentration was due to fish metabolism. The accumulation of trace metals in the liver could be traced to the metabolic processes and enzyme catalysed reaction taking place in the liver. The

liver might have also played an essential role in detoxification, and this detoxification in the two fish species may be by sequestration rather than elimination by excretion (Gbem et al., 2001). Also, the entry of metals into fish occurred either through the gill membrane or through ingestion, and this may have accounted for the high concentration of trace metals in the gills of both fish species (Chatterjee et al., 2006).

Another factor that also could cause of decreasing heavy metal was bio-accumulation. The present study showed that metal accumulation was low in muscles of *Haruan*. It might be attributed to the growth factor as growth may dilute toxicant concentration if growth is faster than accumulation (Gbem et al., 2001)

5.5 Dietary health risk assessment based on the levels of heavy metal lead, arsenic and cadmium accumulated from up taking *Channa Striatus* of fish

Generally, if a person was having high BMI value and aging, they were more likely to have health problems. A study by N. Kragh (2019) showed that there was a significant association between BMI and health-related quality of life found after controlling factors such as gender, age, and obesity-related comorbidities.

As people started to age, the body system becomes weak, creating and causing health problems. Immunosenescence could be simplified to the changes that occur in the immune system with an increasing age (PN Bryman, 2014). The article Immune function in older adults by A. Azar (2018) stated that, as the immune system ages and these capabilities decline, there is increased susceptibility to infections and cancer and an increased incidence of autoimmune disorders.

The results showed that factors such as BMI and age categories did not contribute to Hazard Quotient and Lifetime Cancer Risk that exceeds the limit. This

evidence could tell the villagers that the consumption of contaminated *Haruan* was not causing health problems as long as they followed the guidance. Even though the HQ value and LCR value showed that consumption of fish was safe, there are many factors that cause heavy metal entering the body. Exposure from one source may not be affecting the health of the body. However, exposure from other exposure routes such as inhalation or direct contact by skin, possibly having health problem was higher in the long-term period. Consumption of contaminated water also become the primary concern of health prom, Arsenic, for instance, the exposure to arsenic utilising air, food and water. Drinking water polluted with arsenic is one of the significant causes of arsenic toxicity around 30 countries. (Chowdhury et al., 2000).

Villagers from *Simpang Lima* village should consider the frequency of fish consumption because based on table 4.11 which is individual Hazard Quotient show that the HQ exceeds the limit if they consume more than 48 times in 365 days. Because metabolism might control villager health condition. Females need to concern more about their body condition because they have weak metabolism compared to male. A metabolism, known as biotransformation, is the enzyme-catalysed conversion of one chemical into another that may be more or less toxic (Lech and Vodcnik, 1985). During this process, lipid-soluble compounds converted into water-soluble ones, excreted in the bile and urine. Lead, for example, will be eliminated from the body by both urine and faeces (Yabe J, 2012). Once our metabolism process becomes slow, it will slow down excretion, which is removing the foreign substance and lead to the heavy metal trap in the body until it becomes dependent on its half-life.

The first half-time of lead in blood after the cessation of exposure is 35-40 days, while for the lead in long bones; the half-time is around 20 yr. (Zenz, 1994).

For arsenic, the half-life of inorganic arsenic in humans is about 10 hours (Rossman 2007) and for cadmium, it depended on the heavy metal accumulated. The kidney takes about 6-38 years, but in the liver, it takes 4-19 year to disappear. (ATSDR, 2008).



CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Overall, the consumption of contaminated fish in *Simpang Lima* trenches was considered safe for the villagers especially during harvesting season. The low level of heavy metal in fish muscle indicated that consumers might not have any acute symptom, but they should consider the long-term effect from it. There were few factors that could harm people even if the level of trace element was low. From the HQ calculation to estimate the potential hazard of polluted food, it can be seen that factors such as frequency of eating and body weight played a main role whether consumption could cause health problem or if it was safe to consume. Sungai Besar villager need to take into consideration all possible causes of health problems.

6.2 Study limitation

As the pesticide exposure to the water and aquatic life in the paddy field was considering non-point source, it is hard to estimate if the pesticide only from paddy field is the main cause of pollution. In this study, housewife also had used the pesticide during the planting activity like the flower and vegetable. They tend to have their own mini crop or subsidiary farming to support their food production for their

family. Besides, as the pre- observation during site visit, most of the villager likes to catch the *Channa Striatus* in different trench. But, the fish sample of the study only covers several trenches in *Simpang Lima* due some limitation. So, the result from the sample cannot represent the population in the Sungai Besar.

6.3 Recommendations

Channa Striatus gives a lot of benefit the health especially to heal people that having wound when they consume it. But consumption to *Channa Striatus* that contaminated with heavy metal may cause harm to body. Even the concentration level of the heavy metal inside the fish is consider low by comparing to Food Act 1983, but continuously eating the contaminated fish could build the effect on long term exposure.

Farmer or other people that worked with pesticide should take precaution to prevent from direct exposed to the heavy metal. Excessive use of the pesticide not only harming the people, but also can pollute the environment. Dispersion of heavy metal to environment causes a lot of pollutant such as air, water, animal and plant. The effect was not only being taken by adult but also to the children.

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APPENDIX A

- RESPONDENT'S INFORMATION SHEET AND INFORMED CONSENT FORM
- QUESTIONNAIRE



**JAWATANKUASA ETIKA UNIVERSITI UNTUK
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FORM 2.4: RESPONDENT'S INFORMATION SHEET AND INFORMED CONSENT FORM

Please read the following information carefully and do not hesitate to discuss any questions you may have with the researcher.

1. STUDY TITLE :

Health Risk Assessment of Heavy Metal via Dietary Intakes of *Channa striatus* Fish Body from Paddy Field, Sabak Bernam.

2. INTRODUCTION:

In Malaysia, rice is the primary source of food and has since increased in demand of the staple food for the nation that lead to multiple cropping of paddy plant. To ensure the production of the plant is in the control, government already take a few step which are, in 1960s, green revolution programme was developing by government to introduce the double- cropping system. This system included the package programme of new high-yield variety seeds, input of chemical fertilizer and use of agricultural machinery. These post-independent programmes (with subsidy), has enabled the farmers to produce more rice for sale, and thus improved their income. Along the way to improve the paddy plant, a lot of pesticide was used and cause water contamination in paddy field. aquatic plant like fish has been one of victim of the pesticide contamination. End of the process, human is the one that eating all the contamination produce and this lead the health problem such as brain, heart and other organs.

3. WHAT WILL YOU HAVE TO DO?

You are required to sign a registration form and respondents express an interest to participate in this study. It can be done after you read and understanding the content of this description. Entry form must be returned to the researcher respondents before the interview and test will be conducted. Tests to be carried out is the weight and height.

4. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

Respondent from the local village in Sungai Besar must living in the village at least 20 year and having experience eating the fish *Anabas Testudineus* and *Channa striatus* for 7 year and more. Besides, the respondent age also only for 25 to 60 year old. Those who not having the criteria are not allowable to participate.

5. WHAT WILL BE THE BENEFITS OF THE STUDY:

(a) TO YOU AS THE SUBJECT?

This study objective is to determine the concentration of heavy metals (lead, cadmium and zinc) that accumulate in the fish body part of *Anabas Testudineus* and *Channa striatus* from paddy field water and its dietary human health risk to paddy agricultural villager. By doing the study, we can identify if there is any significant of the fish contamination with the health risk of the person who eat the freshwater. Based on the data, we can give any recommendation suitable with the fish intake of the villager.

(b) TO THE INVESTIGATOR?

Data collected will help the researchers to study the effect of eating the contaminated fish that living in the paddy field. From the data, researchers will identify the possible chronic health effect to the eater if the concentration of the heavy metal; lead, cadmium and zinc was eaten exceed the recommended intake. The data also can be the use as a guide to help overcome health problem related to eating contaminant fish

6. WHAT ARE THE POSSIBLE RISKS?

The procedure are safe and not risk because onlu use the weight and height as well as questionnaire form. The process does not going any drug or painful procedure.

7. WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?

Information and identification used in this study will remain confidential

8. WHO SHOULD YOU CONTACT IF YOU HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

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Please initial here if you have read and understood the contents of this page _____

9. CONSENT

I Identity Card No.
address.....

.....hereby voluntarily agree to take part in the research stated above *(clinical /drug trial/video recording/ focus group/interview-based/ questionnaire-based).

I have been informed about the nature of the research in terms of methodology, possible adverse effects and complications (as written in the Respondent's Information Sheet). I understand that I have the right to withdraw from this research at any time without giving any reason whatsoever. I also understand that this study is confidential and all information provided with regard to my identity will remain private and confidential.

I* wish / do not wish to know the results related to my participation in the research

I agree/do not agree that the images/photos/video recordings/voice recordings related to me be used in any form of publication or presentation (if applicable)

* delete where necessary

Signature
(Respondent)

Signature
(Witness)

Date :.....

Name :.....

I/C No. :.....

I confirm that I have explained to the respondent the nature and purpose of the above-mentioned research.

Date

Signature
(Researcher)



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 Logam Berat melalui Pengambilan Pemakanan dari *Channa Striatus* isi ikan dari sawah padi, *Sabak Bernam*.

2. PENGENALAN

Di Malaysia, beras adalah sumber utama makanan dan telah meningkat dalam permintaan makanan ruji untuk negara yang membawa kepada pelbagai tanaman padi. Untuk memastikan kuantiti pengeluaran padi berada di dalam kontrol, pemungut telah mengambil beberapa langkah yang pada tahun 1960-an, program revolusi hijau telah dibangunkan oleh kerajaan untuk memperkenalkan sistem penanaman dua kali. Sistem ini termasuk program pakej benih varieti hasil tinggi baru, input baja kimia dan penggunaan jentera pertanian. Program pasca-bebas (dengan subsidi), telah membolehkan petani menghasilkan lebih banyak padi untuk dijual, dan dengan itu meningkatkan pendapatan mereka. Sepanjang jalan untuk meningkatkan mutu dan kuantiti tanaman padi, banyak racun perosak digunakan dan menyebabkan pencemaran air di sawah padi. Haiwan akuatik seperti ikan telah menjadi mangsa pencemaran racun makhluk perosak. Ini kerana, ikan yang tinggal di kawasan sawah cenderung makan makanan yang sudah tercemar dengan racun perosak. Ikan mempunyai keupayaan untuk mengumpul logam berat di tisu mereka dengan penyerapan di sepanjang permukaan insang dan buah pinggang, hati dan usus dinding ke tahap yang lebih tinggi daripada kepekatan alam sekitar. Akhir proses, manusia adalah yang makan semua pencemaran menghasilkan dan ini membawa kesihatan masalah seperti otak, jantung dan organ lain.

3. APAKAH YANG PERLU ANDA LAKUKAN?

Responden dikehendaki menandatangani borang pendaftaran dan mereka perlu menyatakan minat untuk mengambil bahagian dalam kajian ini. Ia boleh dilakukan selepas mereka membaca dan memahami kandungan keterangan dalam soal selidik. Borang penyertaan mesti dikembalikan kepada responden penyelidik sebelum wawancara dan ujian akan dijalankan. Pengukuran yang akan dilakukan ialah berat dan ketinggian sebagai data semasa mengisi sesi soal selidik ..

4. SIAPA YANG TIDAK BOLEH MENYERTA KAJIAN INI?

Responden yang tidak tinggal di kampung setempat di Sungai Besar tidak diminta untuk menjawab soalan tersebut. Sekurang-kurangnya 20 tahun tinggal di kawasan sampel dan mempunyai pengalaman makan *Channa striatus* selama 7 tahun dan lebih diperlukan dalam sesi kuesioner. Selain itu, umur responden juga hanya berusia 20 hingga 70 tahun. Mereka yang tidak mempunyai kriteria tidak dibenarkan untuk mengambil bahagian.

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?

Objektif kajian ini adalah untuk menentukan kepekatan logam berat (plumbum, kadmium dan arsenik) yang terkumpul di dalam badan ikan *Channa striatus* dari air medan padi dan risiko kesihatan manusia untuk penduduk padi pertanian. Dengan melakukan kajian ini, kita dapat mengenal pasti jika terdapat sebarang pencemaran ikan yang signifikan dengan risiko kesihatan orang yang makan ikan *Channa striatus*. Berdasarkan keputusan dari analisis makmal, kami boleh memberikan cadangan yang sesuai dengan pengambilan ikan penduduk kampung untuk mencegah dan bahaya kesihatan jika logam berat dikesan dalam bahagian badan ikan.

b) KEPADA PENYELIDIK?

Data yang dikumpul akan membantu para penyelidik mempelajari kesan makan ikan yang tercemar yang tinggal di sawah padi. Dari data, para penyelidik akan mengenal pasti kesan kesihatan kronik yang mungkin ke pemakan jika kepekatan logam berat; plumbum, kadmium dan arsenik dimakan melebihi pengambilan rekomendasi. Data ini juga boleh digunakan sebagai panduan untuk mengatasi masalah kesihatan yang berkaitan dengan makan ikan tercemar.

6. ADAKAH IA BERISIKO?

Prosedur ini selamat dan tidak berisiko kerana hanya menggunakan berat dan ketinggian serta borang soal selidik. Proses ini tidak akan berlaku sebarang ubat atau prosedur yang menyakitkan.

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?

Dr Vivien How
Jabatan Kesihatan Persekitaran dan Pekerjaan
Fakulti Perubatan dan Sains Kesihatan
Universiti Putra Malaysia
43400 UPM Serdang
Nombor Telefon: 0166193697
Nombor faks: 0389472585
E-mel: vivien@upm.edu.my

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 Helaiian 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
(Responden)

Tandatangan
(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)



Borang Soal Selidik Kajian Penilaian Risiko Kesehatan Logam Berat melalui Pengambilan Pemakanan ikan puyu dan ikan haruan di sawah padi, Sabak Bernam.

Fakulti Perubatan dan Sains Kesehatan, Universiti Putra Malaysia.

Nama responden	
Alamat responden	
Nombor telefon	

rahan untuk soal selidik:

Saya dengan ini menjemput anda sebagai salah seorang responden dalam kajian penyelidikan yang mengkaji hubungan antara nutrien ikan puyu dan ikan haruan dalam sistem pengairan sawah padi melalui penilaian risiko kesehatan. Oleh itu, saya meminta perkhidmatan yang baik daripada anda untuk menjawab soalan-soalan yang terkandung dalam borang kaji selidik ini dengan tepat dan jujur. Sebarang maklumat yang diterima dari syarikat anda akan dirahsiakan. Kerjasama anda amat dihargai.

Borang soal selidik ini merangkumi beberapa bahagian. Antaranya:

1. BAHAGIAN - A (MAKLUMAT UMUM PENDUDUK KAMPUNG)
2. BAHAGIAN - B (GAYA HIDUP)
3. BAHAGIAN - C (PENGUNAAN RACUN PEROSAK)
4. BAHAGIAN - D (PENGAMBILAN IKAN)
5. BAHAGIAN - E (STATUS PERUBATAN)

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

BAHAGIAN - A (MAKLUMAT UMUM PENDUDUK KAMPUNG)

A1	Jantina			
	Lelaki		Perempuan	
A2	Umur			
	_____ Tahun			
A3	Tahap pendidikan			
	Sekolah rendah		Sekolah menengah	
			diploma	
				ijazah
	Lain-lain			
A4	Status pekerjaan			
	bekerja		Tidak bekerja	
			pelajar	
				pesara
A6	Pekerjaan			
	Petani		Bukan petani	
A7	Status pendapatan			
	<RM3000		>RM3000	
A8	Berat (kg)			
A9	Tinggi (cm)			

BAHAGIAN - B (GAYA HIDUP)

B1	Adakah anda seorang perokok			
	Ya		Tidak	
B2	Jika ya, berapa kerap anda merokok setiap hari			
	_____ Batang rokok			
B3	Berapa umur anda mula merokok secara teratur?			
	_____ Umur			
B4	Jika anda telah berhenti merokok, berapa umur anda berhenti merokok?			
	_____ Umur			
B5	Adakah anda mengambil minuman keras (alkohol)?			
	Ya		Tidak	
B6	Berapa banyak botol untuk diminum dalam sehari?			
	_____ botol			

BAHAGIAN - C (PENGUNAAN RACUN PEROSAK)

C1	Adakah anda menggunakan sebarang racun makhluk perosak di sawah padi anda?		
	Ya		Tidak
C2	Adakah anda membeli racun perosak dari satu atau lebih pembekal lain?		
	Ya		Tidak
C3	Adakah anda membeli jenama racun serangga yang sama untuk digunakan di ladang anda, atau anda sering membeli jenama yang berbeza?		
	Ya		Tidak
C4	Berapa kerap anda menggunakan racun perosak untuk penanaman padi setiap tahun? _____ setahun		
C5	Senaraikan jenis racun perosak yang digunakan untuk penanaman padi.		
Bil	Keadaan padi (peringkat)	Jenis racun	Kuantiti penggunaan sepanjang penanaman
1.			
2.			
3.			
4.			
5.			
6.			
7.			
C6	Adakah anda sedar kehadiran racun perosak memberi kesan kepada kawasan tanaman?		
	Ya		Tidak
C7	Pernahkah anda menyaksikan kematian secara besar-besaran dalam ikan di sawah padi anda?		
	Ya		Tidak
C8	Adakah ikan menunjukkan apa-apa tanda yang tidak normal sebelum kematian?		
	Ya		Tidak
C9	Adakah anda bimbang tentang kesan racun perosak kepada kehidupan akuatik? Terangkan.		
	Ya		Tidak

BAHAGIAN - D (KONSUMSI IKAN)

D1	Adakah anda atau rumah anda menangkap ikan dari 12 bulan yang lalu?																							
	Ya	Tidak																						
D2	Di mana anda biasanya menangkap Puyu atau Haruan?																							
	Parit	Sawah																						
D3	Adakah anda atau rumah anda memakan ikan dari 12 bulan yang lalu?																							
	Ya	Tidak																						
D4	Dalam 12 bulan yang lalu, adakah anda pernah makan Puyu atau Haruan?																							
	Ya, Haruan	Tidak																						
	Ya, Puyu	Kedua-dua.																						
D5	Berapa kerap dalam tempoh 12 bulan yang lalu anda makan Haruan atau Puyu apabila berada pada musim? _____ (Hari, minggu, tahun)																							
	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr><td>Tidak pernah</td><td></td></tr> <tr><td>1-6 kali setahun</td><td></td></tr> <tr><td>7-11 kali setahun</td><td></td></tr> <tr><td>1 kali sebulan</td><td></td></tr> <tr><td>2-3 kali sebulan</td><td></td></tr> <tr><td>1 kali seminggu</td><td></td></tr> <tr><td>2 kali seminggu</td><td></td></tr> <tr><td>3-4 kali seminggu</td><td></td></tr> <tr><td>5-6 kali seminggu</td><td></td></tr> <tr><td>1 kali sehari</td><td></td></tr> <tr><td>2 atau lebih kali sehari</td><td></td></tr> </table>		Tidak pernah		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		1 kali sehari		2 atau lebih kali sehari	
Tidak pernah																								
1-6 kali setahun																								
7-11 kali setahun																								
1 kali sebulan																								
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1 kali seminggu																								
2 kali seminggu																								
3-4 kali seminggu																								
5-6 kali seminggu																								
1 kali sehari																								
2 atau lebih kali sehari																								
D6	Bagaimanakah anda memasak ikan puyu dan haruan?																							
	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr><td>Steam/Wap</td><td></td></tr> <tr><td>Panggang</td><td></td></tr> <tr><td>Menggoreng</td><td></td></tr> <tr><td>Merebus</td><td></td></tr> </table>		Steam/Wap		Panggang		Menggoreng		Merebus															
Steam/Wap																								
Panggang																								
Menggoreng																								
Merebus																								
D7	Apakah punca yang mempengaruhi anda untuk menangkap ikan? _____																							
D8	Mana-mana bahagian ikan yang anda suka makan?																							
	insang	isi																						
D9	Berapa banyak ikan yang anda makan dalam satu hidangan ? _____ satu hidangan																							

BAHAGIAN - E (STATUS PERUBATAN)

E1	Adakah anda mempunyai apa-apa rekod perubatan kesihatan anda?									
	Ya	Tidak								
E2	Adakah anda mempunyai pengalaman satu isu kesihatan selepas makan Puyu atau Haruan									
	Ya	Tidak								
E3	Adakah anda mempunyai sekatan yang diperintahkan oleh doktor mengenai kebiasaan makan? Terangkan.									
	Ya	Tidak								
E4	Adakah anda mempunyai keadaan jantung-seperti kegagalan jantung kongestif atau penyakit jantung koronari?									
	Ya	Tidak								
E5	Adakah anda mengalami gangguan paru-paru-seperti asma atau COPD?									
	Ya	Tidak								
E6	Adakah anda mempunyai diabetes/kencing manis?									
	Ya	Tidak								
E7	Adakah anda mempunyai tekanan darah tinggi?									
	Ya	Tidak								
E8	Adakah anda mempunyai penyakit ginjal atau adakah dialisis?									
	Ya	Tidak								
E9	Adakah anda mempunyai kanser?									
	Ya	Tidak								
E10	Nyatakan keadaan kesihatan anda yang lain:									
E11	Bilakah kali terakhir anda mendapatkan rawatan daripada doktor?									
	<table border="1"><tr><td>Seminggu yang lalu</td><td></td></tr><tr><td>Dua minggu lalu</td><td></td></tr><tr><td>Sebulan yang lalu</td><td></td></tr><tr><td>Dua bulan lalu</td><td></td></tr></table>		Seminggu yang lalu		Dua minggu lalu		Sebulan yang lalu		Dua bulan lalu	
Seminggu yang lalu										
Dua minggu lalu										
Sebulan yang lalu										
Dua bulan lalu										

Saya telah membaca perjanjian ini dengan teliti dan memahami istilahnya,

Tandatangan peserta

Nama:

Tarikh:

Hubungi No:

Alamat rumah:

Saya menghargai dan berterima kasih atas kerjasama anda dalam menjawab soalan ini. Semua jawapan anda sangat dihargai dan akan menjadi maklumat penting dalam menjalankan kajian ini. Sekali lagi, saya ingin menjamin kesulitan respons dan tindak balas anda. Jawapan anda dan respon akan kami rahsia dan tidak akan diedarkan ke mana-mana pihak atau digunakan untuk tujuan lain. Terima kasih.

Untuk maklumat lanjut sila hubungi:

ERNNEY SHUHEIDA BINTI KHAMARUDIN / NURUL NADIAH BINTI

MOHKHTAR@MOKHTAR

E-mel: ernneyshuheidakhamarudin@gmail.com / shenadia7@gmail.com

Hubungi: 013-3513813 / 012-7477116

APPENDIX B

- **LABORATORY PERMISSION LETTER**
 - ✦ **Institute of Bioscience, UPM**
 - ✦ **Faculty of Environmental Studies, UPM**



FAKULTI PERUBATAN DAN SAINS KESIHATAN
Faculty of Medicine and Health Sciences

Institute of Bioscience,
Universiti Putra Malaysia,
43400 UPM Serdang,
Selangor, Malaysia.

Puan,

**MEMOHON KEBENARAN MENGGUNAKAN MAKMAL DAN ALATAN BAGI
PENYELIDIKAN TAHUN AKHIR KURSUS EOH4999A&B (PROJEK ILMIAH TAHUN
AKHIR)**

Dengan hormatnya perkara di atas adalah dirujuk.

2. Sukacita dimaklumkan bahawa pelajar Tahun 4 program Bacelor Sains (Kesihatan Persekitaran dan Pekerjaan), Fakulti Perubatan dan Sains Kesihatan, UPM, dibawah ingin memohon kebenaran untuk menggunakan makmal bagi tujuan aktiviti makmal proses pencernaan ikan daripada jabatan bagi tujuan melaksanakan penyelidikan akhir tahun.
3. Tujuan Projek Ilmiah Tahun Akhir ini dijalankan adalah bagi memenuhi salah satu syarat untuk pelajar bergraduati. Berikut adalah senarai butiran pelajar dan alat peminjaman yang diperlukan.

Pelajar 1

Nama pelajar : Nurul Nadiah binti Mohkhtar@Mokhtar

No. Matrik : 183737

Tajuk Kajian : Dietary Health Risk Assessment and Determination of Heavy Metals in Fish from Paddy Field in Sabak Bernam

Pelajar 2

Nama pelajar : Ernney Shuheida binti Khamarudin

No. Matrik : 184575

Tajuk Kajian : Health Risk Assessment of Heavy Metal via Dietary Intakes of Anabas Testudineus and Channa Srtiatus Fish Body Parts from Paddy Field, Sabak Bernam



Zairi bin Ismai,
Penolong Pegawai Kesihatan,
Faculty of Environmental Studies,
Universiti Putra Malaysia,
43400 UPM Serdang,
Selangor Darul Ehsan.

Tuan/Puan,

MEMOHOM MENGANALISA LOGAM BERAT BAGI PENYELIDIKAN TAHUN AKHIR KURSUS EOH4999A&B (PROJEK ILMIAH TAHUN AKHIR)

Dengan hormatnya perkara di atas adalah dirujuk.

2. Sukacita dimaklumkan bahawa pelajar than 4 program bachelor sains (Kesihatan Persekitaran dan Pekerjaan), Fakulti Perubatan dan Sains Kesihatan, UPM, dibawah ingin memohon menganalisa logam berat menggunakan Inductively-Coupled Plasma Mass (ICP-MS) spectrometry di UPM lab bagi tujuan melaksanakan penyelidikan akhir tahun.
3. Tujuan projek ilmiah tahun akhir ini dijalankan adalah bagi memenuhi salah satu syarat untuk pelajar bergraduati. Berikut adalah tarikh menganalisa logam berat yang telah ditetapkan.

Pelajar 1

Nama pelajar: Nurul Nadiah Binti Mohkhtar@Mokhtar

No. matrik: 183737

Tajuk kajian: Dietary Health Risk Assessment and Determination of Heavy Metal in Fish from Paddy Field in *Sabak Bernam*.

Pelajar 2

Nama pelajar: Ernney Shuheida Binti Khamarudin

No.matrik: 184575

Tajuk kajian: Health Risk Assessment of Heavy Metal via Dietary Intake of *Channa Striatus* Fish from Paddy Field, *Sabak Bernam*.

The image features a large, faint watermark of the Universiti Putra Malaysia (UPM) logo in the background. The logo is a shield-shaped emblem with a red and white color scheme. At the top of the shield, the letters 'UPM' are written in white on a red background. Below this, there is a stylized white book with an open cover. The shield is flanked by vertical lines and has a red base. The text 'APPENDIX C' is centered over the logo in a large, bold, black serif font.

APPENDIX C

- ETHIC APPROVAL

The image features a large, faint watermark of the Universiti Putra Malaysia (UPM) logo in the background. The logo is a shield-shaped emblem with a red and white color scheme. At the top left of the shield, the letters 'UPM' are written in white on a red background. In the center, there is a white book with an open cover. Below the book, there are several vertical white lines of varying heights. The entire shield is set against a light grey background.

APPENDIX D

- ICP-MS LABORATORY RESULT

Quantitative Analysis - Summary Report

Sample ID: 1

Sample Date/Time: Monday, January 28, 2019 14:38:04

Sample Description: Fish sample

Solution Type: Sample

Blank File:

Number of Replicates: 3

Peak Processing Mode: Average

Signal Profile Processing Mode: Average

Dual Detector Mode: Dual

Dead Time (ns): 55

Sample File: C:\Elandata\Sample\Emney Shuheida.sam

Method File: C:\Elandata\Method\emney shuheida.mth

Dataset File: C:\Elandata\DataSet\Emney Shuheida\1.008

Tuning File: C:\Elandata\Tuning\Default.tun

Optimization File: C:\Elandata\Optimize\Default.dac

Calibration File: C:\Elandata\System\Emney Shuheida.cal

Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	12528	3.215	131.445	3.667
Pb	208	335984	0.445	103850.873	3.047
Cd	111	1570	1.940	8109.467	4.669

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	12396.078	6.042	0.20	3.2	ppb
Pb	208	232133.236	9.147	0.06	0.6	ppb
Cd	111	-6539.109	-2.679	0.01	0.5	ppb

Replicates

Repeat 1

Analyte	Concentration
As	5.862086
Pb	9.079822
Cd	-2.683958

Repeat 2

Analyte	Concentration
As	6.013805
Pb	9.187681
Cd	-2.687645

Repeat 3

Analyte	Concentration
As	6.251525
Pb	9.174610
Cd	-2.664428

Quantitative Analysis - Summary Report

Sample ID: 3

Sample Date/Time: Monday, January 28, 2019 14:40:01

Sample Description: Fish sample

Solution Type: Sample

Blank File:

Number of Replicates: 3

Peak Processing Mode: Average

Signal Profile Processing Mode: Average

Dual Detector Mode: Dual

Dead Time (ns): 55

Sample File: C:\Elandata\Sample\Ernney Shuheida.sam

Method File: C:\Elandata\Method\ernney shuheida.mth

Dataset File: C:\Elandata\DataSet\Ernney Shuheida\3.009

Tuning File: C:\Elandata\Tuning\Default.tun

Optimization File: C:\Elandata\Optimize\Default.dac

Calibration File: C:\Elandata\System\Ernney Shuheida.cal

Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	12359	1.840	131.445	3.667
Pb	208	263361	2.334	103850.873	3.047
Cd	111	824	1.443	8109.467	4.669

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	12227.344	5.960	0.11	1.9	ppb
Pb	208	159510.596	6.286	0.24	3.9	ppb
Cd	111	-7285.541	-2.984	0.00	0.2	ppb

Replicates

Repeat 1

Analyte	Concentration
As	5.916429
Pb	6.097909
Cd	-2.981600

Repeat 2

Analyte	Concentration
As	5.877947
Pb	6.199977
Cd	-2.981668

Repeat 3

Analyte	Concentration
As	6.086292
Pb	6.558991
Cd	-2.990066

Quantitative Analysis - Summary Report

Sample ID: 4

Sample Date/Time: Monday, January 28, 2019 14:41:58

Sample Description: Fish sample

Solution Type: Sample

Blank File:

Number of Replicates: 3

Peak Processing Mode: Average

Signal Profile Processing Mode: Average

Dual Detector Mode: Dual

Dead Time (ns): 55

Sample File: C:\Elandata\Sample\Emney Shuheida.sam

Method File: C:\Elandata\Method\emney shuheida.mth

Dataset File: C:\Elandata\DataSet\Emney Shuheida4.010

Tuning File: C:\Elandata\Tuning\Default.tun

Optimization File: C:\Elandata\Optimize\Default.dac

Calibration File: C:\Elandata\System\Emney Shuheida.cal

Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	11876	1.211	131.445	3.667
Pb	208	342233	0.941	103850.873	3.047
Cd	111	973	2.652	8109.467	4.669

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	11744.308	5.725	0.07	1.2	ppb
Pb	208	238382.599	9.394	0.13	1.4	ppb
Cd	111	-7136.415	-2.923	0.01	0.4	ppb

Replicates

Repeat 1

Analyte	Concentration
As	5.645778
Pb	9.259928
Cd	-2.927181

Repeat 2

Analyte	Concentration
As	5.749008
Pb	9.408449
Cd	-2.911408

Repeat 3

Analyte	Concentration
As	5.779513
Pb	9.512517
Cd	-2.931482

GANTT CHART AND MILESTONE

	2018					2019				
Project/ activities	September	October	November	December	January	February	March	April	May	June
Determine Research/title										
Proposal writing										
Proposal submission										
Proposal presentation										
Order/ book for equipment										
Data collection										
Data analysis										
Thesis writing										
Thesis submission & VIVA										