FYP FSB

POTENTIAL HEALTH RISK OF METAL CONCENTRATION IN FISH SPECIES TO BATEK TRIBE IN MERAPOH PAHANG.

By

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A report submitted in fulfillment of the requirements for the degree of Bachelor of Applied Science (Sustainable Science) with Honours

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THESIS DECLARATION

I hereby declare that the work embodied in this Report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

……………………………………..

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I certify that the Report of this final year project entitled Potential Health Risk Consequences of Metal Concentration in Fish species to Batek Tribe in Merapoh Pahang by Mohammad Azlan Bin Rosman, matric number E17A0015 has been examined and all correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Sustainable Science) with Honors Faculty of Earth Science, University Malaysia of Kelantan.

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Potential Health Risk Consequences of Metal Concentration in Fish species to Batek Tribe in Merapoh Pahang.

ABSTRACT

This study aims to evaluate the distributions of metals elements in fish species (*Cyclocheilichthys apogon*, *Barbodes lateristriga*, *Pristolepis groot*, *Rasbora elegans* and *Hemibagrus* sp.) sampled from three different river in Merapoh, Pahang, and to assess the safety of the fish consumption among Batek Tribe. The concentrations of Cd, As, Ni, Pb, Zn and Cu in those samples were determined using Atomic Absorption Spectroscopy (AAS) with acid digestion method which have been verified with a certified reference material (ERM BB422). The results revealed that the metal variability in the sampled fish originated from human activities around the sampling sites, in addition to natural causes. Such variations were also reflected on the metal accumulation pattern in the sampled fish, although the variations between different sampled fish were mainly subjected to their sampling sites location. In risk and safety assessment, the metal concentrations in the edible muscle of fish samples were found to be below the established limits (Malaysian Food Act,1983; Food and Agricultural Organization, USEPA), however target hazard quotient of As and Cd in *Cyclocheilichthys apogon* from Sungai Jalang and Sungai Kalong approached unity. Thus, it is advisable to regulate intake of the fish *Cyclocheilichthys apogon* to reduce the risk of deleterious health effect among Batek Tribe people.

Potensi Risiko Kesihatan akibat Kepekatan Logam pada spesies Ikan kepada Suku Batek di Merapoh Pahang.

ABSTRAK

Kajian ini bertujuan untuk menilai taburan unsur logam pada spesies ikan (*Cyclocheilichthys apogon, Barbodes lateristriga, Pristolepis groot, Rasbora elegans* dan *Hemibagrus sp.*) Yang diambil sampel dari tiga lokasi sungai yg berbeza di Merapoh, Pahang, dan untuk menilai keselamatan bagi mengkosumsi ikan di kalangan Suku Batek. Kepekatan Cd, As, Ni, Pb, Zn dan Cu dalam sampel tersebut ditentukan menggunakan Spektroskopi Penyerapan Atom (AAS) dengan kaedah pencernaan asid yang telah disahkan dengan bahan rujukan yang diperakui (ERM BB422). Hasil kajian menunjukkan bahawa kebolehubahan logam pada sampel ikan berasal dari aktiviti manusia di sekitar lokasi pengambilan sampel, selain penyebab semula jadi. Variasi semacam itu juga tercermin pada pola pengumpulan logam pada sampel ikan, walaupun variasi antara sampel ikan yang berlainan terutama tergantung pada lokasi sampel diambil. Dalam penilaian risiko dan keselamatan, kepekatan logam dalam sampel ikan yang dapat dimakan didapati berada di bawah had yang ditetapkan (Akta Makanan Malaysia, 1983; Organisasi Makanan dan Pertanian, USEPA), namun risiko bahaya As dan Cd di dalam spesis ikan *Cyclocheilichthys apagon* dari Sungai Jalang dan Sungai Kalong menghampiri perpaduan. Oleh itu, disarankan untuk mengatur pengambilan ikan *Cyclocheilichthys apogon* untuk mengurangkan risiko kesan kesihatan yang merugikan di kalangan orang Batek Tribe.

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LIST OF ABBREVIATIONS

As	Arsenic
AAS	Atomic Adsorption Spectrometry
Cu	Copper
Cd	Cadmium
EDI	Estimated Daily Intake
EF	Exposure Frequency
FAO	Food and Agriculture Organization
Ni	Nickel
Pb	Plumbum
RfD	Reference Dose
THQ	Target Hazard Quotient
WAB	Average Body Weight
WHO	World Health Organization
Zn	Zinc

LIST OF SYMBOLS

%	Percentage
° C	Degree Celsius
±	Plus, Minus

CHAPTER 1

INTRODUCTION

1.1 Background of study

As the human population began to grow, fish demand is expected to increase. Fish provides proteins, vitamins and minerals and it is known to be essential for human growth and development. Therefore, several studies have been conducted on the benefits of fish consumption to human health. Mineral elements like iron, manganese, copper, and zinc are important nutrients for the diet of the fish. Nonetheless, when the mineral elements are present in the diet in an extremely high or low concentration, it will cause adverse effect on both human and fish. (Okoye et al., 2011).

Around three billion people across the globe rely on both wild and farmed fish as their primary source of protein (WWF, 2016). Indigenous people are known for their lifestyle which rely on natural resources for survival. The Bateks, which is one of the aboriginal people found in the Peninsular Malaysia are categorized as Negrito. In Malaysia, the Negritos are the smallest aborigine's group found with approximately 3%

of the whole indigenous population (Fatanah et al., 2012). The Bateks are known as a group of egalitarian hunters and gatherers (Endicott, 2008). They live a nomadic lifestyle in Peninsular Malaysia's forests. They also love the river compared to land. There are two groups of Batek Tribe that can be found in Pahang: BatekIga and BatekTe' (Lye, 2005). Several settlement or villages of the Bateks can be found at present in and around the boundaries of Taman Negara (Nik, 1997).

1.2 Problem Statement

Based on the potential health risk perspective, the existing condition of the river due to active development may distort the quality of the fish, especially due to metal accumulation in the fish tissues. This study is in need for the calculation of the metal concentration in the fish muscle that will yield the result of the potential health risk to the Batek tribe in Merapoh Pahang. This study is very important as Batek tribe mainly depends on fish hunting for their livelihood. The Batek tribe was chosen for the study because they still live in traditional ways, even in this modernized era. Merapoh Pahang has been gazette for the national treasure of conserved forest in Malaysia. It is important to monitor the quality of the river for the conserving purposes and to secure our food especially to the Batek tribe. Human health can be affected if their food source composition has been contaminated with heavy metal. Therefore, it is important to undertake monitoring to ensure food sources of Batek tribe and their quality stay within the acceptable limits.

1.3 Objectives

The main objective of this study is to identify and assess the potential health risks of Batek Tribe in consuming wild caught freshwater fishes. This can be achieved in the following manner:

i. Determine the concentration of metals (Pb, Cd, Cu, As, Zn, Ni) in the muscle tissues of *Cyclocheilichthys apogon, Barbodes lateristriga, Pristolepis groot, Rasbora elegans* and *Hemibagrus* sp.
ii. Analyze the potential risk of human health by using Estimated Daily Intake (EDI) and Target Hazard Quotients (THQ).

1.4 Scopes of study

This study generally focused on identifying heavy metal accumulation in the tissues of fish muscles. The fish (*Cyclocheilichthys apogon, Barbodes lateristriga, Pristolepis groot, Rasbora elegans* and *Hemibagrus* sp.) were donated by the Batek tribe which are wild caught from Sungai Yu and Sungai Jalang in Hutan Simpan Kekal Tanum, Sungai Kalong in Hutan Simpan Kekal Persit. In order to analyze the heavy metals concentrations in the fish muscles tissues, wet digestion method was used. The concentration of Plumbum, Cadmium, Copper, Arsenic, Zinc, and Nickel in those samples were determined using Atomic Absorption Spectrometer (AAS) which have been verified with a series of certified reference material. In risk and safety assessment, estimated daily intake (EDI) and Target hazard quotient (THQ) were used to evaluate the potential risk.

The data then be compared with the permissible limit of heavy metal consumption to human by World Health Organization (WHO).

1.5 Significant of the study

A variety of environmental component, such as water, soil and biotic life, are protected by the measures of environmental quality. For example, fish is one of the most important diet to human and animals. Details on the quality of the aquatic environment through knowledge of its physical, chemical, and biological characteristics is very beneficial for water use. Therefore, it is very important to implement monitoring to ensure the water usage and food sources of Batek Tribe and their quality stays within the acceptable limits. Each of the fish species can act as an indicator to evaluate the presence of the heavy metal. As the fish is one of the most frequent diet of the Batek tribe, the research has been conducted in order to determine the presence of heavy metals and assess the potential health risk to the Batek tribe. There are still lack of awareness on the potential health risk due to metal accumulation. As the research result obtain, the data were compared with the permissible limit of heavy metal consumption to human by World Health Organization (WHO, 2006). As the study of the accumulation of heavy metal in the fish muscle tissues is being proposed, it can also evaluate the quality of the river.

CHAPTER 2

LITERATURE REVIEW

2.1 General concept of Heavy Metal

Heavy metal is defined as the metal group and metalloids with density greater than $4g/cm^3$ or 5 times or more than water (Duruibe et al., 2007). Some of these metals are biologically necessary even at low concentrations are toxic to living organisms, while at relatively high concentrations, some will become toxic. Heavy metals combine with the biomolecules of the body, such as proteins and enzymes, when this metal is absorbed in large amounts, they form stable biotoxic compounds that mutilate the structures and interfere with the bioreaction processes (Duruibe et al., 2007).

2.1.1 Effect of the heavy metal

Heavy metals are potentially life-threatening due to their toxicity and potential for accumulation, as heavy metals cannot be degraded, deposited, assimilated or incorporated into humans, aquatic animals and the environment (Linnik and Zubenko 2000). Even at the lowest concentration, each of the heavy metal element brings effect to their surroundings, especially to human health. For examples Cd, Cr and Pb are non-essential metal that are well known for their toxicity, while Cu, Na and Fe are essential metal which is toxic to human in higher concentration. There is other element that exists and being studied for their affect to human and environment (Turkmen et al., 2009).

2.1.1.1 Lead (Pb)

Lead has a density of 11.3g/cm^3 atomic number 82 and exists in various oxidation state, where most lead is bio-accumulated by aquatic organisms (Akan et al., 2009). Lead is particularly concerned by its toxicity and ability to bioaccumulate the aquatic ecosystems, as well as its persistence in the natural environment. Lead does not undertake any essential function on the human body and can cause adverse effects on human beings and the environment (Hudson et al., 2001). Lead can come from sources like food, water, soil, dust, utensil, and leaded gasoline (CDC, 2004).

2.1.1.2 Cadmium (Cd)

Cadmium can oxidize and form inorganic compounds mostly a water-soluble compound. Cadmium can be found in soils under agriculture from insecticides, fungicides, sludge and commercial fertilizers. Ingestion of cadmium can cause adverse effect to human health. As a reference, Itai-itai disease in Japan in year 1955. The diseases was identified among people living in cadmium-polluted areas in Fugawa (Dural et al., 2007) for the causes of consuming livestock which have been contaminated by the heavy metals cadmium as the wastewater are discharge from the nearby mining pollute the water and eventually pollute all the resources around it including bivalves, fish and rice. Eventually as they consume the livestock, metal pollution destroys their organs liver, placenta, kidneys, lungs, brain and bones (Reilly, 2002).

2.1.1.3 Copper (Cu)

Copper has an atomic weight of 63.55 with a specific gravity of 8.96. It is well known in the electrical industries for the wire making. In hemoglobin synthesis and in metabolic mechanism catalysis, copper is an important component of the metalloenzymes needed by living organisms. It is toxic to the aquatic organisms when present in excess in the environment (Akan et al., 2009). In fact, copper is not enlarged in the body or bio-accumulated in the food chain, unlike other artificial materials (Greany, 2005).

2.1.1.4 Arsenic (As)

Arsenic is a chemical element that occurs in many minerals and known to be a metalloid. It is also classified as carcinogen and pose threat to human life. Arsenic can be found in air, food and in water. Ground water resources are well known water resources for the human population. Ions in the ground pollute the groundwater with unhealthy level of arsenic where about 300 million of the human population uses groundwater in their daily lives (Winkel et al., 2008). With excessive intake or exposure, heavy metal arsenic may be a potent toxic and bring adverse effect to human and environment (Hudson et al., 2001).

2.1.1.5 Zinc (Zn)

In the prenatal and postnatal, zinc is very important essential mineral for their development (Hambidge et al., 2007). Zinc is very important to human and environments. As they are essential requirement for vitality, they can also cause harm to human health and highly toxic to plants and aquatic animal when excess (Muyssen et al., 2006). Zinc can accumulate in certain species of fish that can be found in the zinc polluted area. When the heavy metal zinc polluted the fish, the effect could biomagnifies the food cycle while the groundwater can contaminate water-soluble zinc that is in soils. Due to the zinc accumulation in the soil, plants may also be killed. Finally, zinc can disrupt the fertility of the soil and affect the activity of the soil ecosystem, thereby delaying organic matter breakdown. (Greany, 2005).

2.1.1.6 Nickel (Ni)

Nickel with density of 8.9 g/cm3 readily forms alloys and can be dissolved in dilute acids (Reilly, 2002). Skin allergies, cardiovascular system poisoning, and lung fibrosis are well known threat from the nickel pollution to human (Kasprzak et al., 2003). The mobility of nickel is known to be low in the atmosphere under neutral and alkaline conditions, nickel is bound to sediment or soil particles by adsorption in surface complex formation and absorption in ion exchange sites, or by precipitation or co-precipitation extracted from the liquid phase(Cempel and Nikel, 2006).

2.2 Heavy metal contamination

Heavy metal contamination in aquatic ecosystem have becomes a global concern. The heavy metal contamination may occur naturally or by the anthropogenic sources (human activities). These elements exist at low concentrations in the natural aquatic ecosystem. Contamination of this heavy metal element are non-degradable, deposited, incorporate with water and assimilated the e ecosystem which result in pollution of the river by the heavy metal element. (Abdel-Baki et al., 2011).

2.2.1 Metals in fish

Faced with the exponential growth of fisheries and aquaculture in order to satisfy the greater need for human consumption of seafood, the current quality of the environment, fish stocks and climate issues have also raised concerns about seafood products, supply chains, safety and sustainability (Ziegler et al., 2016). Fish species are one of the most effective environmental indicators for estimating the level of metal pollution due to their rich composition as a livestock for humans (Rashed, 2001).

The fish that widely consumed have been investigated for their threat to wellbeing (Begum et al., 2005). Since the metal element remain undestroyed, living organism will absorb the metal through the food chains from one trophic stage to another (Ali and Khan, 2018). Several studies reported the increase of heavy metal in some food chain over time. The higher the tropic level of the food chain, the higher the risk of the metal accumulation (Barwick and Maher, 2003). Figure 2.1 shows how heavy metal accumulation can affect the food chain by the tropic transfer of heavy metal from freshwater fish to human food chain. Apart from the tropic transfer, the accumulation rate of heavy metal also depends on the ecological characteristics, for example the feed intake habits, age and length of the fish, (Rejomon, 2010).

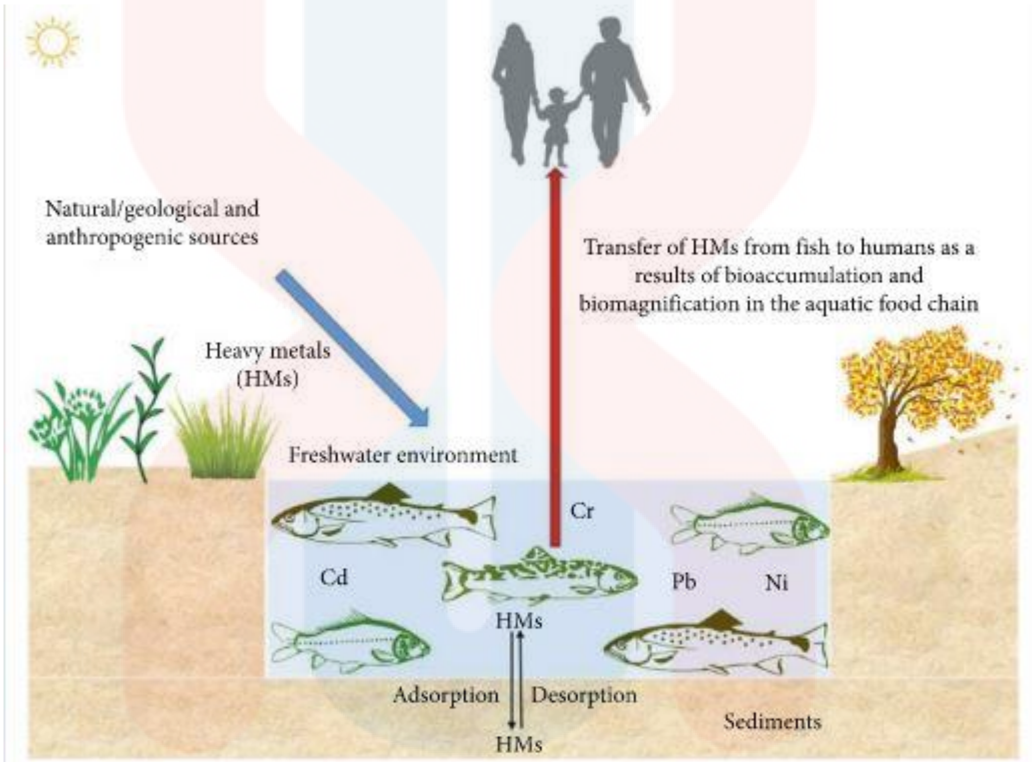

Figure 2.1: The tropic transfer of heavy metal from freshwater fish to human food chain. (Source: Barwick and Maher 2003)

2.2.2 Fish as bio indicator

The sources for the normal metabolism of the fish that have been taken up from the water, food and sediment for their essential metals. Various toxic heavy metals released from natural and anthropogenic sources into the river may threaten the freshwater fish. It has become an issue when the accumulation threatens the fish and eventually will also affect the human (Rahman et al., 2012). In the monitoring of hydrosphere, pollution are recommended to be as complex as possible which means that the levels of the pollution should be known not only in water and sediments but also in the tissues of animals which has been considered to be bio indicators as well even through it sometimes they can be extended to phytoplankton and zooplankton (Zima and Vavrova, 1997).

As the metal accumulation increases in the fish muscle, it can produce toxic effect that will indicate the excessively elevated accumulation of heavy metal in the resources (Tüzen, 2003). This approve that fish are considered as the most significant bio indicator to the aquatic ecosystem.

2.2.3 Candidate species

2.2.3.1 *Cyclocheilichthys apogon*

Cyclocheilichthys apogon is a type of fish species that migrates during high water season and inhabits a range of freshwater environments: rivers, lowland swamps, marshlands, lakes, and reservoirs (Froese et al., 2015). They are also known as Ikan Temperas (Figure 2.2). They are benthopelagic which diet consist of small vertebrates. Usually, they can be found around surfaces, such as trees, leaves, branches, and tree roots, where small planktons and crustaceans are found. This species can be found in the local fish market and well known to be consume by the locals in Asia (Vidthayanon, 2012).

Figure 2.2: *Cyclocheilichthys apogon* (source: Animalspot.net)

2.2.3.2 *Barbodes lateristriga*

Barbodes lateristriga are commonly known as Ikan Bagoh (Figure 2.3) in Malaysia. They are also freshwater fish that are mostly found in the river drainage in Southeast Asia especially in Malay Peninsula where it has been recorded as the most abundance (Vidthayanon, 2015). Froese (2015) suggested that worms, crustaceans, insects and plant matter are the diet of *Barbodes lateristriga*. Plain mountain streams strewn with rocks and boulders are generally inhibit by them and their population mostly located below waterfalls. It is also popular in the aquarium trade, and locally consumed in households (Denaro, 2015)

Figure 2.3: *Barbodes lateristriga* (source: Animalspot.net)

2.2.3.3 *Pristolepis groot*

Pristolepis groot are commonly known as Ikan Patong (Figure 2.4). They are very well known in the local market in Malaysia. The species are mainly located widely in the Mekong and Chao Phraya basins, the Malay Peninsula, Indonesia and Borneo. The species' natural habitat is shallow forest ponds, forest streams, wetlands, bogs, and waterways, including lakes and reservoirs (Kottelat, 2001). As an ornamental fishing industry, Ikan Patong has great potential owing to its sharp body and contours. (Anna Mercy et al., 2003). They also have a relatively cheap prices compared to other fishes mainly, they are sold in bundles depending of their length (Makmur, 2009).

Figure 2.4: *Pristolepis groot* (source: Animalspot.net)

2.2.3.4 *Rasbora elegans*

Rasbora elegans can normally be found in the rivers and streams in Malay Peninsula, Singapore and the Greater Sunda Islands of Borneo and Sumatra (Kottelat, 2001). They are well distributed in the forest streams of Pahang and Johor. This species is also known as Ikan Seluang (Figure 2.5) by the locals. They are also a benthopelagic fish that feed on small plankton, crustacean and small insect.

Figure 2.5: *Rasbora elegans* (source: Animalspot.net)

2.2.3.5 *Hemibagrus* sp.

Hemibagrus sp, is normally known as Ikan Baung by the locals where they are generally found in the lakes and rivers of Southeast Asia (Usmani et al., 2003) (Figure 2.6). In most inland water bodies, this catfish species is commonly established and forms a dominant species in Malaysian reservoir fisheries (Khan et al., 1995). This species is cultured in a large quantity in certain region for a fish stock in order to meet the river freshwater cage system opportunities (Molnar et al., 2006).

Figure 2.6: *Hemibagrus* sp. (source: Animalspot.net)

2.3 Human health risk assessment

Risk assessments are meant as qualitative or quantitative assessment of the risk to human health and the environment resulting from the current or prospective use of particular contaminants (USEPA, 2000). Even through the risk assessments can also conducted on non-pollutant agents, it can be also defined as the idea of risks to the environment to human health. Heavy metals that was consumed cannot be degradable or destroyed (Castro-Gonzalez et al., 2008). The accumulation of the heavy metal threatened the human life for its toxicity. As it is now could be a global problem because of the certain cases, heavy metal is considered as the most dangerous pollutant category in the biodiversity. For example, the cases of heavy metal that have already occurred in the early year of 1952 where Mina Mata diseases originated in the area of Mina Mata's Japanese fishing harbor. The epidemic is caused by the consumption of livestock that was contaminated by the heavy metals’ methyl mercury due to the wastewater discharged by the factories (Dural et al., 2007, Chen et al., 2001).

CHAPTER 3

MATERIALS AND METHODS

3.1 Research methodology flowchart

Figure 3.1 summarized the methodology of the study. Three locations were selected as the sampling sites for the collection of water and sediment samples. Certified Reference Materials (CRM) has been used to validate the acid digestion method by Plesis. The analysis was carried out using Atomic Absorption Spectroscopy (AAS) in the risk assessment whereas THQ and EDI were used for health risk assessment.

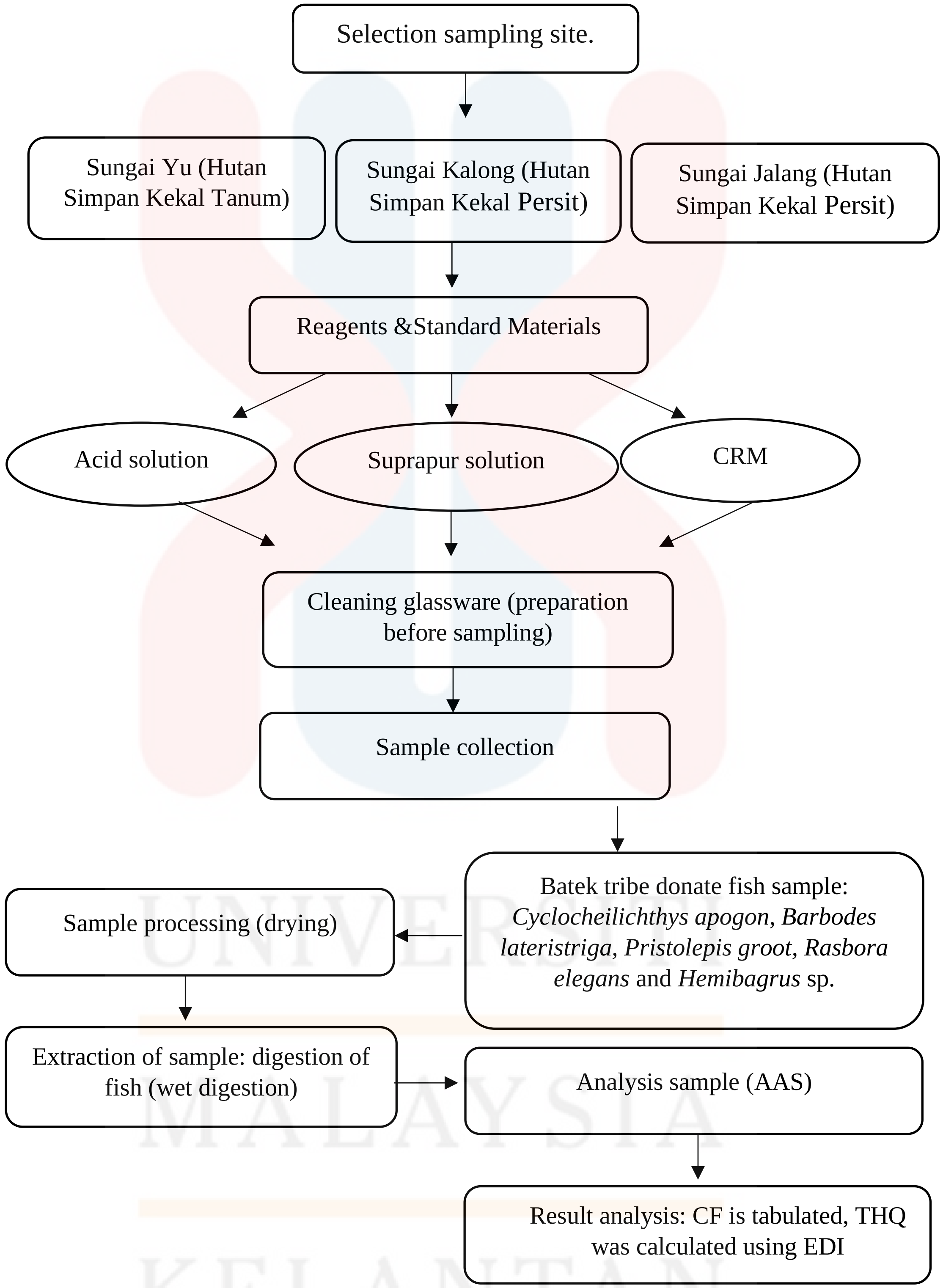

Figure 3.1 Research methodology flowchart

3.2 Sampling Site

The study area for this research was at Merapoh Pahang where Sungai Kalong located in Hutan Simpan Kekal Persit also Sungai Yu and Sungai Jalang located in Hutan Simpan Kekal Tanum as in (Table 3.1). Figure 3.2 shows the sampling area which are surrounded by reserved forest. The river in the forest are the main sources of food for the Batek tribe that live at the nearby area. Those rivers are the place where the fish sampled have been wild caught by the Batek tribe. Since the river have been reserved, they are rich in biological resources where the target species can be found.

Table 3.1: Study area

	Sungai Kalong, Hutan Simpan Kekal Persit	Sungai Yu, Hutan Simpan Kekal Tanum	Sungai Jalang, Hutan Simpan Kekal Tanum
Coordinates	4°37'09.6"N 101°58'46.4"E	4°32'14.9"N 101°59'27.9"E	4°42'14.9"N 101°59'44.9"E

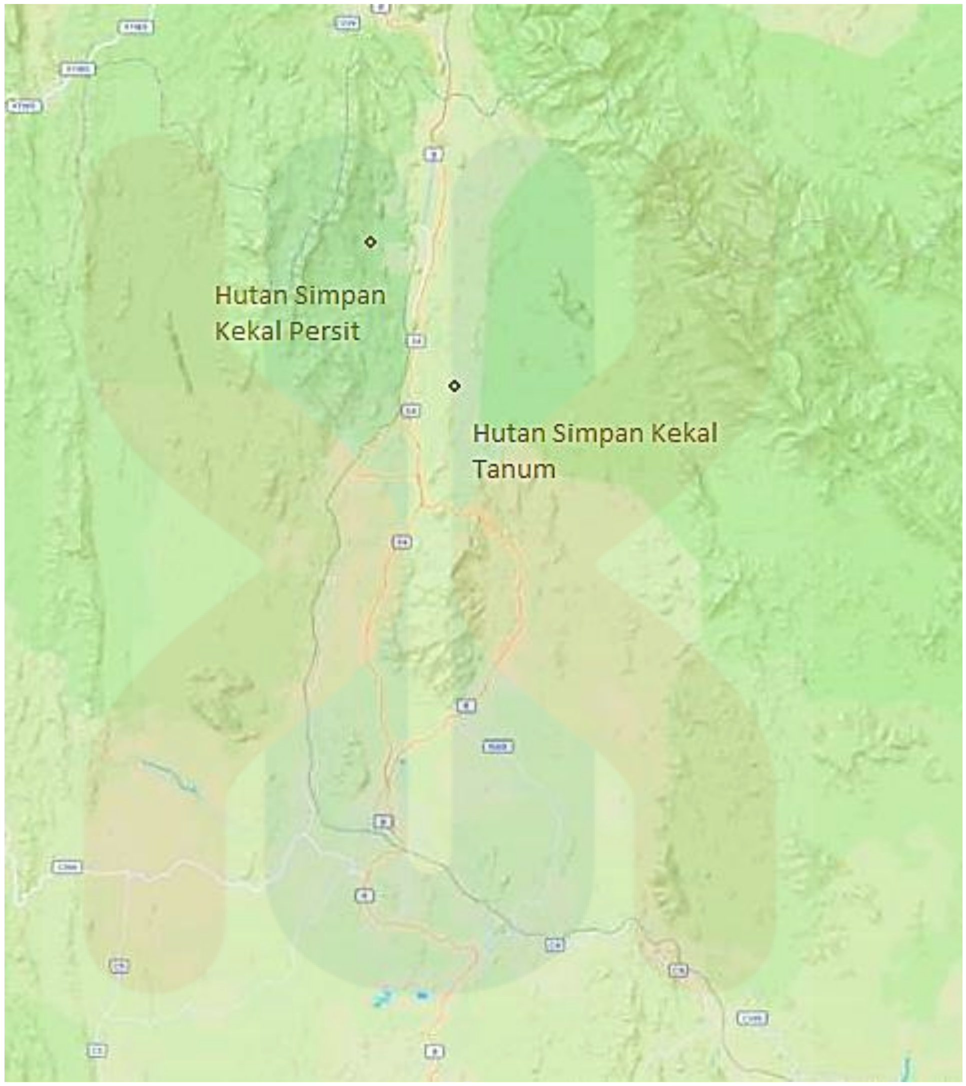

Figure 3.2: The map showing the sampling site in Hutan Simpan Kekal Tanum and Hutan Simpan Kekal Persit (Source: Google map 2020).

3.3 Reagents and standard materials

Nitric acid (HNO_3) of Suprapur® quality were used. Certified reference materials (CRM) for trace metals are used to demonstrate the validity of methods. They were fish muscle (ERM BB422).

3.4 Cleaning glassware

To prevent cross-contamination and decrease any potential of decontamination, all apparatus was washed and soaked overnight in dilute 10% HNO3.Then they were rinsed thoroughly using distilled water and dried before used.

3.5 Sample Collection and preparation

3.5.1 Sampling of fish

The fish were donated by local people (Batek tribe), which were wild caught from Sungai Jalang, Sungai Kalong Hutan simpan kekal Persit and Sungai Yu in Hutan simpan kekal Tanum, Merapoh, Pahang. The *Cyclocheilichthys apogon*, *Barbodes lateristriga*, *Pristolepis grooti*, *Rasbora elegans* and *Hemibagrus* sp., were donated by Batek Tribe people which were caught from the mentioned study area (Table 3.2). The fish specimens were wrapped individually in polyethylene bag, kept in icebox then were transported to the laboratory in University Malaysia Kelantan on the same day. The total fish length and weight were recorded before storing under -20 ºC until dissection was performed. The length is measured as the distance from the tip of snout to the tip of the caudal fin as shown in (Figure3.3).

Table 3.2: Fish Species

LOCAL NAME	BATEQ NAME	SCIENTIFIC NAME
Ikan Temperas	Biakang	*Cyclocheilichthys apogon*
Ikan Bagoh	Bill	*Barbodes lateristriga*
Ikan Patong	Kon	*Pristolepis grooti*
Ikan Seluang	Cajeng	*Rasbora elegans*
Ikan Baung	Baung	*Hemibagrus* sp.

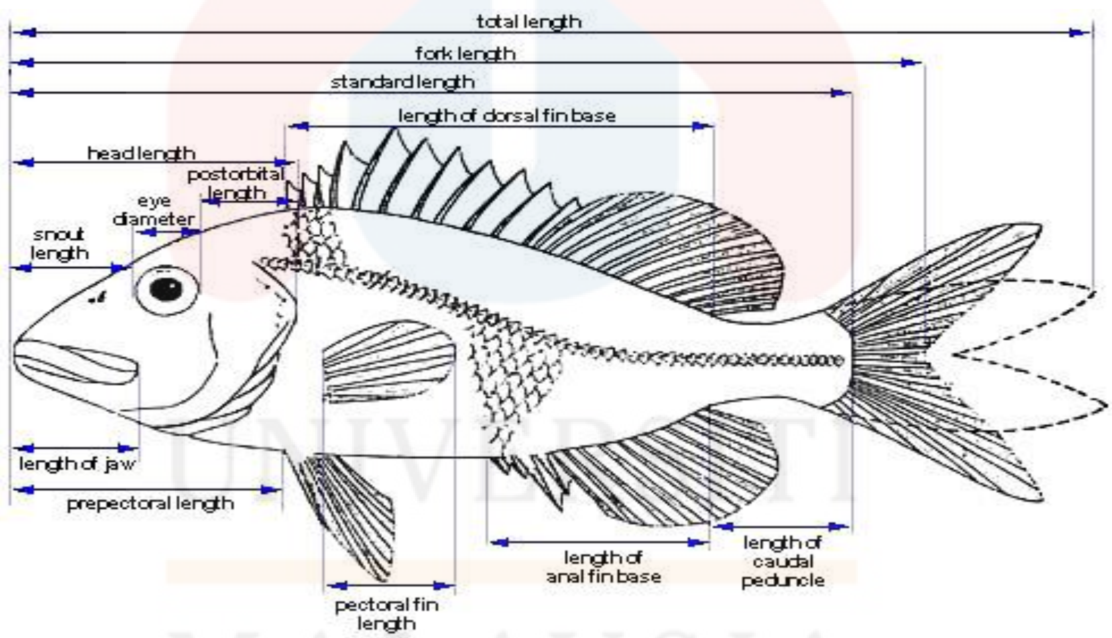

Figure 3.3: Fish measurement (Source: Hindawi)

3.6 Sample processing

After the fish samples was partially thawed, dissecting kits was used to remove tissues of muscle and was cleaned multiple times using distilled water. In order to improve sample homogeneity, each dissected sample was grinded using mortar and pestle. Then, these tissue samples were dried in the oven at 110°C to achieve constant weight. The samples were then further homogenized using mortar and pestle. Last step of the sample processing was acid digestion. Before undergoing acid digestion, dried samples were kept in amber jars under the desiccator.

3.7 Digestion of fish

The acid digestion method was used based on the analytical method for flame atomic absorption spectrophotometer (AAS). Fish sample (1.0 g) was placed in a 50 ml beaker. Afterwards, about 10 ml of concentrated HNO_3 (analytical grade, 69 %; w/w) was poured into the beaker. A watch glass was placed at the mouth of the beaker and the beaker was placed on a magnetic stirrer/hot plate. Initially, the temperature was kept at about 40 °C for one hour to prevent vigorous reactions. Then the temperature was maintained at 140 °C for another 3 hours. Once the digestion was completed all tissue samples were completely dissolved in the acid. Then the mixture was cooled to room temperature. Double distilled water was added into the 250 ml Volumetric flask to dilute the mixture for AAS detection of heavy metals. The sample was filtered by filter paper (Whatman No.1 grade) and syringe into the 15 ml centrifuge tube using 40 μm filter. The filtrates were stored at 4 °C until the metal determination by AAS (Plessis, 2012).

3.8 Risk Characterization

3.8.1 Morphometric data of fish

The condition factor (CFs) was used to quantify the condition of fish which include the degree of maturity and nourishment (Williams, 2000). CFs can be estimated by the following equation:

$$CF= [\frac{W}{L^3}] \text{ x } l00 \qquad (3.1)$$

where W is the weight of fish body in grams, L is the length of fish in centimeters. Condition factor (CF) is used to quantify the condition of fish which include the degree of maturity and nourishment (Williams, 2000).

3.8.2 Estimated Daily Intake (EDI)

The daily intake of metals depends on both the metal concentration in fish muscle tissues and the daily fish consumption. In addition, the body weight of the human can influence the tolerance of contaminants. The EDI are a concept introduced to take into account these factors. The EDI is calculated as follows:

$$\text{EDI: } \frac{(C \ x \ FIR \ x \ ED \ x \ EF)}{WAB \ x \ TA} \text{ x } 10^{-3} \qquad (3.2)$$

where, C is the average content of metal in fish (mg kg-1 wet weight), FIR is the fish ingestion rate, 71 g day -1 person-1, ED is the exposure duration; 70 years, average lifetime, EF is the exposure frequency; 365 day year-1 (Agusa, 2007; FAO, 2005, 2008, 2009), WAB is the average body weight; 64 kg, the reference weight were derived from numerous local Malaysia studies (Lim, 2000), TA is the average time of exposure; 365 day year-1 multiplied by ED (Wei et al., 2014)

3.8.3 Target Hazard Quotient (THQ)

The target hazard quotient (THQ) is an estimate of the risk level (non-carcinogenic) due to pollutant exposure. To estimate the human health risk from consuming metal contaminated fish, the target hazard quotient (THQ) was calculated as per USEPA Region III Risk-Based Concentration Table (USEPA 2011). The equation used for estimating THQ was as follows:

$$\text{THQ: } \frac{(\text{EF x ED x FIR x C})}{(\text{RfD x WAB x TA})} \text{ x}10^{-3} \qquad (3.3)$$

where, EF is the exposure frequency; 365 days year-1, ED is the exposure duration; 70 years (Agusa, 2007; FAO, 2005, 2008, 2009), FIR is the fish ingestion rate; fish: 71 g day-1 person-1 (FAO, 2008), C is the metal concentration in muscle of studied fish; edible fish part (mg kg-1; wet weight), RfD is the oral reference dose (USEPA, 1997, 2000) as stated in (table 3.3), WAB is the average body weight; 64 kg, the reference weight were derived from numerous local Malaysia studies (Lim, 2000), TA is the average time of exposure; 365 day year-1 multiplied by ED (Wei et al., 2014). As a conclusion, there is no potential risk found related to studied metal if their hazard quotient less than one (THQ <1).

Table 3.3: Oral reference dose of heavy metals

Heavy metal	Cadmium (Cd)	Lead (Pb)	Copper (Cu)	Arsenic (As)	Zinc (Zn)	Nickel (Ni)
RfD (mg/kg-day)	1×10^{-3}	4×10^{-3}	4×10^{-2}	3×10^{-4}	3×10^{-1}	1×10^{-2}

(source: USEPA, 2000)

CHAPTER 4

RESULT AND DISCUSSIONS

4.1 Acid digestion method verification

The analytical performance of the acid digestion/extraction of fish, were checked with the certified reference material (CRM), namely, Certified Reference Material ERM®- BB422 from European Commission, Joint Research Centre Institute for Reference Materials and Measurements (IRMM) Geel, Belgium.

Tables 4.1 shows, there are good agreements between the AAS measurements and the certified values. In all cases, the recoveries were found to be in the range of 60 % - 116 %, within the traditionally accepted results for the certified values; which means that the results of the present study can be considered to be accurate with respect to the true concentrations in the samples (Mziray & Kimirei, 2016).

Table 4.1: Analysis of Certified Reference Material ERM®- BB422

	ERM®- BB422		
	Certified / mg/kg	Found / mg/kg	Recovery / %
Ni	-	-	-
Cu	1.67	1.0	60
As	12.7	12.82	100
Zn	16.0	18.7	116
Pb	-	-	-
Cd	0.0075	-	-

Note: Pb and Ni not certified

4.2 The Morphometry of Fish

Weight, length and lifespan could be related to the concentrations of accumulated contaminations in a fish. (Agusa et al., 2005). For freshwater fish, however, the difference in size seldom shows a major association with the metal content, as internal metal concentrations in freshwater fish are often biologically controlled. (Yi & Zhang, 2012). To overcome the potential variability associated with size variation in this study, an effort was taken to sample fish with comparable size and their morphometric data are summarized in Table 4.2 and Table 4.3 displays the possible classification to Condition Factor values as suggested by Barnham and Baxter (2003); the higher the CF value, the better is the fish condition.

Table 4.2: Biometric information related to *Cyclocheilichthys apogon*, *Barbodes lateristriga*, *Pristolepis grooti*, *Rasbora elegans* and *Hemibagrus* sp. (Mean value ± SD)

	Sungai Kalong				Sungai Jalang				Sungai Yu
	Barbodes lateristriga	*Cyclocheilichthys apogon*	*Rasbora elegans*	*Pristolepis grooti*	*Barbodes lateristriga*	*Cyclocheilichthys apogon*	*Rasbora elegans*	*Pristolepis grooti*	*Hemibagrus* sp.
Length (cm)	26±0.41	37±0.3	11±0.3	14±0.34	29±0.24	17±0.8	5±0.8	10±0.1	16±0.33
Weight (g)	11±0.1	14±0.3	10±0.8	10±0.1	12±0.7	10±0.5	7±0.2	7±0.9	51±0.1
Condition Factor (g/mm3)	1.0±0.8	1.0±0.3	0.9±0.05	1.0±0.5	1±0.5	1±0.3	1±0.5	2±0.5	1.0±0.2

Table 4.3: Condition factor (Barnham & Baxter, 2003)

CF	Comments
1.60	Excellent condition, trophy class fish
1.40	A good, well-proportioned fish.
1.20	A fair fish, acceptable to many anglers.
1.00	A poor fish, long and thin.
0.80	Extremely poor fish, resembling a barracouta; big head and narrow, thin body.

4.3 Metal distribution in fish tissues.

Table 4.3 indicate the level of metals in muscle tissue of *Cyclocheilichthys apogon, Barbodes lateristriga, Pristolepis grooti, Rasbora elegans and Hemibagrus* sp in mg/kg on dry weight basis. Based on the Table 4.3, all the reading for Pb concentration was reported as undetected by the AAS. The heavy metal concentration of the metal element As also appears to be undetectable by most of the sample except for *Cyclocheilichthys apogon* with the value of mean concentration of 8.4 mg/kg in Sungai Kalong sample and 50.9 mg/kg in Sungai Jalang Sample. In compliance with the 1985 Food Legislation, the acceptable amount of arsenic in fish and their product is 1.0 mg/kg. In the two fish samples submitted for the test, the mean reading for arsenic was much higher than the acceptable amount. This happen as the stream and river are polluted and the significant amount of As found in the fish muscles tissues could be linked with the nearby logging and gold mining site (Faiz, 2017). The accumulation of metals in fish tissues has been documented to generally rely on the physiological function of fish, although fish species can originate from the same location (Dhaneesh et al., 2012).

Table 4.4: The metal distribution in fish tissues of *Cyclocheilichthys apogon, Barbodes lateristriga, Pristolepis groot, Rasbora elegans and Hemibagrus* sp. (Mean value ± SD)

	Sungai Kalong				Sungai Jalang				Sungai Yu
	Barbodes lateristriga	*Cyclocheilichthys apogon*	*Rasbora elegans*	*Pristolepis grooti*	*Barbodes lateristriga*	*Cyclocheilichthys apogon*	*Rasbora elegans*	*Pristolepis grooti*	*Hemibagrus* sp.
Lead (Pb)	-	-	-	-	-	-	-	-	-
Copper (Cu)	0.7±0.05	2±0.08	0.5±0	1±0.25	-	9±0.3	1±0.08	0.6±0.07	1±0
Nickel (Ni)	7±0.25	2±0.08	1±0.3	11±0.7	-	11±0.9	7±0.17	4±0.08	1±0.63
Zinc (Zn)	63±0.7	140±0	66±0.8	77±0.4	56±0.7	145±0.5	92±0.8	69±0.42	69±0.25
Cadmium (Cd)	0.8±0.03	1±0.25	0.6±0.07	1±0.08	0.6±0.07	1±0.25	0.4±0.02	1±0	0.4±0.02
Arsenic (As)	-	8±0.4	-	-	-	50±0.9	-	-	-

Note: All reported values are referred to dry base (mg/kg) n = 30

(-) undetected

The results demonstrated the concentration of hazardous metals As in *Cyclocheilichthys apogon* tissue which reflect the influence of anthropogenic activities. This suggest the potential of *Cyclocheilichthys apogon* is an important valuable bioindicator of pollution in the monitoring and the assessment of the environmental quality.

In this work, it is found that Zn was reported the highest concentrations in all studied fish species due to this element in one of the essential metals for human needs and the environment (Hambridge et al., 2007). According to Greany (2005), even though this element is important, running in excess may cause fatality to human, plant and animal as it can bioaccumulates in the waterways and sediment. It is noticed that Cd was demonstrated in low concentration for all studied fish species. Cadmium can be found naturally in the environment or since some findings have shown that cadmium contamination in the water ecosystems is triggered by industrial processes and agricultural practices. (Cambier *et al.*, 2010). Since the fish was captured in reserved forest, it may be the least polluted by the human and Cd detected only accumulates naturally from the environment (WHO 1992).

The element Copper and Nickel are relatively in optimum concentration where the values are low in the fish muscle tissues. The *Barbodes lateristriga* fish muscles tissues in Sungai Jalang appear to be undetected for the concentration of the Cu and Ni element. This result is maybe due to the lower concentration of Cu and Ni element in Sungai Jalang and the lower potential of the *Barbodes lateristriga* fish muscles tissues to accumulates that metal element.

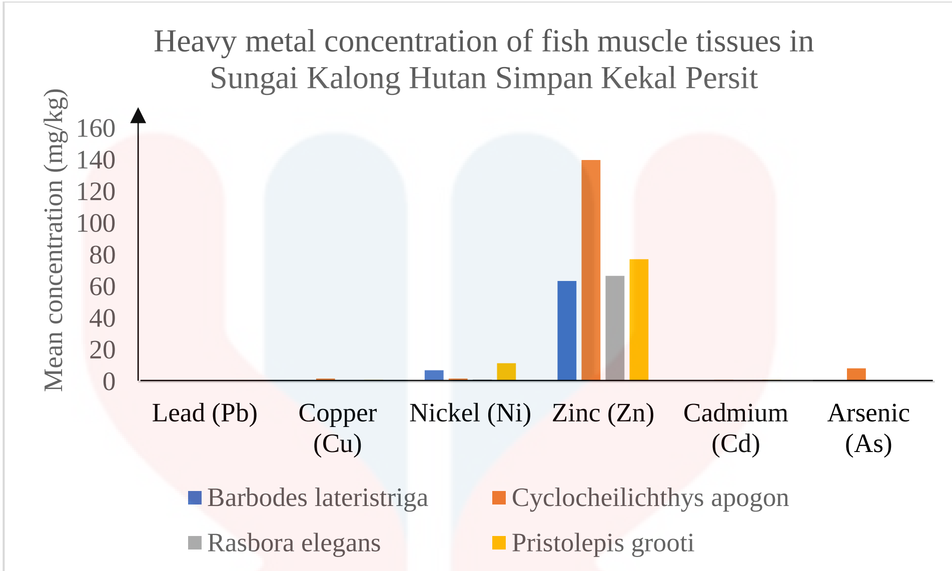

Figure 4.1: Mean concentration of heavy metal in fish muscle tissues in Sungai Kalong

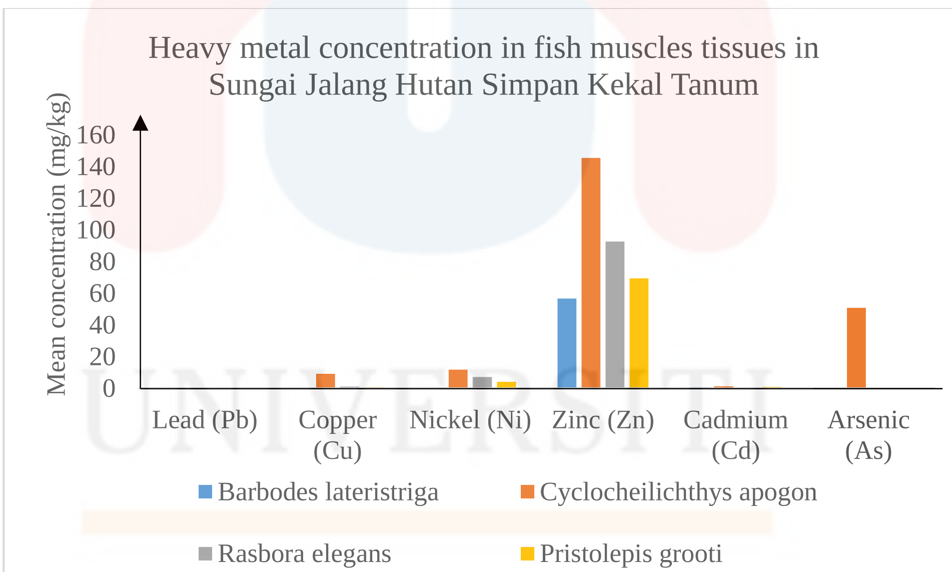

Figure 4.2: Mean concentration of heavy metal in fish muscle tissues in Sungai Jalang

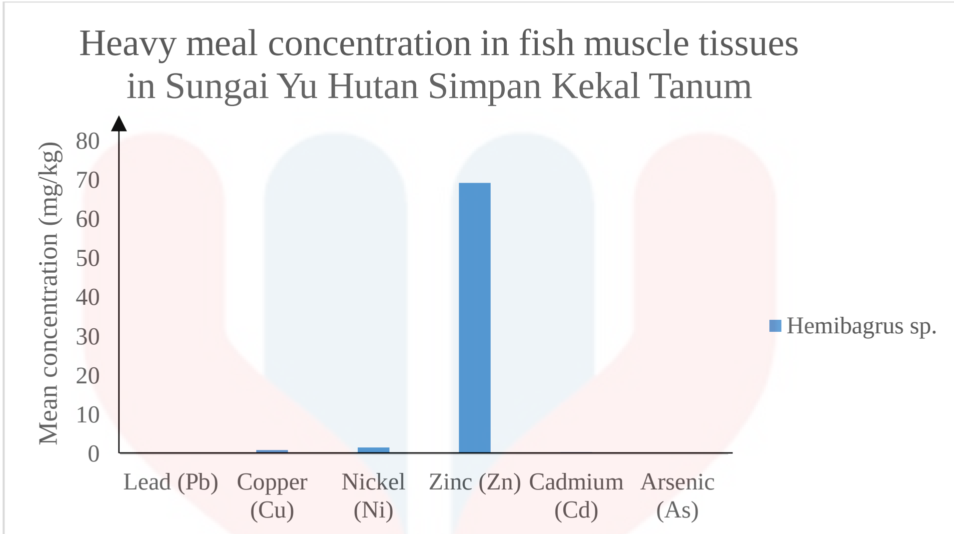

Figure 4.3: Mean concentration of heavy metal in fish muscle tissues in Sungai Yu

4.4 Chronic Human Health Risk Assessment

Hemibagrus sp., *Cyclocheilichthys apogon, Barbodes lateristriga, Pristolepis groot*,and *Rasbora elegans* well known in Southeast Asia and becoming one of the popular freshwater fish that being consumed by the community. Several studies about this species have been publish as a concern for the heavy metal accumulation and metal exposure to human consumption. The metal concentrations in the edible muscle tissues are structured on the basis of wet weight for comparative analysis in Table 4.4 together with established limits. Tables 4.5, 4.6, 4.7 and 4.8 shows the comparison of the metal concentration in muscle tissues of different species of fish in different river and together compared with Malaysian Food Act,1983; Food and Agricultural Organization, 2008; USEPA, 2000.

In fish muscles tissues of *Cyclocheilichthys apogon* from Sungai Kalong and Sungai Jalang from the Figures 4.1 and 4.2 there was the unexpected result where there is a higher significant level of heavy metal As. The level of the As heavy metal was more than the legal limit compared with Malaysian Food Act,1983; Food and Agricultural Organization, 2008; USEPA, 2000. Other heavy metal such as Cd, Cu, Zn, Ni were lower than the compared legal limit.

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Table 4.5: Metal concentration in muscle tissues of *Hemibagrus* sp. (Mean value ± SD)

	This study	Previous study			
Metal conc. (mg/kg)	Hutan simpan kekal Tanum, Merapoh, Pahang	Selangor River (Idris et al., 2017)	Legal limits		
	Sungai Yu	River	MFA	FAO	USEPA
Cu	1±0	0.8	30	30	54
Zn	69±0.25	23	100	40	410
As	-	2.3	1	-	0.41
Ni	1±0.63	0.11	-	-	6.8
Cd	0.4±0.02	-	1	-	1.4
Pb	-	<0.03	1	0.5	-

Note: Malaysian Food Act,1983; Food and Agricultural Organization, 2008; USEPA, 2000

(-) undetected and not reported

Table 4.6: Metal concentration in muscle tissues of *Cyclocheilichthys apogon* (Mean value ± SD)

Metal conc. (mg/kg)	This study	This study	Legal limits		
	Hutan simpan kekal Persit, Merapoh, Pahang	Hutan simpan kekal Tanum, Merapoh, Pahang			
	Sungai Kalong	Sungai Jalang	MFA	FAO	USEPA
Cu	-	-	30	30	54
Zn	2±0.08	9±0.3	100	40	410
As	2±0.08	11±0.9	1	-	0.41
Ni	140±0	145±0.5	-	-	6.8
Cd	1±0.25	1±0.25	1	-	1.4
Pb	8±0.4	50±0.9	1	0.5	-

Note: Malaysian Food Act,1983; Food and Agricultural Organization, 2008; USEPA, 2000

(-) undetected and not reported

Table 4.7: Metal concentration in muscle tissues of *Barbodes lateristriga* (Mean value ± SD)

Metal conc. (mg/kg)	This study Hutan simpan kekal Persit, Merapoh, Pahang Sungai Kalong	This study Hutan simpan kekal Tanum, Merapoh, Pahang Sungai Jalang	Legal limits MFA	 FAO	 USEPA
Cu	-	-	30	30	54
Zn	0.7±0.05	-	100	40	410
As	7±0.25	-	1	-	0.41
Ni	63±0.7	56±0.7	-	-	6.8
Cd	0.8±0.03	0.6±0.07	1	-	1.4
Pb	-	-	1	0.5	-

Note: Malaysian Food Act,1983; Food and Agricultural Organization, 2008; USEPA, 2000

(-) undetected and not reported

Table 4.8: Metal concentration in muscle tissues of *Pristolepis groot* (Mean value ± SD)

Metal conc. (mg/kg)	This study Hutan simpan kekal Persit, Merapoh, Pahang	This study Hutan simpan kekal Tanum, Merapoh, Pahang	Legal limits		
	Sungai Kalong	Sungai Jalang	MFA	FAO	USEPA
Cu	-	-	30	30	54
Zn	1±0.25	0.6±0.07	100	40	410
As	11±0.7	4±0.08	1	-	0.41
Ni	77±0.4	69±0.42	-	-	6.8
Cd	1±0.08	1±0	1	-	1.4
Pb	-	-	1	0.5	-

Note: Malaysian Food Act,1983; Food and Agricultural Organization, 2008; USEPA, 2000

(-) undetected and not reported

Table 4.9: Metal concentration in muscle tissues of *Rasbora elegans* (Mean value ± SD)

Metal conc. (mg/kg)	This study Hutan simpan kekal Persit, Merapoh, Pahang	This study Hutan simpan kekal Tanum, Merapoh, Pahang	Legal limits		
	Sungai Kalong	Sungai Jalang	MFA	FAO	USEPA
Cu	-	-	30	30	54
Zn	0.5±0	1±0.08	100	40	410
As	1±0.3	7±0.17	1	-	0.41
Ni	66±0.8	92±0.8	-	-	6.8
Cd	0.6±0.07	0.4±0.02	1	-	1.4
Pb	-	-	1	0.5	-

Note: Malaysian Food Act,1983; Food and Agricultural Organization, 2008; USEPA, 2000

(-) undetected and not reported

4.5 Estimation of potential health risk

To estimate the potential health risk of consumption of contaminated fish species to Malaysians, the analysis of heavy metal was studied and the formulation of Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) analysis was used.

4.5.1 Estimated Daily Intake (EDI)

Daily metal intake was estimated based on the average metals concentrations in fish muscle and the daily fish consumption rates. Current metal intake was compared with the respective provisional tolerable weekly intake (PWTIs). An international regulation does not set concentration threshold in consumption of fish species for studied metals, but the World Health Organization suggest the use of PWTI as shown in Table 4.9

The EDI was shown in Table 4.9 with average body weight; 64 kg, the reference weight was derived from numerous local Malaysia studies (Lim, 2000), The estimated daily intakes show that the fish is generally less susceptible to environmental pollution, as its safety has been presumed. Hence, an estimation of the daily intakes of metals which reflect the metal exposures through fish consumption must be carried out.

Table 4.10: The estimated daily intakes (EDI) of metals (mg/kg bw /day) through consumption of freshwater fish species by adult people (assuming 64 kg person)

Estimated Daily Intake (EDI)

	Sungai Kalong				**Sungai Jalang**				**Sungai Yu**	**PWTI**	**RfD**
	Barbodes lateristriga	*Cyclocheilichthys apogon*	*Rasbora elegans*	*Pristolepis grooti*	*Barbodes lateristriga*	*Cyclocheilichthys apogon*	*Rasbora elegans*	*Pristolepis grooti*	*Hemibagrus* sp.		
Pb	-	-	-	-	-	-	-	-	-	0.025	4×10^{-3}
Cu	8.3×10^{-4}	2.3×10^{-3}	5.5×10^{-4}	1.3×10^{-3}	-	4.7×10^{-3}	1.2×10^{-3}	7.4×10^{-4}	1.1×10^{-3}	-	4×10^{-2}
Ni	8.0×10^{-3}	2.3×10^{-3}	1.5×10^{-3}	1.3×10^{-2}	-	1.3×10^{-2}	2.4×10^{-3}	4.5×10^{-3}	1.8×10^{-3}	-	1×10^{-2}
Zn	7.0×10^{-2}	1.6×10^{-1}	7.4×10^{-2}	8.6×10^{-2}	6.3×10^{-3}	1.6×10^{-1}	1.02×10^{-2}	7.7×10^{-2}	7.7×10^{-3}	-	3×10^{-1}
Cd	9.3×10^{-3}	1.4×10^{-2}	7.4×10^{-3}	1.2×10^{-2}	7.4×10^{-3}	1.4×10^{-2}	4.6×10^{-3}	1.1×10^{-2}	4.6×10^{-3}	0.007	1×10^{-3}
As	-	9.2×10^{-3}	-	-	-	5.6×10^{-2}	-	-	-	0.015	3×10^{-4}

Note: (-) Undetected

4.5.2 Target Hazard Quotient (THQ)

The risk of non-carcinogenic health risks associated with fish consumption are assessed based on target hazard quotients (THQs). If the assuming THQ result are less than one THQ <1, there or no threats to human life as there are no adverse effect from the heavy metal exposure compared to the THQ >1, the higher the THQ values mean a higher chances of experiencing long term non-carcinogenic effects (Wei et al., 2014).The THQ values in this work are given in Table 4.6.

Based on the table 4.11, the THQ for Zn, Ni, and Cu was relatively lower than 1. Therefore, the fish muscle tissues does not accumulate these metals resulting the river to be free from heavy metal pollution of these metal. Thus, there will be less risk to the consumer especially to the Batek tribe. As for the Pb, the THQ cannot be calculated due to the concentration that was not detectable by the AAS analysis before.

Based on the result, the heavy metal As and Cd metal concentration level found to be abundance resulting the THQ to be more than 1. Thus, they will be health risk to the consumer. Heavy metal As and Cd bring adverse effect to human health. As the human expose to this heavy metal, over the time this heavy metal especially Arsenic can cause cancer and other diseases. Apart from higher health risk, this also shown that that river was polluted by heavy metal As and Cd

Table 4.11: The Target Hazard Quotient (THQ) in consuming of *Cyclocheilichthys apogon, Barbodes lateristriga, Pristolepis groot, Rasbora elegans and Hemibagrus* sp.

	Target Hazard Quotient (THQ)								
	Sungai Kalong				**Sungai Jalang**				**Sungai Yu**
	Barbodes lateristriga	*Cyclocheilichthys apogon*	*Rasbora elegans*	*Pristolepis grooti*	*Barbodes lateristriga*	*Cyclocheilichthys apogon*	*Rasbora elegans*	*Pristolepis grooti*	*Hemibagrus* sp.
Pb	-	-	-	-	-	-	-	-	-
Cu	0.02	0.06	0.01	0.03	-	0.005	0.03	0.02	0.03
Ni	0.4	0.12	0.07	0.7	-	0.7	0.04	0.2	0.002
Zn	0.24	0.52	0.25	0.3	0.21	0.54	0.34	0.26	0.26
Cd	0.92	1.4	0.74	1.2	0.74	1.4	0.46	1.1	0.46
As	-	>1	-	-	-	>1	-	-	-

Note: (-) Undetected

CHAPTER 5

CONCLUSION AND RECOMENDATION

5.1 Conclusion

Based on the result obtained, it can be concluded that the heavy metals accumulation on the fish muscles tissues of fish *Cyclocheilichthys apogon*, *Barbodes lateristriga*, *Pristolepis groot*, *Rasbora elegans* and *Hemibagrus* sp. were result from the both natural and anthropogenic sources in the water or sediment from the river. Besides, the tropic level of fish also contributed to the level of heavy metals in the fish muscles tissues. The level of the heavy metal was determined by using acid digestion method and AAS.

The analysis of the level of heavy metals were then compared with the permissible limit to evaluate the possible human health risk using Target Hazard Quotients. By calculating the THQ, the fish in the river were mostly saved because the

THQ obtained are less than 1, but some of the metal element shown a value of more than 1 which is As and Cd. So, by the result, it can be concluded that over the time there will be a carcinogenic effect to the human health as Arsenic can cause cancer. As the increase in awareness and technological advance, the study of heavy metal accumulation in wild caught fish muscle tissues is very important as fish is consumed by the local especially the Batek tribe in Merapoh Pahang. In addition, different area of study could give a varies of result of heavy metal accumulation to determine the polluted area for the treatment of the water and the sediment.

5.1 Recommendations

The needed of the study of the heavy metal in different fish species is important so that heavy metal level in fish can be determine for an analysis of the potential health risk could be developed to ensure there is zero risk in consuming fish stock.

Besides, future study could include the tropic level that can be related to the accumulation of heavy metals. The awareness of potential health risk assessment through the consumption of wild caught fish to the Batek tribe is also important to ensure the safety of their tribe from heavy metals pollution

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