ASSESSMENT OF HEAVY METAL VARIATION IN CATFISH FROM TWO DIFFERENT HABITATS

by

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DECLARATION

I declare that this thesis entitled "Assessment of Metal Variation In Catfish From Two Different Habitat" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : _____

Name : _____

Date : _____

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ABSTRACT

Catfishes is one of the protein source that commercially known by many people in Malaysia. It also has highly demand from community day by day. However, the concern on heavy metal pollution in fish has become a great issue nowadays since it will give a bad effect to human health. Therefore, in this study, heavy metal concentration from two different breeding habitats of catfish which is soil pond and tank were evaluated by using target hazard quotient (THQ). The aim of the research is to determine the target hazard quotient of catfish from two different cultivated habitats. The fish samples undergo acid digestion process before analysed by using Atomic absorption spectrophotometer (AAS) to determine the heavy metals concentration. The element that tested are cadmium(Cd), iron(Fe), nickel(Ni), zink(Zn), lead(Pb) and mercury(Hg) in catfish's gills, liver, muscle, water and soil of the pond. Heavy metals concentrations in catfish from both habitats are under permissible limit stated by FAO except for the concentration of Fe in soil pond. However, THQ values for both habitats are <1, which is can be concluded that the fish from these two areas are safe to be consume by human.

Keywords : Heavy metals, catfish, THQ, AAS.

ABSTRAK

Ikan keli merupakan salah satu sumber protein yang dikenali secara komersial oleh ramai orang di Malaysia. Ia juga mempunyai permintaan yang tinggi dari masyarakat pada hari ke hari. Walau bagaimanapun, keprihatinan terhadap pencemaran logam berat dalam ikan telah menjadi isu besar pada masa kini kerana ia akan memberi kesan buruk kepada kesihatan manusia. Oleh itu, dalam kajian ini, kepekatan logam berat dari dua habitat pembiakan ikan keli yang merupakan kolam tanah dan tangki dinilai menggunakan bahaya bahaya sasaran (THQ). Tujuan kajian ini adalah menentukan bahaya bahaya sasaran ikan keli dari dua habitat yang berbeza. Sampel ikan dari kedua dua habitat menjalani proses pencernaan asid sebelum dianalisis dengan menggunakan spektrofotometer penyerapan Atom (AAS) untuk menentukan kepekatan logam berat. Unsur yang diuji ialah kadmium (Cd), besi (Fe), nickel (Ni), zink (Zn), lead (Pb) dan merkuri (Hg) dalam insang 'ikan. Kepekatan logam berat dalam ikan keli dari kedua-dua habitat adalah di bawah had yang dibenarkan oleh FAO kecuali nilai bagi Fe di dalam kolam tanah. Namun begitu nilai Thq bagi kedua dua habitat adalah <1, yang dapat disimpulkan bahawa ikan dari kedua-dua kawasan ini selamat dimakan oleh manusia.

Kata kunci : Logam berat, ikan keli, THQ, AAS.

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrophotometer
Cd	Cadmium
EPA	Environmental protection agency
Fe	Iron
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
Hg	Mercury
HI	Hazard index
HNO_3	Nitric cid
Ni	Nickel
Pb	Lead
THQ	Target hazard quotient
Zn	Zinc
HI	Hazard index

CHAPTER 1

INTRODUCTION

1.1 Background of study

Fish are one of the famous protein sources to human. The fish contain low saturated fat and adequate omega of fatty acid which important in helping human to gain good health (Hashim, Song, Muslim & Yen, T. P, 2014). Besides protein, fish also important sources in supplying vitamins, minerals and others benefits to the human. Malaysian also takes either marine or freshwater fish daily as one of their protein source. Freshwater fish also has a high market among the Malaysia. Info Fish International (2010), stated that the demand for freshwater fish has increased 37% from 2003 to 2007 which is from the statics proved that demand for freshwater fish getting higher day by day and this situation might because of the inconsistent supply and price compared to marine fish (Saalah., Shapawi, Othman & Bono., 2009).

Freshwater fish has high protein content which is 15–20% (Babji, Daud & Yusop., 2013). It is also proved that fish are better protein supplier than meat as it contains high protein and low-fat content as mention by Babji *et al* (2013). Certain freshwater fish was reported containing Docosahexaenoic acid (DHA) which is use as one of the precursor in prostaglandin and used to reduce the ache and inflammation (Chedoloh, Karrila, Taewee & Pakdeechanuan. P., 2011). The famous

freshwater fish with high demand from the community are catfish, tilapia and patin as mentioned by Malaysian Fisheries Institute (2013). The aquaculture production in 2013 are calculated to 260,773 tonnes, with major species being catfish, 28.4%, tilapia is 31.5% and patin is 11.2 %. From the percentage mentioned before, the value shows that the catfish are one of the fish that highly demand and has high market in all over the Malaysia. One of example we can see, the catfish will be one of the main dish that served in any cafeteria or restaurant everyday and the consumers will choose the catfish to consume. So, to fulfilled the demand from the customers, agriculture entrepreneurs started to breed commercially the fish as the demand from consumers are high and the business are profitable to run. Catfish were breed broadly in Malaysia whether in pond or tank to supply the demands. The breeding of catfish takes place widely and also practiced in Kelantan. In Kelantan, catfish also be the one of the famous food that consumer looking for so the breeding of catfish also occur widely there. Thus, freshwater catfish has played important role as one of main animal protein sources in Kelantan.

Catfish (*Clarias Macrocephalus)* or is known as Ikan Keli Bunga by the community. The demand for this type of fish is really high and worldwide (Malaysian Fisheries Institute, 2013). The specialty of this kind of fish is been able to be underlying in mud by using its special pectoral sines and sinuos movement during dry seasons and can move on to land for few hundred meters away (Reimchen & Temple., 2004). They also consist of gills which is enable the atmospheric air-breathing and could make they survive in hypoxic (lack of water) habitats such as swamp, dried pools, and rainforest (Pouyaud, Sudarto & Paradise, 2009). Catfish

also has ability to leave water for specific times and move to another body of water (Van Wassenbergh *et al.*, 2006).

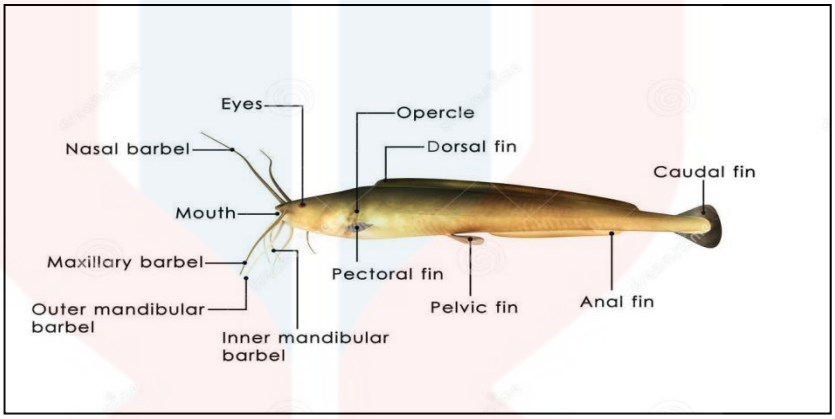

Figure 1.1 : Parts of catfish

(Source : *https://thefishsite.com/articles/channel-catfish-life-history-and-biology*)

But nowadays, the fish consumed by the human is no longer in good quality and it might affect the human health and life due to the heavy metal contamination as it is said that heavy metal be the most toxic substance that pollute aquatic organism (Pandey G & Madhuri S., 2014). This issue not only worried in our country but also known as a worldwide issue (Malik & Bharti., 2011). The increasing quantity of heavy metals in resource at present being worried since there a large number of industries nowadays discharge metal contain effluent into some fresh water sources without any proper treatment before releasing them (Malik & Bharti., 2011). Heavy metal is the symbolic, significant of a pollutant, or anthropogenic has contaminated some area and will cause many adverse effects on human health (Jaishankar *et al*, 2014).

Heavy metal is classified into two classifications, which are essential and non-essential heavy metal (Ali, Khan.E & Sajad., 2013). Essential heavy metals

perform as a nutrient that needs by living organism in certain quantity for vital physiological and biochemical function (Cempel & Nikel., 2006). Example of essential heavy metals are zinc (Zn), iron (Fe), calcium (Ca), nickel (Ni) and manganese (Mn). (Arshad *et al.,* 2013).Whereas, non essential heavy metal is the unwanted or unneeded element by living organism in the environment for any purpose neither physiological or biochemical. The example of non-essential heavy metals is lead (Pb), chromium (Cr) and cadmium (Cd) (Arshad *et al.*, 2013).

Jaishakar (2014) mentioned that the level of toxicity of heavy metal would increase the significance of ecological, evolutional, nutritional and environmental reasons. Heavy metal can be a reason to adverse effects and other severe problems even at very low concentrations (Arshad *et al*., 2014) by the formation of free radical, heavy metal can cause oxidative stress (Mudipalli A., 2008). Oxidative stress enhancing in generate the reactive oxygen species (ROS), that can lead to cell damage or the worst situation it may cause death or fatal (Mudipalli A., 2008). The level of Zn, Cd, Ni, Pb and Cu observed in the previous study which is caused detrimental effect on the health of people that consume contaminate catfish (Agusa *et al.*, 2005).

Therefore, this study was proposed to investigate the level of heavy metals concentration in two different habitats of catfish, which in soil pond and in culvert tank, which is represent two different habitat of catfish. Thus, by determining the level of heavy metal contained in two different habitats of catfish fish, the target hazard quotient (THQ) of catfish from two different habitats can be determined. THQ is the assessment tools that used to determine the health risk that might the consumer expose when eating the heavy metal contamination of catfish. THQ is use for non-carcinogenic risk. Non-carcinogenic risk is the exposure that faced by the

consumers in terms of health or other adversse effect besides the cancer. Non carcinogen is the ratio between the exposure and reference dose (Khoshnood R, Jaafarzadeh, Khoshnood, Ahmadi & Teymouri., 2014). THQ is the index for the evaluation of the exposure risk that might the consumer face by consumed the heavy metals contaminated food (Khosnood *et al.*, 2014) in long term. The severity of the damage on human depend on the value of THQ. If the THQ value is high, so the probability of the hazard risk to human health also high. So, based on THQ value, the risk exposure face by human by consuming the contaminated food can be determined (USEPA., 2000). Value THQ must less than 1 to ensure that there are no risk exposed to the consumers that consume the fis in long term but if the value of THQ are higher than 1, means that there is some harm to the consumer that takes the contaminated food as their dietary meals (EPA., 2000).

The muscle, gills, and liver of the fish from soil pond and culvert tank were tested for Cadmium (Cd), Lead (Pb), Zinc (Zn), Nickel (Ni), Iron (Fe) and Mercury (Hg). Cd, Ni, and Fe are represented for non-essential heavy metals and for non-essential heavy metals are represented by Pb and Zn. Besides that, the water quality of each breeding habitat was also analysed.

1.2 Problem Statement

Heavy metals contamination in fish is considered as one of the most significant sources to human health risks. Globally, the increasing of contaminated aquatic ecosystem by heavy metals contributed to the health risk due to the direct consumption form contaminated area especially in developing country. As an example, the most significant sources of foodborne diseases are from microbiological and chemical hazards such as heavy metals (Khosnood *et al.*, 2014) The heavy metals were widely use in the industry has direct to increase the release of harmful heavy metals into the aquatic environment directly (Agusa *et al.,* 2005). As a result, the demand for food safety research has increased about concerning the risk related to consumption of food that contaminated by heavy metals (Mansour *et al.,,* 2009). The previous study also proves that the rise in pollution of a certain type of heavy metal in the river (Hashim *et al.,* 2014). Then, result pattern from the previous study showed that the safeness to consumption of fish threatened as river is polluted by heavy metal.

The catfish is one of the fish that has high worldwide demand mentioned by Malaysian Fisheries Institute (2013). So, the catfish were picked as the focused species in this study as it is high demand from community generally. Catfish that sold in the market generated from two different habitats, which are natural habitat and cultivated habitat (the fish were breed in the tank or soil pond.) So, the level of the heavy metal contains also must be different in different habitat of catfish (Özer & Olguner., 2014). The safety for human consuming the catfish from these two habitat also would be different but which one that has most secure are not be determined yet.

Heavy metal can give adverse effect to human if the consumption last in long term. Metals can get through control mechanisms such as homeostasis, transport, compartmentalization, and required to bind with other definite cell constituents, then they contain of toxic and might cause fatal effects (Arif *et al.*, 2015).

1.3 Objectives of the study

1. To investigate the level of heavy metals concentration lead (Pb), zinc (Zn), cadmium (Cd), nikel (Ni) ,iron (Fe) and mercury (Hg) in gills, liver, muscle of the catfishes.
2. To determine the target hazard quotient (THQ) of two different habitats of catfish which are natural habitat and cultivated habitat to human health.

1.4 Significance of study

This study focused on the heavy metal contamination between the fish from soil pond and culvert tank. The fish tested for 6 element of heavy metal which are Cd, Pb, Ni, Zn, and Fe. Cd, Ni, and Fe are represented for non-essential heavy metals and non-essential heavy metals are represented by Pb and Zn. The heavy metal proven can cause many illnesses to human as example the exposure to cadmium fumes or particles can be severe, and although acute pulmonary effects and lethal are uncommon, however there are random cases still happen and also may cause kidney damage (Lars J, 2003).

In this study, absorption atomic spectrophotometer (AAS) will be used to detect the concentration of heavy metals. From the reading of AAS, the non-carcinogenic risk can be determined using THQ equation. Then, both of the samples from soil

pond and culvert tank tested for the level of the each heavy metal contained in the fish sample, which are gills, muscle and liver. Besides, the level of concentration in water and soil from study site also analysed to make sure if there is any contamination detected. After the sample were tested and analyse by using AAS, the value of the concentration heavy metals that obtained from the result will be used to generate THQ value for each of the parts of the fish. THQ depend on value of heavy metal concentration as if the concentration changes the value of THQ for the sample also changed.

By determining the level of THQ in the catfish, the health risk due to the heavy metal can figure out. The test will be done in two different habitats of the catfish so the concentration of heavy metal may different from the different habitat. The present study is very important to determine the concentration of heavy metals in the catfish in two different of habitat. By the study, which habitat of catfish consist of higher contamination of heavy metal and give higher health risk to human the fish directly can be determined. Then, from the result obtained, monitoring step can be taken on the style of breeding are which one are safer to consume by human nowadays.

Generally, the community demands for catfish in market are getting higher day by day. Thus, the entrepreneurs are good in taking the advantage to carry out the aquaculture and start to breed catfish in order to fulfil the demand from community. The high demand for catfish in market actually is a good things and profitable for the entrepreneurs. However with the issues of heavy metal issues that disturb thought widely inspired some ideas to test the safety of consuming those breed fish. In addition, there are many type of catfishes were breed such as in soil pond and culvert

tank. The level of heavy metal contamination from those two habitats might different and which one will be more secure to be consumed by human will be determined. The comparison of the THQ contributes to helping the community to make a decision to choose the least impact or least heavy metal content in their food.

Fish are commonly stated as one of the main aquatic living things in the aquatic food chain and in previous study also mentioned that normal metabolism of fish may accumulate large amounts of certain metals from food, water, and sediment (Idris *et al.*, 2015). The heavy metals impact to human has a big and long-term effect. The heavy metal levels may be different due to the food that the fish consume, the water and environment of the habitat that the fish live in (Shakya & Khwaounjoo, 2014).The justification for this research is being able to give least impact human health due to the consumer behavior. Next, the heavy metal impact on human due to the direct consumption also can be reduced. So, this study is very important to figure out and determine the level of heavy metals contamination in two different habitats of catfish.

The consumption of contaminated food can seriously deplete essential nutrients in the body which is will end up causing a decrease of immunological defenses, disabilities associated with malnutrition (Canli & Atli., 2003). The most save catfish between two are the catfish from natural habitat which is they might not contaminate by the food and consumer behavior. As for the cultivated, it is might be contaminated from the food and also might because of the management of the habitat itself by the owner (Chedoloh & Pakdeechanuan., 2011).

CHAPTER 2

LITERATURE REVIEW

2.1 Catfish

Catfish is freshwater fish type with whisker like barbel around the mouth, typically bottom dwelling. There are a few previous study that investigate the biological of catfish. Mentioned by Townsend & Winterbourn (1992), catfish species can survive and live in wide range of freshwater habitat such as ponds, lake, stream, and river. They also found in high sendiment loads aquatic habitat which are the sendiments load are high in the water however they also can survive in clear water condition (Townsend & Winterbourn., 1992).

Stated by Nelson (1994) catfish is rare species of fish because did not have scaled skin. They consist of slimy skin and usually have an adipose fin and often spine-like rays. In addition, Lunberg & Fiel (2003) stated that some catfish species able to stings by using their spines if they harmed or feel any pressure. The stings consist of poisonous venom which is produced from poison gland in the epidermal tissue of fish that covers the spine. The content of catfish poison are complex hemolytics and dermanecrotic composition (Worral *et al.*, 2003) where can cause the oedema, erythrena and pain to the human when get stung.

2.1.1 Catfish demand

From the previous study , it is mentioned that Asian and the Western Europe contributed to the highest range for fish production around the world, meanwhile the last contributor in fish production are sub Sahara (FAO., 2013). Despite the low aquaculture production in sub-Saharan Africa, some improvements are taking place, when compared to the past production trends (Gabriel *et al.*, 2007). However, in Malaysia, aquaculture production trend are increasing and there were recorded on aquaculture production where always been dominated by aquatic plants (seaweeds), which represent 51.5% of the total aquaculture production of 2015 but the rest are from fish source (Akinrotimi., 2011). This trend shows that fish is one of the aquaculture product that has high demand and important source in Malaysia.

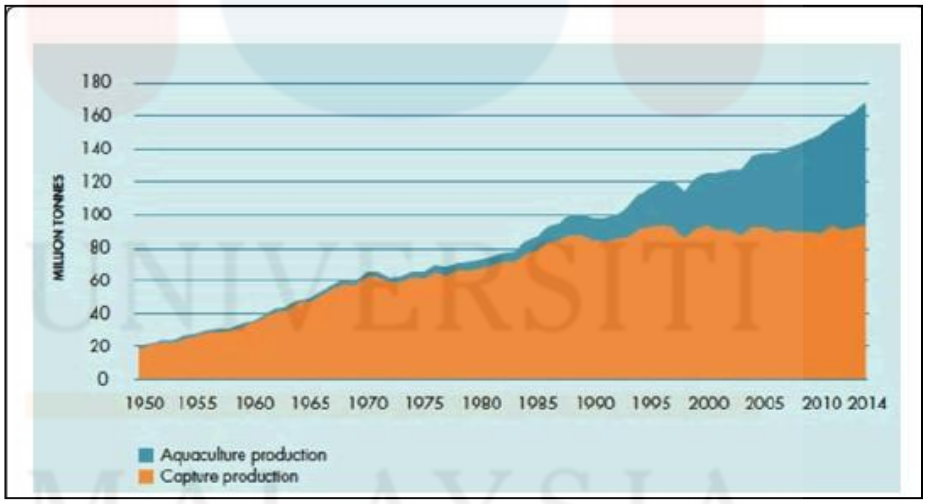

Figure 2.1: World capture fisheries and aquaculture production

(Source:*http://www.viableplans.com/9-must-know-things-about-starting-a-fish-farming-small-business.*, nd)

Demand for fish in market is increasing day by day over than a decade ago in Asian due to the rapid growth of the population, urbanization and expansion in per capita income that is currently being experience in the region. (Ibhadon *et al.,* 2014). The demand is getting higher day by day to fulfill the need and food source of human in Malaysia as one of the protein source. It is proved that catfish has the highest demand in Kelantan form previous studies. Anon (2003) mention that the highest production of freshwater fish in Kelantan is dominated by catfish. Catfish also being one of the important protein supply in community. The figure 2.1 shows the aquaculture production and capture production in Malaysia which is the product are mostly come from plant and fish.

2.1.2 Catfish nutritional value

Catfish anciently known as the great protein supplier by human but it is now proven with the study that confirmed the catfish is very high in protein. As mention by Dale (2001), the protein values in catfish more closely approximated those of menhaden meal (made from whole fish) than tuna meal (which does not include fillets). As between 75 and 80% of catfish processed at the present time are filleted, protein content might have been expected to be somewhat lower than the 60% found in the previous study.

The nutritional value contain in the catfish has been analyse by the previous studies. The nutritional value of the catfish are included the contain of proximate composition such as crude protein, ether extract, nitrogen corrected true metabolize energy and crude fibre (Dale, 2001). The study from Dale (2001) also proved the existence of amino acid, minerals, vitamins and fatty acids composition in catfish.

The results of the analysis of metals in other research its shows that different fish organs show that different metal levels in their tissues due to variations in feeding habits, habitats and behaviours (Tanee *et al.*, 2013).

2.1.3 Catfish feed behaviour from two different habitats.

Catfish is an omnivorous type of fish. The dietary protein requirement for catfish are about 40%, their energy requirements range between 13 and 17 kJ.g-' (Van *et al.,* 1995). Catfish also famous regarding to their ability to provide protein to the consumer that consume. The muscle nutrient profile shows that catfish is highly nutritious – high in protein, low in fat, and a good source of certain vitamins and minerals. The liver nutritional profile may be useful in assessing the nutritional (Ibhadon *et al.,* 2014). The catfish nutritional values were different in two different habitats due to the feed behaviour of the fish (Abalake S.E., 2013). It is proved from the previous study that the cultivated catfish contain of higher nutrient characteristics of catfish muscle and liver (Schram *et al.*, 2001). It is also mentioned that catfish contain of phosphorus are high in cultivated catfish compare to the catfish grow naturally in natural habitat.

In cultivated catfish there many kind of food feed to fish one of them are chicken guts. However the Oke, Abou, Adite, & Kabre (2016) mentioned that catfish that feed with chicken intestine were higly in protein and also low and cost compared to the fishes that given fish feed. Next, in the same study it is also stated chicken guts could incorporated up to 30% in catfishes diet which is proved that it contain good nutritional value in catfishes diet without adverse effect on growth, feed utilization and body protein content. So, the behaviour of catfish might slightly different

between two habitat as the feeding habitat also changes (Gupta, Gupta, & Monica., 2013).

2.2 Heavy metal

Metals and metalloids are from natural and anthropogenic existence sources where then constantly enter the environment and cause crucial and serious threat to human and ecological health because of their chemical properties that will contribute to the toxicity, long persistence, bioaccumulation, and biomagnifications in the food chain (Copat *et al,*. 2011). Human body need some the presence of essential element metals such as Fe for enzyme systems, haemoglobin formation and vitamin synthesis function for human (Jaishankar *et al.,* 2014). On the other hand, the excess of these essential metals can affect in metabolic and disturb its function (Hina , Rizwani & Naseem., 2011). Heavy metals accumulate in crucial organs in the human body such as the kidneys, bones, and liver where can cause kind of severe health effects such as neurotoxic and carcinogenic effects (Duruibe *et al.*, 2007). In addition, non-essential metals such as Pb, Ba, Cd, Hg, Cr, and As also give adverse effect on human health where their presence in human body will disturb a biological system (Canli, M., & Atli, G., 2003). It is also have been identified as toxic heavy metals and their permissible limit has been recommended and residual levels have been decided for humans (FAO 1983) to avoid any adverse health on human due to the exceed toxicity from heavy metal contamination.

2.2.1 Lead (Pb)

Lead (Pb) is a chemical element where widely known with symbol Pb. Lead is the element consist of high resistant to rust but tarnished upon expose to the air (Nelson., 1994). The isotope of Pb are the end products of each of the three series naturally occurring radioactive element (William., 2015). Lead is severe substance that consist of also highly toxic metal that may cause widespread in environmental contamination and adverse health effect to the human (Monisha, Tensin, Naresh, Blessy B & Krishnamurthy N.A., 2014). From the previous study, it is mentioned that source of Pb are come from industrial process, smoking behaviour and other domestic sources such as gasoline, house paint which is widely used in daily activity such as plumbing pipes and batteries (Jaishankar *et al*., 2014). From the previous study run by Sharma *et al* (2012) it is proved that the plant also can be contaminated by lead which is can affct the growth process and end up the plant that consist of high lead concentration, will produce the production of reaction oxygen species (ROS) faster than the plant that did not contaminated by lead. This situation cause the lipid membrane membrane of the plant which is can give more severe impact to the plant by damaging the chlorophyll and photosynthesis process and effect the overall production of the plant (Sharma *et al*., 2012)

2.2.2 Zinc (Zn)

Zinc (Zn) is chemical element with symbol Zn and one of the frequent elements found in earth crust. It is found in air, soil, water and present in many kind of food. Zinc is is an essential element in our diet which is lack of zinc can cause

some problem to human health but exceed amount of Zn also end up sacrificing human body (Ra

chel N., 2017). Zinc is essential element used in biological system of human.

The presence of Zn involved in many biochemical process where can help in system of immunity, sexual, neursensory and many more Nriagu, J. (2011). However it is proved in many of previous study that stated Zn can give adverse effect on human health if consume exceed the allowed value. As mention by Nriagu J. (2011) the long term health effect caused by consuming excessive Zn are anaemia, lymphadenopathy problems, neutropenia problem. Exceed limit of Zn consumption can cause adverse health problems, such as stomach cramps, skin irritations, vomiting, nausea and anaemia (Nriagu J.,2011) . In addition, very high levels of Zn can affect the pancreas and also disturb the protein metabolism, and cause arteriosclerosis. Extensive exposure to Zn can cause respiratory disorders.

2.2.3 Cadmium (Cd)

Cadmium (Cd) is one chemical element that well known with symbol Cd and atomic number 48. Cadmium is a lustrous, silver-white, ductile and very malleable metal (Ibhadon *et al*., 2014**).** Cd is element that soluble in acids but not in alkalis an it is similar in many respects to Zn but form in more complex compound. From the previous studies, mentioned that Cd can cause renal dysfunction, characterized by tubular proteinuria in human body if exposed in long term of time. Cd also caused more severe impact such as lead to the lung disease because of the death lining of the lung tissue **(**Yu. J., 2008). Besides that, Zn also might cause other chronic diseases which are defection of bone, osteoporosis and spontaneous fractures.

2.2.4 Nickel (Ni)

Nickel (Ni) is a silver-white metal with properties that assist the formation of nickel-iron alloys (Javed M *et al*.,2013). In difference to the soluble nickel salts (chloride, nitrate, sulfate), metallic nickel, nickel sulfides, and nickel oxides are poorly water-soluble (Cirovic N *et al*., 2015). From previous study it state that nickel compounds will accumulate in the food chain by entering the environment (Bashir *et al*, 2011). Nickel is not a cumulative toxin in animals or in humans however the element is important for proper functioning of the immune system because Ni is a general sensitizing agent and is also reported to induce embryotoxic, teratogenic and carcinogenic effects (Samal & Mishra., 2011).However, exceed limit of Nickel in body can caeuse adverse effect on human health (Coogan et al., 1989).

From previous study, it is mentioned that Nickel would not be destroy throughout the body, but the chemical form may be altered due to some reaction. The metabolism of Nickel can be seen by the presence of light where it is binding to form ligands and then will be transport throughout the body (Coogan *et al.,* 1989). In addition, many of the toxicity of nickel may be related to the intervention with the physiological processes of manganese, zinc, calcium, and magnesium (Coogan *et al.,* 1989).

2.2.5 Iron (Fe)

Iron (Fe) is one of 94 naturally occurring element. It is metallic material with silver grey colour and metallic lustre. The atomic number for Fe is 26 and the symbol is Fe. There are many from previous study said that Fe found from all part of the planet. The Fe are actually essential in dietary of human but excessive consumption of Fe can also cause some problematic to human health. The excessive iron consumption can cause many chronic diseases such as high blood, gastrointestinal tract damage, heart and liver failure (Olatunji O.S., 2012) . It is happen because Fe react with peroxide to produce radicals, which are highly reactive and can corrode the internal organ (Olatunji O.S., 2012).

2.2.6 Mercury (Hg)

From the previous study by Bernhoff R.A (2013) it is mentioned that Mercury (Hg) is a toxic heavy metal which is widely distributed in nature and the most human exposure results are from fish consumption. Mercury occurs in several chemical forms where are in compound pharmacokinetics. Mercury is widely used in cosmetics sector where acts as one of the effective substance to lighten the (WHO) however, mercury has been found to be a causative agent of various sorts of disorders, including alterations of motor function and neuroendocrine secretion at very low exposure levels of mercury. Mercury also proved as a cause to chronic disease such as alzheimer, anxiety, asthma, hyperactive disorde, auto immune disease, polar disorder and personality disorder (Jain, R., Singh, S. K., Advani, U., Kohli, S., & Sharma, N., 2013)

2.3 Heavy metal contamination in fish

Heavy metal existence in fish composition can change the nutritional values contain in the fish. The toxicity from heavy metals can contribute to individual growth rates, physiological functions, mortality and reproduction changes in fish (Afshan *et al*, 2014). There are three probable customs to heavy metals enter the fish body where are through gills, digestive track and body surface (Afshan *et al*, 2014). The gills are considered as the significant site for direct accumulation of metals from the water (Wong C, Wong P & L Chu., 2001) and it is stated that outer surface of fish were estimated to be the minor possibility to involved in uptake the heavy metals into the fish (Wong *et al*, 2014). Accumulation of heavy metal in fish caused by the food source, where it can possibly leading to bio-magnification, the augmentation of toxins up in the food chain. As a source of protein to human, fish are considered as an one of the good source of polyunsaturated fatty acids (predominantly omega-3 fatty acids), protein, Zn, Fe and calcium(Ca). Rauf A, Javed M & Ubaidullah M (2009) also reported that the fish liver have the highest tendency to accumulate heavy metals. The liver also plays an important role in accumulation and detoxification of heavy metals. Induction of metallothioneins (MTs) in the liver is the main metabolic process to act as a form of storage and detoxication of heavy metals in fish (Romeo, M., Gnassia-Barelli, M., Mathieu, A., & Lafaurie, M., 1995).

2.3.1 Effect of heavy metal on human health

Heavy metal toxicity has proven to be a major threat and there are several health risks occur due to the presence of it. The toxicity effects of these heavy metals are even though they do not have any biological role, the presence of heavy metals in any form are harmful for the human body and its proper functioning. Heavy metals sometimes act as a pseudo element of the body while at certain times they may even interfere with metabolic processes (Jaishankar *et al*, 2014) and cause other adverse health effect. As example, the main symptoms of acute lead poisoning are headache, irritability, abdominal pain and various symptoms related to the nervous system. Lead encephalopathy is characterized by having hard time to sleep and rest. In severe cases of lead contamination, the person whom contaminated by lead will suffer to spontaneous affect such as acute psychosis, confusion and reduced consciousness (Jarup.L., 2003). Direct heavy metal consumption also in a long time can cause cancer (Mudgal, V., Madaan, N., Mudgal, A., Singh, R. B, & Mishra, *S.*, 2010).

Heavy metal is one of the crucial anthropogenic in environment (Yu, R., Yuan, X., Zhao, Y., Hu, G., & Tu, X., 2008). The heavy metal cause dangerous hazard risk to the world component because of their toxicity, persistence and bioaccumulation characteristics (DeForest, D. K.,Brix, K. V., & Adams, W. J., 2007). Stated by Fu & Wang (2011) many heavy metals ions are known to be toxic or carcinogens to human. Heavy metals also contribute to disturb marine ecosystems by reducing species diversity and abundance through accumulation of metals in living thing and food chain (Hosono *et al*., 2011). Anthropogenically, heavy metal

can be released to the environment by variety of sources such as industries, wastewatersand domestic effluent (Subba *et al*., 2012).

Stated in previous study by Balpınar, N., Arslan, M., Çelik, N., & Bingöl, Ü. (2018), heavy metal has strong effect on central nervous system and may cause the human to lost memory and lost ability to speak if the heavy metal were consume over the permissible limit in long term. It is also stated the essential heavy metals like Hg can cause membranous nephropathy and ight end up affecting the embryo if the pregnant mother consume the food contaminated by heavy metal. In other study, it is stated that heavy metals affected the chronic illness such as anaemia, bone narrow hiperplacy and cardiac hypertrophy (Ikeda. M, Zhang.W & Higashikawa ., 1990) and it is cause because the heavy metals deplete the formation of metabolism of essential tracing element strived directly on the human cells (Ikeda *et al*.,1990).

2.4 Bioaccumulation of fish

From the various of previous study, it is mentioned that the heavy metals might alter or change the physiological activities and biochemical parameter both in tissue and blood (Basha & Arani,200). These heavy metal could reach food chain through various biochemical process as mentioned by Ozer & Olguner (2011) the process involve are bioaccumulation in various trophic level and eventually threaten the health of human by consume the contaminated food.

Mentioned by Rajeshkumar & Li (2018) the concentrations of heavy metals in fish have been broadly studied over the past several decades. The past studies shown that excessive of accumulation of heavy metals in fish is dependent on the metal types, fish species, and the tissues respectively (Korkmaz et al ., 2013). In addition, water chemistry (Farag., 1998) directly affects the accumulation of heavy metal in fish. Sediment is also known as main factor of heavy metal accumulation in fish, as it is measured as the major source of contaminants for bottom dwelling and bottom feeding aquatic organisms (Meche *et al*.,2010), where it will in represents the concentrated source of metals in the fish diet.

Therefore, many international monitoring programs have been recognized in order to involve in assess the quality of fish for human consumption and to monitor the health of the aquatic ecosystem (Meche *et al*., 2010). According to the literatures, metal bioaccumulation by fish and subsequent distribution in organs is greatly inter-specific. In addition, many factors can influence metal uptake like sex, age, size, reproductive cycle, swimming patterns, feeding behaviour and living environment.

2.5 Permissible limit of metal consumption

To ensure the safety human on consuming the metals, the permissible guideline of heavy metal that allowed to be taken that stated by FAO and WHO. In the table 2.4 stated about the permissible limits recommended by FAO 1983 and thesese value also used in various of previous study.

Table 2.1 : Guideline for permissible level of heavy metals according to FAO mg/kg

Heavy metals in fish	Pb	Zn	Cd	Ni	Fe	Hg
Permissible value for fish	0.5	30	0.05	0.5	0.5	0.00015

(Source : FAO,1983)

The permissible value limit for fish were commonly used in many of previous study. Stated by Tariq & Mott (2006) the level of heavy metal concentration in fish should be under permissible limit value of the of the fish so, the adverse health effect toward human health can be reduced.

2.6 Target Hazard quotient

The target hazard quotient (THQ) is one of the way to assessed the non-carcinogenic health hazards for each individual metal through fish consumption. The THQ value will results total up and sum of THQ are known as hazard index (HI) (USEPA 2011). The THQ are assumption of a level of hazard exposure below which it is unlikely for even sensitive populations to experience adverse health effects due to the consumption of certain food. On the other hand, HI indicates the combined hazard of all metals. For carcinogens, Target risks (TR) were estimated as the incremental probability of an individual to develop cancer over a lifetime, as a result of exposure to that potential carcinogen (i.e., incremental or excess individual lifetime cancer risk (USEPA., 2009).

THQ widely used in previous study worldwide to estimate the impact on human health by consuming certain food such as vegetables and seafood. THQ also used to estimate the severity consuming the heavy metal contaminated food. In Nigeria, there were studies conduct by Agusa *et al*,(2005) focus in survey of heavy metal in catfish. But in Malaysia, the estimation of THQ on catfish were did not started yet but the study on the other type of fish already recorded such as tilapia and swamp eel by Ahmad A.K *et al* (2015).

The general formulas of THQ that used to calculate the non-carcinogenic effects are :

$$THQ = \frac{(EFr \times ED \times IR \times C)}{(RfD \times BW \times AT)} \times 10^{-3} \qquad \text{Eq. (2.1)}$$

From THQ equation the EFr represents the exposure frequency which was in this study (365 days/year). ED represent the expose duration the human face the exposure where is it is that comparable to the average human life time (70 years). IR is the ingestion rate for person (g person^{-1} d^{-1}). C is the metal concentration in samples. BW average body weight of the person that consume the contaminated food and for this study, average body for Malaysian was used (adult: 63 kg). AT is the averaging time for non-carcinogens 365 d year^{-1} wills the multiple with the number of exposure years, assuming 70 years). RfD is the oral reference dose (mg kg^{-1} d^{-1}) where RfD are different for each element (USEPA.,2005). If the THQ value is less than 1, the exposed population is may not experience any adverse health hazard. On the other hand, if the THQ value higher than 1, there is a potential health risk to human that consume that food (Wang *et al*,2005) and related interventions and protective measurements should be taken to the community that consume the contaminated food.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The materials that have been used in the laboratory throughout the study were listed in table 3.1.

Table 3.1: List of chemicals and reagent

Chemicals and reagent	Brands
Concentrated nitric acid (HNO_3)	H1009-Sigma
Hydrogen peroxide (H_2O_2)	Hp 502
Distilled water	-
Hydrochloric acid (HCl)	MSI H3950
Sulphuric acid (H_2SO_4)	S-1200

3.2 Instruments

Table 3.2 shows the instruments that used throughout the study.

Table 3.1: List of instruments

Instruments	Brands
Atomic Absorption Spectophotometer	Ice 3000 series
YSI Multiparameter	YSI

3.3 Apparatus

The apparatus that have been used throughout the study were listed in Table 3.3. In this present study the apparatus were handled carefully to obtain actual result.

Table 3.3 List of laboratory apparatus

No	Apparatus
1	Volumetric flask 50 ml
2	Hot plate
3	Stainless kit
4	Knife
6	Pestle and mortar
7	Test tube
8	Dropper
9	Filter funnel
10	Filter paper
11	15ml falcon tube
12	Polyethylene bag
13	Blade
14	Syringe filter

3.3 Methodology

3.3.1 Sampling site

The sampling sites are located in Jeli and Tanah Merah. First site is located in Batu Melintang which located about 15 km away from UMK Jeli Campus. The sampling area is bordered by latitude of 5^0 41'30"N and $5^0$44'35''N for latitude. For cultivated habitat, the fish catch directly from the commercial fish farm which is their breed place for sale located in Jeli. The second habitat is the fish breed in culvert tank which is located in Tanah Merah. The sampling area for second location are 5^0 45' 7''N for latitude and 102^0 4' 30''E for longitude

3.3.2 Sampling methods

The catfish caught using the net at the tank. Then, the sample was rinsed with clean water at the point of collection, label each of the sample and the sample was preserved immediately by acidifying with 10 ml of concentrated nitric acid to pH < 2. The fish samples were kept in polyethylene bag and ice box to sustain the freshness before transported to the laboratory for further step and process.

3.3.3 Sample preparation

The length and weight for each catfish were measured and the data were recorded (Hashim *et al.*, 2014). The samples were rinsed with water to eliminate dust and dirt. The samples were dissect and only the dorsal muscle (edible tissue) of fish were taken from each sample by using stainless steel kit or clean knife (Ahmad &

Sarah., 2014). The glassware and other apparatus that used were soak in diluted nitric acid (HNO_3) for 24 hours and rinsed with distilled water before use to minimize the contamination during the fish dissection (Hashim *et al.*, 2014). The samples were dried at 60^{o}C for 48 hours in laboratory oven. Then, after the sample fully dried, the sample was mashed and pound by suing laboratory mortar until the sample from into powder to produce in homogenous tissue (Ahmad & Sarah *et al.*, 2014). The powder sample were keep in polyethylene bag and keep at -20^{o}C in freezer.

3.3.4 Sample digestion

About 0.5 gram of dry sample was put in the 50 ml beaker with 5 ml of HNO_3 and 5 ml of H_2S0_4 and wait till the reaction between the chemical and fish tissue stop. Then the beaker putted on the hot plate and heated at 60^{o}C for 30 minutes. The beaker allowed to cool before 10 ml of HNO_3 were added to beaker then the beaker were put on the hotplate again and reheat at 120^{o}C. The temperature increased to 150^{o}C after a while and the beaker was removed after the sample turned black.

Then, let the sample cool before H_2O_2 were added and wait until the sample was clear. The beaker content then transfer into a 50 ml volumetric flask and fill in the distilled water until reached the mark. The entire step performed in a fume hood. The above procedures in this section followed the guidelines from the Analytical Methods for Atomic Absorption Spectroscopy (Perkin Elmer., 1996).

3.3.5 Analytical procedure

About 5 ml of sample pipeted from previous volumetric into another volumetric flask and deionised water were added until reach the mark and mix thoroughly (Tyagi *et al*, 2014). The sample was labelled for each heavy metal. Analyze the digest sample three time for metals Cd, Pb, Ni, Zn, Fe and Hg using AAS. The instrument were calibrate with chemical standard solution that prepare from commercially available chemical. The reading of the prepared sample solution will be take and record directly from the instruments (Bhavtosh *et al*, 2014). Analytical blank run in the same way as the sample and the concentration will be determine using the standard solution prepare in the same acid matrix.

3.3.6 Calculation of THQ

The target hazardous quotient (THQ) represents a complex parameter which is introduced by the US Environmental Protection Agency (EPA 1989). It is used commonly for the assessment of the potential of non-carcinogenic risks associated with long term exposure to contaminants, such as heavy metals from food such as fish and water.

The general formulas that used to calculate the non-carcinogenic effects (THQ) are :

$$THQ = \frac{(EFr \times ED \times IR \times C)}{(RfD \times BW \times AT)} \times 10^{-3} \qquad \text{Eq (3.1)}$$

Where ,

EFr = exposure frequency (365 days/ year)

ED = exposure duration equivalent to the average human life time

IR = ingestion rate for person

C = metal concentration for each heavy metal

RfD = oral reference dose

BW = average body weight

AT = number of exposure years

CHAPTER 4

RESULT AND DISCUSSION

4. 1 Size of fish

The weight and length of raw catfish were taken and recorded to use as a morphoric data. The morphoric data of the species were listed in Table 4.1

Table 4.1 : Average weight and length of raw fish from two different habitat

Catfish	Average weight (g)	Average length (cm)
Soil pond	89.40	25.6
Culvert tank	60.70	20.5

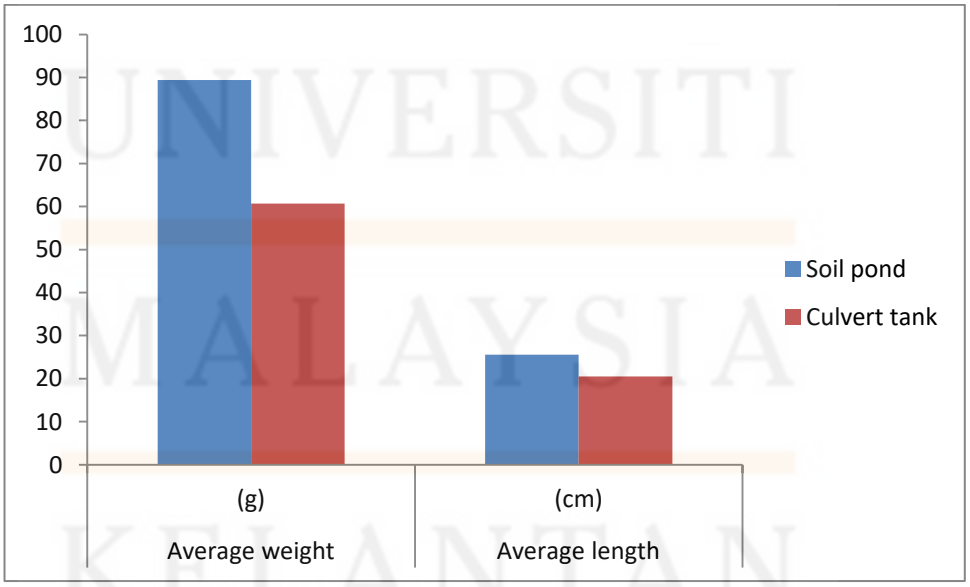

Figure 4.1 Graph of average weight and length of raw fish from two different habitat

Table 4.1 shown the average weight and length of the catfish from two different places which are the Jeli area shows that the catfish from that area are 89.40g and 25.6 cm for length. Meanwhile in Tanah Merah the average weight and length are 60.70g and 20.5cm. The size of fishes for both places are in standard range as the standard size for catfish in Malaysia are 19.8cm – 33.6 cm for length and 60.0g to 260g for weight (M.F.Yusof *et al.*, 2011). The fishes are in good and suitable condition to consume by the human. But we can see here the size of the catfish breed in Jeli are bigger than in the Tanah Merah same things with the length. This things might happen because of the feed habit used by the owner of the farm. In Jeli farm, the owner supply the fish with own-made food made from raw chicken intestine. The raw chicken intestine will be boil until half cook then distributed directly into the fish pond once a day. So, we can assume that fishes from Jeli's pond were given enough food supply that affects the size of the fish itself. Meanwhile for the catfishes that breed in culvert in Tanah Merah only given fish food bought in ordinary market. So, the needs of the fishes needed to grow quite limited and only depend on the nutrient supply from the fish food and effect the size of the fishes as well but both of fishes breed in soil pond and culvert are reached standard size for catfish to be sold in market. From previous study run by Latif M (2006) it is stated that chicken boiled intestine give better growth for the fish and boiled chicken intestine could be used as main protein source to replace up to 50% of ordinary fish feed.

4.2 Physico-chemical properties of water from two different places

Physicochemical properties of water for both habitat were recorded tested by using YSI Multiparameter then the result were recorded in Table 4.2.

Table 4.2 : The physico-chemical properties of water from two different habitat

Place	pH	Temperature (°C)	Dissolve oxygen(%)	Salinity %	BOD mg/l
Soil pond	6.82 – 7.0	29.0- 30.2	26.9 – 30.1	0.02	2.42 – 2.50
Culvert tank	7.35 - 7.4	26.35 -26.55	34.8 -39.3	0.13-0.14	2.78 – 3.00

4.2.1 pH

From Table 4.2, the result of physic-chemical properties of water from both places show slight differences. PH for pond in Jeli showed lower value than Tanah Merah which are the pH value for soil pond are 6.82 to 7 while for Tanah Merah are 7.35 to 7.4. Value of pH in Jeli pond is constant in natural state which is good state for fish's growth. Meanwhile the pH values for culvert tank are slightly alkaline. So, the pond are consist of optimum pH for catfish breeding as mention by Ibhadon *et al* (2014) the optimum pH for catfish are in range 6 to 7 which is in natural standard. But for Tanah Merah culvert also did not reach or exceed any the extreme pH for catfish survival as for the extreme pH for catfish are 4 and 10 which is high acidity and high alkalinity (Uzoka *et al*., 2015). pH value of the water actually relate and

effect other physic-chemical properties in the water as it is reported that the lower pH will end up with low DO value in the water (Yee *et al*.,2012). It is happen because of the consumption of oxygen are high during the organic matter breakdown process which is come from excess feed and fish waste.

4.2.2 Temperature (°C)

Temperature is one of the important physic-chemical properties in water. From the Table 4.3 , temperature in soil pond are higher than culvert tank which is in range 29°C to 30.2°C while for culvert tank are 26.35°C to 26.55°C. The temperature in Jeli higher because of the depth of the pond is higher than in Tanah Merah because the size of culvert is small and the depth also low. Nyanti *et al* (2013) mentioned that if the depth of the pond increase the temperature will be decrease because the water at the surface will absorb heat from solar energy and cause the density less than the water in bottom of the pond.the depth for soil pond are about 5 feet while for culver tank are 1 meter .Temperature also affect the rate of metabolism, breeding pattern, feeding pattern and rate of enzymatic activities in fish occur in the water (Ibhadon S *et al*., 2014).

4.2.3 Dissolve oxygen (%)

Vital role in physicochemical properties of water are actually shown by the dissolve oxygen value. Dissolve oxygen are produced from the diffusion of surrounding air. Table 4.2 showed that in soil pond there are 26.9 to 30.1 and culvert tank are 34.8 to 39.3. The values for both habitats are in considerable condition and capable to support the process occur in the water. Dissolve oxygen in water cannot lessen as it is important and main role of water properties. Dissolve oxygen must sufficient and needed to ensure the quality of water, the growth of aquatic organism and also need by microorganism to decompose waste in the water (Islam M *et al*, 2010).

4.2.4 Biochemical oxygen demand mg/l

Biochemical oxygen demand also one of the vital role in properties of water which is defined the measure of organic pollutant extent in the water. The values of BOD for both habitats are 2.42 to 2.40 mg/l for soil pond and 2.78 to 3.00 mg/l for Culvert tank. Both places are considered as clean and in optimum range for BOD as stated by WHO the value must not exceed 5mg/L (Nwankwo C *et al*, 2014). If the BOD are exceed range 5 mg/l it defined that the water are started to pollute by the heavy metal or other substance.

4.3 Heavy metal concentration in fish sample (gills, muscles and livers), water and soil in two different place.

Heavy metal concentration of fish, water and soil tested by AAS were listed in Table 4.3 and Table 4.4 for soil pond and culvert tank**.** Heavy metal concentration in each sample would determine whether the samples were contaminated by the heavy metals or not.

Table 4.3. : Heavy metal concentration of parts of the fish (gills, muscle and liver) , water and soil from soil pond mg/L (mean value)

Heavy Metals	**Cd**	**Fe**	**Ni**	**Pb**	**Zn**	**Hg**
Gills	0.551	0.406	0.585	0.372	0.108	0.0000083
Muscle	0.076	0.246	0.419	0.079	0.116	0.0000087
Liver	0.072	0.363	0.379	0.075	0.1	0.0000047
Water	0.055	1.393	0.1	0.175	0.065	0.0000038
Soil	0.004	0.447	0.28	0.362	0.363	0.0000450

Table 4.4 : Heavy metals concentration in part of the catfish (gills, muscle and liver) , water and soil from culvert tank mg/L (mean value)

Heavy Metals	**Cd**	**Fe**	**Ni**	**Pb**	**Zn**	**Hg**
Gills	0.0	0.449	0.0	0.284	0.33	0.0000031
Muscle	0.0	0.0	0.0	0.258	0.155	0.0000048
Liver	0.0	0.179	0.0	0.296	0.197	0.000001
Water	0.0	0.0	0.0	0.220	0.287	0.0000042

4.3.1 Cadmium (Cd)

The data presented in Table 4.4 shows that fish muscle,gill and liver, water and soil from the soil pond in Jeli are detected certain amount of trace metal. Cadmium detected in the fish samples which are 0.551 in gills, 0.076 in muscles and 0.072 in liver. The heavy metals detected in fish body are highest in gills followed by muscles and liver. The differences of value are not huge but metal also trace in water which is 0.055 mg/l. Here we can see that cadmium detected in all of the sample tested. This implies that the level of Cd in water also absorbed into the fish bodies as both result also traced the existence of the Cd. However the concentration of Cd in muscles and liver pond are still under permissible value state by FAO (2003) which is the content of Cd should under range 0.50 mg/l while the concentration in gills are exceed the permissible value. This can be concluded that the fishes from soil pond are faced potential of Cd contamination. Meanwhile for culvert tank habitat, the Cd in all of the sample were not detected. This can conclude that the fish from culvert tank habitat are free from Cd contamination.

In addition, there were recorded from various of previous study that the concentration of Cadmium are always detected low in the fish muscles. The result are same with the study did by Azlan *et al* (2015). There were no detected of Cd concentration in the catfishes sample and the result were negative as there are no Cadmium in the fish sample at all. The same result also obtained by the study by Squadrone *et al* (2013) which is reported Cadmium muscle in all parts of fish are in legislation limit and it is also proved that Cd concentration are shown the lowest concentration compared to other heavy metal.

4.3.2 Iron (Fe)

Iron plays vital role in haemoglobin transportation of fish which is act as colouring agent of the blood. Then it is expected Fe existence in the fish body. As the Table 4.3 presented the concentration of Fe are highest in gills which are 0.406 while 0.246 mg/l for muscle and 0.363 in liver. It is shown that the least concentration of Fe detected in muscle. It is norm when the heavy metal in gills are highest because of the previous study of the bioaccumulation in fish also recorded the highest concentration are in gills of the fish. Fe also trace in the water sample with concentration 1.393 which is quite high. The concentration of Fe are exceed the permissible value fixed by WHO (2003) which is it is stated that the content of Fe in fish must not exceed 0.5 mg/l. So, the Jeli pond is severe and face exceed concentration of Fe. This condition is not good as it can give impact to the human health in future. One of the impact of Fe to human health is can cause conjunctivitis (Khosnood *et al*,2014).

The concentration of Fe in culvert tank habitat are quite high detected in gills and also liver. However in the muscle of fish there were not detected any of Fe. However the limits are still under permissible and safe to be consumed. The trend of the result might different with other previous study which is when the metals were detected in other part of fish, it is also detected in muscle of fish (Zahra *et al*, 2014).

4.3.3 Nickel (Ni)

Nickel is the heavy metal that can cause severe health effect on human if the consumption occur in long term. The disease that cause by consumption of Ni are cancer (Javed M *et al*, 2013) and it is also mentioned that consumption of Ni in huge quantity may cause respiratory failure, birth defects and heart disorders. From the Table, the result shown that the highest concentrations of Ni were traced in gills which is 0.585 mg/l. Meanwhile the concentration in liver are 0.419 mg/l and muscle are 0.379 mg/l. The pattern of the result are same with other heavy metal which is the gill parts consist of the highest concentration of Ni and the lowest part that contain of heavy metal are muscle part. The concentration of Ni in water sample are 0.1 mg/l. Based on the result, values of Ni concentration in this pond still under permissible value stipulated by WHO which is 0.5 to 1.0 mg/l. As it is observed that the concentrations were within the permissible limits, implying that the samples obtained were not contaminated by Nickel.

4.3.4 Lead (Pb)

From the Table shown , the highest detected concentration of Pb is in gills which is 0.372 mg/l. Next, concentrations in the muscle are 0.079 mg/l and 0.075 in liver. Whereas the Pb also trace in the water sample as well which are 0.175 mg/l. The concentration in gills is not exceeding the permissible value for the existence of Pb in food which is supposedly uder the range 2.0 mg/l. So the concentration in gills is still far away under the permissible value. Pb is a non-essential element and can be toxic to humans when ingested or inhaled in high doses (Salem *et al*., 2000). Trace metals such as Pb will interfere with essential nutrients of similar characteristics.. Pb

also can give adverse health effect on human such as causes renal failure and liver damage. (Salem *et al*., 2000). In fish, Pb causes decreases in survival, growth, development, behaviour and metabolism, in addition to an increase in the formation of mucus (Hashim *et al*, 2015). This implies that the level of lead in the fish may not cause any effect on its performance and produce effect on human body.

The lead concentration in culvert tank is quite high in all parts of fish. The highest were recorded in liver. The same results were recorded by previous study Nikoo Falahatkar & Rahmani (2010). However the concentration is still under permissible limit stated by FAO.

4.3.5 Zinc (Zn)

Zinc is the heavy metal that play vital role in the enzyme system progress, synthesis of ribonucleic acid which is lead the role in develop the germs and somatic cells of aquatic plant (Milam C *et al.*, 2014). But the high concentration of Zn in one body can disturb growth and change the metabolic and pathological pattern in various organ in fish which is may lead to the health impact to the human that consume the fish (Abdel *et al*, 2011). The Table 4.3 represent the concentration of Zn in all sample are under permissible value stated by WHO. which is 30 mg/l. The concentration of Zinc are 0.108 mg/l,0.116 mg/l, 0.1 and 0.065 in fish gills, muscles, liver and water respectively. Meanwhile, the level of Zn in culvert tank habitat is also low and under permissible limits stated by FAO. The Zn were detected least in muscle of the fish for culvert tank habitat. The trend of the result are similar to the previous study run by Bushra, K., Hizbullah, K., Thariq, K., & Muhammad, S (2012) which is the highest concentration of Zn were found in the gills of fish.

In culvert tank habitat, Nickel were not detected in all of the part of fish, water and soil. This shows that the fish from culvert tank are totally free from Nickel contamination is safe to be consume by human. From the previous study by Abu Hilal & Ismail (2008) reported that the results of Ni concentration in fish parts are not significantly.

4.3.6 Mercury (Hg)

The presence of mercury in one water body can give huge toxicity to the system even in the least amount and threaten human life that consume the contaminated source (Oluwatosin & Edwin, 2015). The toxicity of Hg can cause physiological and histopathological changes to human (E.E Obasohan et al, 2008). However the result from the study shown the very least presence of Hg in all sample tested. The concentration of Hg detected in fish gills, muscle, liver , water and soil are 0.0000083 mg/l, 0.0000087mg/l, 0.0000047mg/l, 0.0000038 mg/l and 0.000045 mg/l respectively. This result shown least of Hg presence in the fish, water and soil and absolutely under the stated value content of Hg that allowed which is 0.00015 mg/l. So, this can be implify that soil pond are free from Hg contamination. Meanwhile for the concentration Hg in culvert tank fish the trend are same with the soil pond which is the muscle part has the highest concentration were detected. Somehow, the result also under permissible limit stated by FAO which is can conclude that catfish from both habitat are safe to be consume.

However, the result trend which is shown the high concentration of Hg are high in muscle of fish were supported by the study run by Monsefrad, Imanpour & Heidary (2012). The trend might because of the presence of metalothionin proteins in

liver with high tendency to bind with other metals meanwhile Hg has high tendency to bind with muscle proteins because melathionin has high tendency to adsorb Hg (Sigel et al, 2009).

4.3.7 The comparison of heavy metal concentration in fish gills, muscle and liver.

The heavy metals concentration in fish gills, muscle and liver are different. Figure 4.3.1 and Figure 4.3.2 shows the graph of heavy metals in gills, muscle and liver in soil pond catfish and in culvert tanks perspectively.

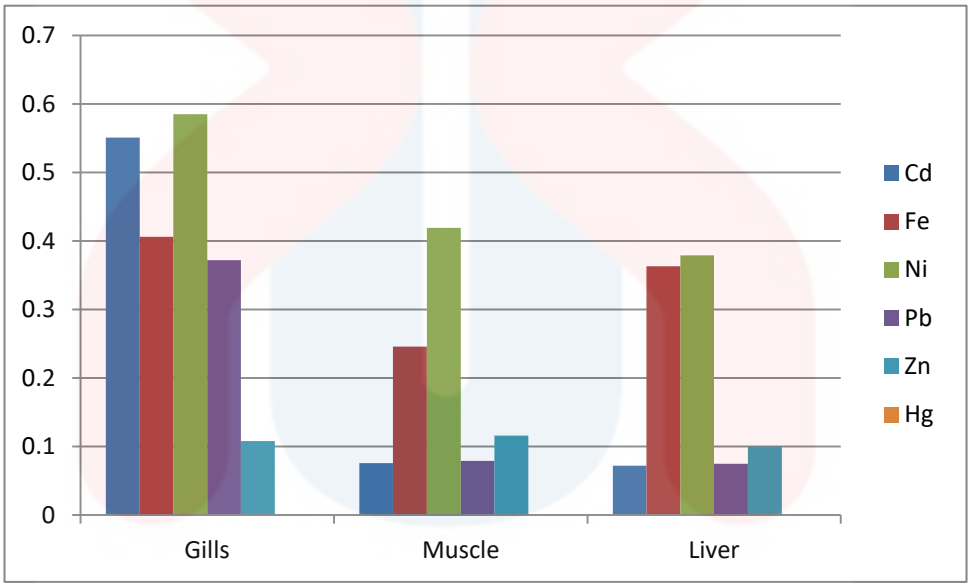

Figure 4.2 : The concentration of heavy metals in gills , muscle and liver in soil pond

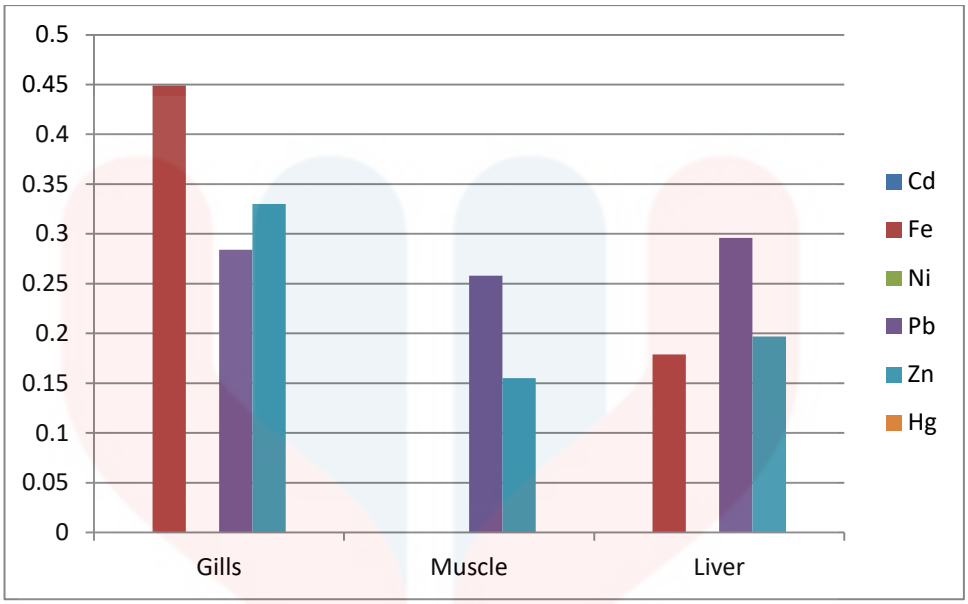

Figure 4.3 : The concentration of heavy metals in gills , muscle and liver in culvert tank

From the both figure shown, the fish organ show variety level of heavy metal concentration in each part. However, soil pond and culvert tank are having same trend of result for heavy metal concentration in the fish parts. This means that each of the parts have varieties in accumulation of heavy metal. The result in three part of fish are different with all element. Gills show the highest concentration of heavy metal in most of the element except for Hg and Zn. Gills surrounded by the epithelial membrane contain of phospholipid and mucus and contribute to has larger surface area than other parts of fish and facilitate the diffusion of toxic metal rapidly (Wepener, V., Van Vuren, J., & Du Preez, H., 2014) and gills surface that negatively charged will acts as the main route of metal ion exchange from water (KH.M *et al*, 2014). The gills are the first parts that water go through when excess into the fish body and considered as the excess point for essential and non essential substance (Vesela *et al*, 2018). Gills also acts play an important role for excretion and homeostasis of the body fluid which will trap the most toxic matter than other parts of the fish (Shovon *et al.*, 2017).

From the figure 4.3 and 4.4 show, the concentration of heavy metals in muscle significantly lower than in the gills. The highest heavy metal concentration that traced in fish muscles are Ni. But, the Ni concentration showed the highest concentration in all part of the fish. The muscles contain less of heavy metals than gills because of the heavy mostly trapped in gills. The concentrations of heavy metals in muscle of the entire examined sample were remarkably below the permissible limits of Food and Agriculture Organization (FAO), the World Health Organization (WHO). The muscle consist of It is well known that the muscle does not actively accumulate metals and seems to have a very fast decontamination rate (Vesela *et al*,2014).

Liver is the part of fish that known as active in storage and uptake of heavy metal and that metallothionein induction takes place in fish liver (Sigel *et al*.,2009). The concentration of heavy metal highest in liver is Fe. Liver of the fish species may be a very rich source of iron for human nutrition and it is normal if the precence of Fe are high as liver is an organ for storage and detoxication of metals (Heier LS *et al.*, 2009). Liver actually act as physiological substance in blood cells and haemoglobin synthesis.

4.4 Human health risk assessment.

Target hazard quotient of the each sample for each element calculated compare which one of these two place consist of higher value. The value extracted by using the THQ equation

$$THQ = \frac{(EFr \times ED \times IR \times C)}{(RfD \times BW \times AT)} \times 10^{-3} \qquad \text{Eq. (4.1)}$$

$$THQ = \frac{(365\ day/year) \times (70) \times (0.17) \times (heavy\ metal\ concentration)}{(oral reference\ dose) \times (63\ year) \times (\frac{365 days}{year} \times 70\ years)} \qquad \text{Eq (4.2)}$$

All of the calculation used the equation but the onl different in calculation are heavy metal concentration for each part and also oral reference dose for each element.

Table 4.5 shows the THQ value for each sample parts of each element for two different habitats which are soil pond and culvert tank.

4.5 : Estimated target hazard quotient (THQ) and HI for each sample from two different habitat.

Elements	Sample	Soil pond	Culvert tank
Cd	Gills	0.0001349	-
	Muscles	0.0002050	-
	Liver	0.0001943	-
Fe	Gills	0.0000016	0.0000017
	Muscles	0.0000001	-
	Liver	0.0000014	0.0000007
Ni	Gills	0.0000080	-
	Muscles	0.0000565	-
	Liver	0.0000565	-
Pb	Gills	0.0002868	0.00022
	Muscles	0.0000609	0.00020
	Liver	0.0000609	0.00023
Zn	Gills	0.0000010	0.000003
	Muscles	0.0000010	0.000001
	Liver	0.0000009	0.000002
Hg	Gills	0.000000075	0.000003
	Muscles	0.000000078	0.000043
	Liver	0.000000042	0.000009
HI (total)		0.00107	0.000731

The calculation of potential health risks for non-carcinogenic risk that consume the fish are determined by target hazard quotient associated with the consumption of fish contaminated with by Cd, Fe, Ni, Pb, Zn and Hg. The calculation of target hazard quotient involved the muscle, gills and liver. The THQ calculated for each of the heavy metal element on fish parts and then will total up called as hazard index. Hazard index is sum or total of target quotient from each element. From Table 4.4 the results present that there are no THQ values higher than 1 through the consumption of fish, suggesting that the health risks associated with heavy metals exposure are insignificant. From the result, it can be simplify that the THQ on catfishes in soil pond are below the stated value where is 1. The catfish are safe to be consumed by human and did not cause any health effect. THQ is one of the important aspects in assessing the risk to human health from potentially harmful chemicals in food is the knowledge that the dietary intake of such substances must remain within determined safe range. The same thing implies on catfish at culvert tank. The THQ value of catfishes below than 1 which is means the catfish are safe to be consume by human.

CHAPTER 5

CONCLUSION AND RECOMENDATIONS

5.1 Conclusion

Based on the result, it can be concluded that heavy metals accumulation in catfishes were result from both habitat which is soil pond and culvert tank. In addition, other environmental condition such as pH, temperature and salinity also contributed to the accumulation of heavy metal in the species. The physic-chemical properties for both habitats are suitable condition for catfish and from the result, there are no unfavourable result for both habitat. For heavy metal concentration, the level of heavy metal concentration in fish parts gills, liver, muscle, water and soil were determined by using acid digestion method and analyse by AAS. The result also shown that there are no contaminations of heavy metals in both of habitat except for the Fe in soil pond habitat where it shown quite high concentration level and exceed the permissible limit for Fe.

Heavy metal concentration results then compared to the permissible limit stated by FAO to evaluate the possible human health risk using Target Hazard Quotient. In conclusion, the element of Ni, Zn, Pb, Cd and Hg are under permissible limit stated by FAO except for the concentration of Fe where it is quite high in soil pond. Next, the heavy metal level for element in culvert tank are all below the permissible limit. However,both catfishes from soil pond and culvert tank are safe to be consume as result of THQ for both habitat are less than 1. The heavy metal

concentration in each parts of fish also under permissible limit stated by FAO. It can be implies that there are absent of non-carcinogenic effect to human health. in addition, the health risk should be taken care seriously as increase in any consumption of species will contribute to the increasing the heavy metal level in human body.

5.2 Recommendation

Throughout this study, there are a few limitation that need to be improved in future study. First of all, the study of heavy metal accumulation in fish species, should be in variation. As example, the different type of fishes may possess different type of behaviour and consume different level of heavy metal due to the physical properties of fish. The fish that can be observed are Patin, Haruan and many more as these fishes has high demand from people. The fish species can be differ in the rate of accumulation of havy metal according to their size and habitat.

In addition, another recommendation for future study is, study in different habitat of catfish which is in natural and cultivated. Habitat of fishes also one of the major contribution to heavy metal accumulation in aquatic species. Natural habitat of fishes may has different sources of heavy metals contaminant such as from industrial waste.

Besides, future study could include the other famous heavy metals that give adverse impact on human health such as arsenic, Cuprum and Manganese. There are many of previous study that include many element in their study to ensure the fishes or other living things that consume are free from heavy metal contamination and safe to be consume by human.

Next, the parameter of the parts in fish test also can be improves. In this study only muscle liver and gills were tested on their heavy metal level. So, to be significant, the other parts of fish such as skin, gonad and bones also should include for future study. So, from the result we could identify more detail on heavy metal accumulation in catfishes.

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APPENDICES

APPENDIX A: Picture of sampling site

Figure A1: Soil pond in Jeli

Figure A2 : Culvert tank in Tanah Merah

APPENDIX B : Sample preparation

Figure B1: Raw catfish sample

Figure B2 : Disected catfish

Figure B3 : Dried catfish sample

APPENDIX C: Sample analysis

Figure C1 : Sample for wet digestion

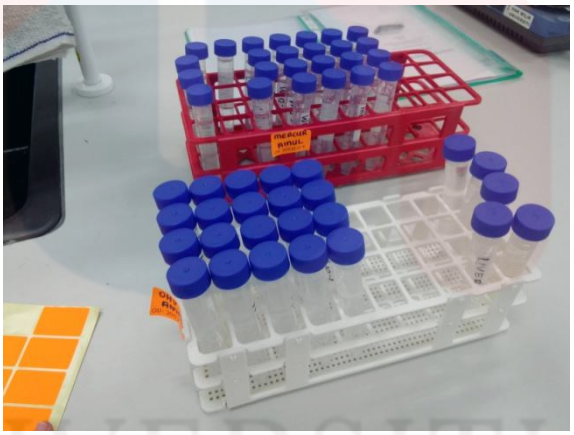

Figure C2 : Sample analysis