SELECTED HEAVY METAL CONCENTRATIONS IN *Lates calcarifer* AND WATER IN AQUACULTURE FARM FROM TUARAN, SABAH

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I hereby declare that the work submitted in this dissertation is the result of my own investigation, except where otherwise stated.

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ABSTRACT

This research was performed to determine the concentration of heavy metals (Cu, Zn, Cd, Fe and Pb) in water and *Lates calcarifer* (gills and muscle) in the aquaculture farm and to investigate the relationship between heavy metals in water and *Lates calcarifer.* The results obtained were compared with the standard permissible values in Food Act 1983. The heavy metal concentration were analysed by ICP-OES after the process of digestion. None of the metal concentration obtained exceeds the standard values in Food Act 1983. Some of the metal concentrations were undetectable in water and gills samples; Cd, Cu and Pb in water and Cd in gills. The concentration for metal content detected in water sample was between 0.0068 mg/kg to 0.0097 mg/kg Pb while there was 0.0008 mg/kg to 0.0033 mg/kg Zn. For muscle sample, Cd range from 0.0800 mg/kg to 0.1150 mg/kg; Cu range from 0.6850 mg/kg to 0.9100 mg/kg; Fe range from 22.4400 mg/kg to 109.5450 mg/kg; Pb range from 0.3900 mg/kg to 0.8685 mg/kg and Zn range from 27.7100 mg/kg to 34.9850 mg/kg. For gills sample, Cu range from 0.0700 mg/kg to 0.9850 mg/kg; Fe range from 101.6100 mg/kg to 119.7550 mg/kg; Pb range from 0.5250 mg/kg to 0.9400 mg/kg and Zn range from 75.0450 mg/kg to 83.9000 mg/kg. Bioconcentration factor calculated indicates that Pb and Zn have accumulated in the gills and muscle of the species selected while the correlation analysis also shown that the heavy metals analyzed in this study has significant effect to the water as well as to the fish species.

KEPEKATAN LOGAM BERAT TERPILIH DALAM *Lates calcarifer* DAN AIR DALAM KAWASAN AKUAKULTUR DARI TUARAN, SABAH.

ABSTRAK

Kajian ini bertujuan untuk mengenalpasti kepekatan logam berat (Cu, Zn, Cd, Fe dan Pb) di dalam air dan *Lates calcarifer* (insang dan otot) di kawasan akuakultur dan untuk mengkaji hubungan antara logam berat dalam air dan spesies *Lates calcarifer*. Keputusan yang diperolehi telah dibandingkan dengan nilai piawai yang dibenarkan dalam Akta Makanan 1983. Kepekatan logam berat telah dikaji oleh mesin ICP-OES selepas proses asid penghadaman. Tiada nilai kepekatan logam berat yang diperolehi melebihi nilai piawai dalam Akta Makanan 1983. Terdapat kepekatan logam yang tidak dapat dikesan dalam sampel air dan insang; Cd, Cu dan Pb dalam air dan Cd dalam insang. Julat kepekatan logam yang dikesan dalam sampel air adalah di antara 0.0068 mg/kg hingga 0.0097 mg/kg Pb manakala 0.0008 mg/kg hingga 0.0033 mg/kg bagi Zn. Bagi sampel otot, julat Cd yang didapati ialah antara 0.0800 mg/kg hingga 0.1150 mg/kg; julat Cu ialah antara 0.6850 mg/kg hingga 0.9100 mg/kg; Fe ialah antara 22.4400 mg/kg hingga 109.5450 mg/kg; Pb ialah antara 0.3900 mg/kg hingga 0.8685 mg/kg dan Zn ialah antara 75.0450 mg/kg hingga 83.9000 mg/kg. Faktor biokepekatan yang didapati telah menunjukkan Pb dan Zn telah terkumpul di bahagian insang dan otot *Lates calcarifer* manakala analisis korelasi menunjukkan bahawa logam berat yang dikaji dalam kajian ini memberikan kesan yang besar terhadap air dan juga spesies ikan tersebut.

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%	Percent
°C	degree Celsius
K	Kelvin
ppm	part per million
μm	micrometer
>	more than
mg/kg	milligram per kilogram
mg/L	milligram per litre
g	gram
mL	millilitre
μg/g	microgram per gram

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometer
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
FAO	Food and Agricultural Organization
FEPA	Federal Environmental Protection Agency
WHO	World Health Organization

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

Aquaculture refers to the breeding, rearing, and harvesting of plants and animals in all types of water environments including ponds, rivers, lakes, and the ocean. The report of FAO shows that world aquaculture production is continues to grow; it has reached a high quantity of production with 90.4 million tonnes in 2012 equivalent to US$144.4 billion (FAO, 2014). The fishery and aquaculture industry has well developed for contribution of fishes which has been the major source of food for humanity. According to FAO member states, aquaculture has to further develop so that it can reduce the impacts of overexploitation of aquatic populations (FAO, 2010). In 2013, China has produced 43.5 million tonnes of food fish and 13.5 million tonnes of aquatic algae (FAO, 2014). Aquaculture in Malaysia has its beginning from 1920's and it has been developing as an important industry and thus research in aquaculture is being given priority in order to ensure the quality and quantity of its production as it is an alternative source of fish supply (FAO, 2014).

There are mainly two types of aquaculture which are brackish water aquaculture and freshwater pond aquaculture. Generally, brackish water aquaculture is the predominant practice where it located mainly in the coastal water. It dominates the aquaculture industry in Malaysia and constitutes more than 70 percent of the total aquaculture production in 2003 (Anon, 2003). Bivalve molluscs are one of the dominant species in brackish aquaculture. There are also several ways to culture such as culture of marine fish in floating net-cages in lagoons and sheltered coastal waters, land-based earthen ponds, hanging method of seaweed culture and etc. Other than that, freshwater aquaculture has also constituted about 30% of total aquaculture production in 2003 (FAO, 2014). One of the culture ways is pond culture area which is now spread throughout in Malaysia (FAO, 2014). It is mainly comprised of red hybrid tilapia, hybrid walking catfish and climbing perch. Other ways of culture included is floating net-cage culture that located in lakes, reservoirs and ex-mining pool.

In recent year, the aquaculture sector has developed into a sustainable industry in Malaysia. Statistics of Department of Fisheries Malaysia showed that aquaculture production has rose 7.93% which is total of 362, 155 mt compared to 333,450 mt in 2009 (Department of Fisheries Malaysia, 2014). Most of the aquaculture production is marketed locally for domestic consumption. Some of the aquaculture production such as crabs, black tiger prawns, white leg shrimps and some freshwater fishes are exported to Singapore, China, Hong Kong, and some of it are exported in block frozen or as value added products to the EU, Japan, USA, and Australia (FAO, 2014). Hence, aquaculture is getting more important in the economic aspect due to the way it increasing fish production. This is also because it has been identified as one of the critical activities to ensure food security in Seventh Malaysia Plan (1996-2000). Generally, famous brackish-water species reared in Malaysia are sea bass, grouper, mangrove snapper and red snapper (Idris *et al.,* 2013). Among these, sea bass has generating the most income in aquaculture sector (Idris *et al.,* 2013). However, threat of diseases, food safety and quality of aquaculture produce has led to the problems to the aquaculture sector.

Hence, proper management is needed to maintain it as a renewable resource to sustain world's population. This is due to aquaculture relies on the rearing facilities and its feeding, others factors such as environmental conditions and culture techniques also determined its yield (FAO, 2010). However, aquaculture can affect the ecosystems and causes environmental problems when if it is being poorly managed. Pollution can occur when pollutants are being released and nutrient output increased which leading to eutrophication, algal blooms or red tides, heavy metals, etc. Human activities especially agricultural and industrial activities are the main cause of the pollution (FAO, 2010).

Pollution is the presence of chemicals in the environment with harmful effects. Pollutants are the chemicals causing environmental harm. Water pollution is one of the main pollutions in the environment. The effects of water pollution could be aesthetic, temperature and pH being altered, deoxygenation, toxicity and eutrophication (Harrison, 2001). Pollutant in high concentration is the main factor of pollution. Marine pollution has been getting attention because the marine environment is the important element in ecosystem which has also contributed to the food supply for human.

Marine pollution is the introduction by man, directly or indirectly, of substances or energy into the marine environment, including estuaries which results or likely to result in deleterious effects to living resources and marine life. There is several human-induced marine pollution which have caused by the discharges from land-based sources, ships, atmospheric deposition, ocean dumping and offshore oil and gas installations (Tan, 2006). Land-based sources contribute the most to the marine pollution which include industrial, domestic and agricultural wastes carried downstream into the sea. Ships discharged oil to the sea whether by intentionally or accidentally could pollute the sea and toxic to marine life (Kasmin, 2010). Substances like oil and grease, suspended solids and *Escherichia coli* (*E. coli*) had been identified as main contaminants of the coastal environment in 1995 and 1996 Environmental Quality Reports of Malaysia. Other than that, heavy metals such as mercury and lead or cadmium could be produced and discharged by industrial activities into any water body which eventually went to the sea also cause marine pollution (Kasmin, 2010).

In fact, water bodies always contain heavy metals where some of it known as trace elements such as copper (Cu), zinc (Zn), and iron (Fe) that essential for aquatic life to maintain body metabolism. However, most of the metals are toxic even at low quantity, for example arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), etc. Heavy metals can be toxic as it is metallic elements with high density. For examples, the presence of Pb can result in detrimental effect even in small amount. The natural source of heavy metals in a water body is usually from the weathering process of rocks. Heavy metal also can be come from anthropogenic source such as industrial waste and agricultural waste. Heavy metal has become the main pollutant because it cannot be removed from the aquatic environment naturally.

Consumption of contaminated fish could cause poisoning (Taweel *et al.*, 2013). The effects could cause human health problems such as renal failure, liver damage, cardiovascular diseases and even death. Thus, the metal pollution has being a problem to both aquatic system and the person who eat sea foods. There are also international monitoring program conducted to assess the quality of fish for human consumption and the health of the aquatic ecosystem (Meche *et al.,* 2010). Furthermore, Food Act 1983 and Regulation Malaysia have set the standard limit of heavy metal concentration in water and food to ensure the safety of water and food consumption.

In recent decades, health risks on food consumption getting more attention from the public. Thus, researches on heavy metal contamination also have started by some researchers as rapid economic and industrial growth in Asian has caused large scale of emission of toxicants (Agusa *et al.,* 2007). Hence, this study is going to carry out in an aquaculture site which is one of the supplies of seafood to the public consumption. Not only the concentration of heavy metal in fishes will be determined, but in water from the same site will be investigated.

1.1 Objectives of Study

There are three objectives in this project:

i. To determine the concentration of heavy metals (Cd, Cu, Fe, Pb and Zn) in water and *Lates calcarifer* (gills and muscle) in the aquaculture farm.
ii. To investigate the relationship between heavy metals in water and *Lates calcarifer* (gill and muscle).
iii. To compare the concentration of heavy metals in the fish samples with the standard limit value in Food Act 1983.

1.2 Significance of Study

Fish is the importance source of protein to human being but most of the public do not know that fish may contain heavy metal which harmful to health. Heavy metal tends to bioaccumulate in the system to high toxic levels which causing undesirable effects (Senthamilselvan *et al.,* 2012). This study is important to provide information about heavy metal (Cd, Cu, Fe, Pb and Zn) concentration contained in *Lates calcarifer* and the water in the study area.

Fishes are often at the top of the aquatic food chain which may have larger amount of metals accumulated in their body. Fish is used as the analysis samples in this study because it is the most common seafood that human consuming. *Lates calcarifer* was chosen for this study due to several reasons. They are considered an economically important fish as food in the tropical and subtropical regions in Asia-Pacific. Hence, they have high economic and ecological importance (Balakrishnan *et al.,* 2014).

Study of metals concentration in fish will be benefit majority of the individuals who lives surrounded by a water body. Five heavy metals are chose to investigate where Cu, Fe and Zn are the trace elements for living organisms while Pb and Cd are toxic metals. The study site is chose because it is one of the aquaculture seafood suppliers in the state of Sabah. Besides, concentration of heavy metals analysed from the study will compared with the permitted value in Food Act 1983 in order to identify the heavy metal level.

1.3 Scope of Study

This project will be carried out in an aquaculture area to determine the concentration of heavy metals in water and *Lates calcarifer.* It is only involved screening process which including sampling and analysis once at the study area. The concentration of (Cd, Cu, Fe, Pb and Zn) will be identified by using ICP-OES. Two samples will be taken for analysis; water sample and *Lates calcarifer* (samples of gill and muscle). *In-situ* parameters such as pH, temperature, dissolved oxygen, salinity and conductivity of the water sample will be measured during sampling process to determine whether the *in-situ* parameters may be the factors affecting the concentration of heavy metals.

The concentrations obtained from the analysis will be used to investigate the relationship between heavy metals in water and *Lates calcarifer.* Furthermore, the concentration of heavy metals in the *Lates calcarifer* will be compared with the permitted value in Food Act 1983. This will help to identify the level of concentration contained in the samples; whether it is above or below the permitted level in Food Act 1983.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metal

Heavy metals are natural constituents of every compartment of the environment. Trace metals such as cobalt, copper and zinc are essential micronutrients in metabolic system. Excessive level of trace metals can cause toxicity (Kachel, 2008). Other heavy metals such as mercury, cadmium and lead have no any benefit on living organisms and these metals are toxic even in small amount (Connel, 2005).

Generally, toxicity of metals is mostly due to bioaccumulation in organisms and the toxicity can cause acute and chronic effects (Govind and Madhuri, 2014; Kachel, 2008). The effects of metals accumulation may cause ecological damage (Sivaperumal *et al.,* 2007). The accumulations of heavy metals in marine environment are commonly found especially in those sedimentary habitats (Kachel, 2008). Govind and Madhuri (2014) have mentioned that the heavy metals stored faster than they are broken down or excreted.

Soluble complexes would form when metals combined with organic and inorganic ligands. Study of Tayab (1991) has mentioned that dissolved organic metals tend to bind stronger with organic ligands compared to inorganic ligands. In fact, ligands are the main factor to cause toxicity because heavy metals in insoluble compounds and metallic form have no toxic effect (Govind and Madhuri, 2014).

Heavy metals are non-toxic if not exceed certain limit; most of the heavy metals are essential to the growth of living organisms. The uptake of essential elements which needed by organisms are usually taken from air, water, soil, and from foods. Deficiency occurs when the uptake of essential elements is low; too much of uptake will lead to toxicity. Each organism has concentration range for each essential element to satisfy its requirements. Hence, there was an Optimal Concentration Range for Essential Elements (OCEE) for each essential element and each living organism shown in Figure 2.1.

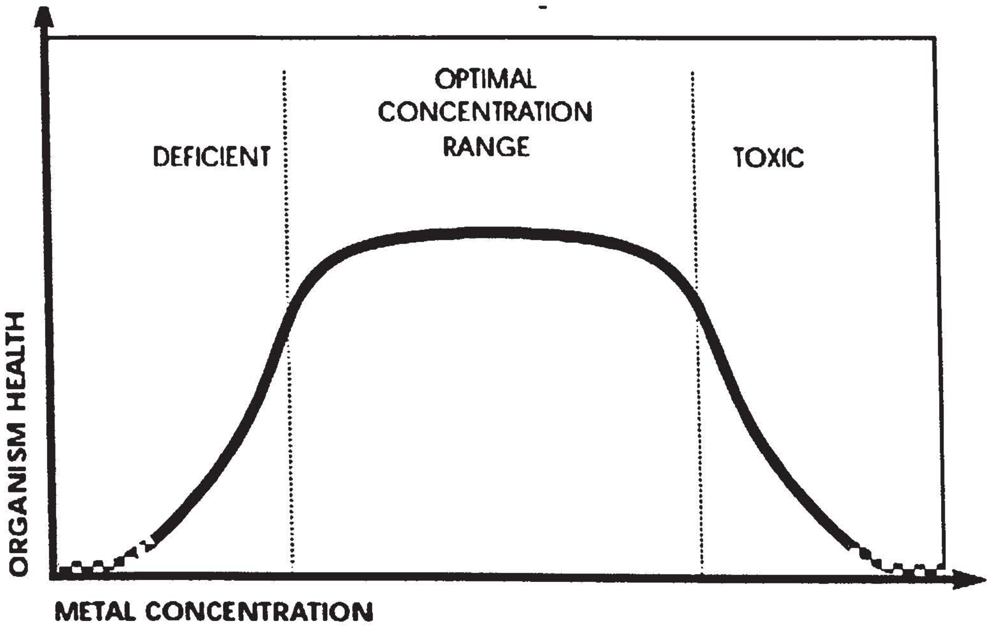

Figure 2.1 Optimal Concentration Range for Essential Elements (OCEE) (Source: Van Assche, 1997)

2.2 Sources and Effects of Heavy Metal

It can be formed through natural weathering processes such as erosion or dissolution, and as a substance that produced by human activities (Tayab, 1991). The metals can transform through precipitation, oxidation or reduction when it entered into aquatic system (Tayab, 1991). The major source of heavy metal is from anthropogenic sources and mining operation is known as the main cause (Duruibe *et al.,* 2007). This is because the metals emitted from mining operation tend to persist in the environment causes metal contamination. Leaching of the metals will be carried by rainwater or runoff to the sea (Duruibe *et al.,* 2007).

An evaluation study of toxic metals on the haematology and DNA damage has been done on this species of fish (Senthamilselven *et al.,* 2012). The results obtained indicated a significant gradual damage of haemoglobin (-35.01 to -24.11) and haematocrit (-35.01 to -31.78) at acute concentration of Cd plus mercury (3.0 ppm) as compared to that control. Thus, this study has proved the presence of harmful effects to fish (Senthamilselven *et al.,* 2012). Industrial wastes, geochemical structure, mining of metals, urban runoff, sewage treatment plants, bating activities, domestic garbage dumps and agricultural fungicide runoff are some of the potential sources of heavy metal pollution to aquatic environment (Alina *et al.,* 2012; Santos *et al.,* 2005). It has been observed that metals that entered into body would lead to cellular level damage, thus it will possibly affect the ecological balance (Govind and Madhuri, 2014).

2.3 Heavy Metals Selected

Generally, heavy metals which are commonly found in fish are potassium, copper, chlorine, phosphorus, calcium, iodine, iron, zinc, manganese, mercury, lead and cadmium (Connel, 1984). Among the heavy metals mentioned, arsenic, cadmium, chromium, lead and mercury are toxic even in a small amount while the others are essentials metals (Irwandi and Farida, 2009). The five heavy metals selected in this study are copper (Cu), zinc (Zn), cadmium (Cd), iron (Fe) and lead (Pb). These heavy metals were chosen because they might be toxic to the fish in high concentration; it could be toxic to human as well as if human consume fish as food (Akan *et al.,* 2012). There are several studies including Akan *et al.* (2012); Bashir *et al.* (2012); El-Moselhy *et al.* (2014) that have had chosen these five metals in their study.

2.3.1 Copper

Copper, the symbol is Cu, with atomic number 29 and atomic weight 63.546. In periodic table, Cu is located in group 11. It is categorized as transition metals same as silver and gold as copper share many characteristics with them. The electron structure of Cu is responsible for its physical properties (Devis, 2001). Cu has high electrical conductivity, chemical stability, and its reddish colour. The ions of Cu include cuprous ion (Cu^{+}) and cupric ion (Cu^{2+}). The melting point of Cu is 1084.88 °C while boiling point is 2595 °C to 2567 °C. Copper is insoluble in both hot and cold water, only slightly soluble in hydrochloric acid and ammonium hydroxide, soluble in nitric acid and hot sulphuric acid (Devis, 2001).

Cu is an essential trace element and a co-factor in many enzyme reactions (Moore *et al.*, 1997). Moore *et al.* (1997) mentioned that there are 75, 000 tonnes of copper is released in to the environment. In this regards, majority of copper is come from anthropogenic sources and only a quarter of it is from natural sources (Moore *et al.,* 1997). The anthropogenic sources include human activities, industrial and agricultural process. Copper is also known as a micro-nutrient which essential for growth of living organisms.

Although Cu is an essential element, it can be toxic when exposures exceed the physiological needs (Moore *et al.*, 1997). Cu is necessary for the synthesis of haemoglobin (Osman and Kloas, 2010). High concentration of Cu cause toxicity as it also may combine with contaminants like ammonia, mercury and zinc which produce addictive toxic effect on fish (Yacoub, 2007). The common features reported are metallic taste in mouth, gastric pain, headache, nausea, dizziness and diarrhoea with massive gastrointestinal bleeding (Moore *et al.,* 1997).

2.3.2 Zinc

Zinc has atomic number of 30 and atomic weight 65.38. The symbol of zinc is Zn. In periodic table, zinc located in group 12 called as transition metal. The melting point of Zn is 419.53 °C while the boiling point is 920 °C (Dalal, 1994). Zn is a greyish white metal which has good conductor of heat and electricity. It will brittle at ordinary temperatures, malleable and ductile at around 120 °C and brittle at beyond 200 °C (Dalal, 1994). Zn has two common oxidation states which are +1 and +2.

Generally, Zn is produced both from ores and from recycled Zn product (IZA, 1997). Zn formed from the natural erosion processes such as weathering and abrasion of rock, soils and sediments by wind and water. Besides, a significance amount of Zn will be transported in the environment through volcanic eruptions, forest fires and aerosol formation above sea. Zn is mostly produced by mining smelting and sewage disposal.

Zn is an essential element for all living organisms due to its bioavailability and specific role in various biological reactions (IZA, 1997). Zn acts as an activator of numerous enzymes present in the liver; hence it will always found high concentration in liver of fish. Zn can be toxic in too high concentration.

2.3.3 Cadmium

Cadmium is a chemical element with symbol Cd. The atomic number of Cd is 48 while its atomic weight is 112.414. In periodic table, it is located in group 12, transition metal. The melting point of Cd 321.07 °C and the boiling point is 767 °C. Cd has two common oxidation states which are +1 and +2 but almost all compounds of cadmium are +2 (IARC, 1993). Cadmium chloride and cadmium sulphate are soluble in water (ATSDR, 2012).

Generally, Cd is emitted from non-ferrous metal mining and refining, manufacture and application of phosphate fertilizers, fossil fuel combustion, waste incineration and disposal. When Cd enters the environment, it can accumulate in aquatic organisms and agricultural crops (ATSDR, 2012).

Cd is a soft, light-coloured metal with high vapour pressure causing it to be rapidly oxidised to cadmium oxide in air. Cd is highly toxic non-essential heavy metal and it does not have a role in biological processes in living organisms. It is toxic even at low concentration. The occurrence of Cd in the marine aquatic environment is usually found in only trace concentrations (ATSDR, 2012). Toxicity of Cd may harmful to some organs such as kidney, lung, bones, brain and also central nervous system (Castro-González and Méndez-Armenta, 2008). Intake of food with high Cd levels can cause severe irritation in stomach, leading to vomiting and diarrhea or even death (ATSDR, 2012). Accumulation of Cd over a long period in kidneys will cause the damage of kidneys. Long period of exposure to low levels of Cd also can cause bones to become fragile (ATSDR, 2012). Furthermore, Cd has been determined as a probable human carcinogen by EPA.

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