ASSESSMENT OF HEAVY METALS IN CULTIVATED FISH *(CHANNA STRIATUS AND OREOCHROMIS NILOTICUS)* FROM AGROPARK UMK, JELI, KELANTAN

by

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DECLARATION

I declare that thesis entitled "Assessment of Heavy Metals in Cultivated Fish (*Channa striatus and Oreochromis niloticus*) from Agropark UMK, Jeli, Kelantan" 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 :

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Date : 28/2/2024

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Assessment of Heavy Metals in Cultivated Fish *(Channa striatus and Oreochromis niloticus)* from Agropark UMK, Jeli, Kelantan

ABSTRACT

Heavy metal pollution has become a major problem that must be addressed. Heavy metal pollution may have direct effects on humans through the food chain as it affects aquatic organisms. By virtue of their position in the aquatic food chain, fish can acquire metals from any sediment, water, or food they meet. Muscle tissues are metabolically active tissues that tend to accumulate large amounts of heavy metals, and this may be important information for this study to provide additional information on environmental health hazards in the studied species. Thus, the objectives of this study are to determine the concentration of metals (Cd, Cu,Pb, and Zn) in *Channa striatus* and *Oreochromis niloticus* and to analyse the possible health risks associated with the accumulation of heavy metals through the consumption of the species by using the Target Hazard Quotient (THQ). Heavy metals concentration in fish samples has been analysed by using Atomic Absorption Spectrophotometer (AAS). The results showed that the concentration of heavy metals such as Cd, Cu, andPb in fish tissue in both species, namely *Channa striatus* and *Oreochromis niloticus*, could not be detected, while only the concentration of Zn could be detected, but was still below the set limit. The findings indicate a heavy metal concentration of Zn of 0.26 mg/kg for *Channa striatus* and 0.18 mg/kg for *Oreochromis niloticus*). The results of the study show that the heavy metal content contained in the fish in the Agropark UMK Jeli pond is very little because of the abundant *Hydrilla verticillata* growth in the pond. Heavy metals in fish might give more helpful information for the monitoring of heavy metals in the aquatic environment and are vital for the improvement of human health.

Penilaian Logam Berat Dalam Ikan Ternakan *(Channa striatus dan Oreochromis niloticus)* dari Agropark UMK, Jeli, Kelantan

ABSTRAK

Pencemaran logam berat telah menjadi masalah utama yang mesti ditangani. Pencemaran logam berat mungkin mempunyai kesan langsung kepada manusia melalui rantai makanan kerana ia menjejaskan organisma akuatik. Berdasarkan kedudukannya dalam rantai makanan akuatik, ikan boleh memperoleh logam daripada sebarang sedimen, air ataupun makanan. Tisu otot adalah tisu aktif secara metabolik yang mempunyai kecenderungan untuk mengumpul sejumlah besar logam berat, dan ini mungkin maklumat penting untuk kajian ini untuk memberikan maklumat tambahan tentang bahaya kesihatan alam sekitar dalam spesies yang dikaji. Dengan itu, objektif kajian ini adalah untuk menentukan kepekatan logam (Cd, Cu, Pb, dan Zn) dalam *Channa striatus* dan *Oreochromis niloticus* dan untuk menganalisis kemungkinan risiko kesihatan yang berkaitan dengan pengumpulan logam berat melalui penggunaan spesies dengan menggunakan Target Hazard Quotient (THQ). Kepekatan logam dalam sampel ikan telah dianalisis dengan menggunakan Atomic Absorption Spectrophotometer (AAS). Keputusan menunjukkan bahawa kepekatan logam berat seperti Cd, Cu, dan Pb dalam tisu ikan dalam kedua-dua spesies iaitu *Channa striatus* dan *Oreochromis niloticus* tidak dapat dikesan, manakala hanya kepekatan Zn yang dapat dikesan, tetapi masih di bawah. had yang ditetapkan. Penemuan menunjukkan kepekatan logam berat Zn iaitu 0.26 mg/kg untuk *Channa striatus* dan 0.18 mg/kg untuk *Oreochromis niloticus*. Hasil kajian menunjukkan kandungan logam berat yang terkandung dalam ikan di kolam Agropark UMK Jeli adalah sangat sedikit hasil daripada pertumbuhan *Hydrilla verticillata* yang banyak dalam kolam tersebut. Oleh itu, logam berat dalam ikan bolehmemberikan maklumat yang lebih berguna untuk pemantauan logam berat dalam persekitaran akuatik dan penting untuk peningkatan kesihatan manusia.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
Cd	Cadmium
Cu	Copper
ED	Exposure Duration
EDI	Estimate Daily Intake
FAO	Food and Agriculture Organization
g	Gram

LIST OF SYMBOLS

°C	Degree Celsius
%	Percentage
±	Plus-minus
<	Less than
×	Multiply

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Heavy metals are metal compounds that are harmful to human health. Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing importance for ecological, evolutionary, nutritional, and environmental reasons (Jaishankar et al., 2014). Human exposure to heavy metals has become a hot issue, in part because of their widespread prevalence. The use of a wide spectrum of metals in industry and our daily lives has caused a significant increase in metal pollution in the environment (Mahurpawar, 2015).

Due to the feeding habits of fish, fish in water contaminated with metals are exposed to toxicological problems (Malik et al., 2010; Morshdy et al., 2019; Ajibare et al., 2022). Exposure of fish to contaminants has several harmful effects on quality, diversity, and human health (Nyantakyi et al., 2021). Heavy metals can enter through the gills, digestive tract and body surface or scales. The body surface is generally assumed to play a minor role in the uptake of heavy metals by fish while the gills are an important site for direct uptake from the water. The liver was analysed because this organ tends to accumulate metals and is involved in detoxification (Selda & Nurşah, 2012). Fish can accumulate heavy metals in their tissues, for this reason they can be used in estimating the pollution status in aquatic ecosystems (Nyantakyi et al., 2021). In addition, the toxic effects of heavy metals can affect fish growth rates, physiological functions, mortality, and reproduction, among other aspects of fish biology(Afshan et al., 2014). Therefore, this study will provide data about the study fish and ascertain the risk in eating it.

1.2 Problem Statement

Heavy metal pollution is a major environmental problem that affects both fish and humans. Heavy metals such as mercury, lead, cadmium, and arsenic are released into Malaysian water bodies because of industrial activities, improper waste management, and agricultural runoff (Department of Environment Malaysia, 2019). Heavy metal exposure changes fish physiology and behavior, causing poor growth, reproductive problems, and impaired immune function (Suresh et al., 2020). These negative impacts not only harm fish populations, but also have far- reaching implications for human health. Humans become susceptible to heavy metaltoxicity when they ingest polluted fish, which can lead to major health problems. Chronic mercury exposure, for example, has been related to neurological illnesses, damage to the kidneys, cardiovascular disease, and developmental abnormalities in children (World Health Organization, 2021). Heavy metal pollution's increasing prevalence necessitates prompt attention and the adoption of appropriate measures to limit its effects, maintain aquatic ecosystems, and protect human well-being. Therefore, heavy metals in fish can provide more useful information for heavy metal monitoring in the aquatic environment and is important for the improvement of human health.

1.3 Objectives

The study focuses on the following objectives:

i. To determine the concentration of heavy metals such as Lead (Pb), Zinc (Zn), Cadmium (Cd), and Copper (Cu) in the *Channa striatus* and *Oreochromis niloticus.*

ii. To analyse the possible health risk associated with heavy metal accumulation via consumption of that species by using Target Hazard Quotient (THQ).

1.4 Scope of Study

In this research, heavy metals accumulated in the tissues of *Channa striatus* and *Oreochromis niloticus* were the main emphasis. *Channa striatus* and *Oreochromis niloticus* were caught from the Agropark pond at UMK Jeli. Fish samples were digested according to the wet digestion method. In risk and safety assessment, Target Hazard Quotient (THQ) and Estimated Daily Intake (EDI), have been used in risk and safety assessment to measure the severity of possible hazards.

1.5 Significance of Study

After the development and cow farming near the pond in Agropark, UMK Jeli may be facing various issues of water pollution. Heavy metal pollution in freshwater systems, increase day by day which always spread in the mass media. Thus, this study can provide information about the presence of heavy metal concentrations *in Channa striatus* and *Oreochromis niloticus* from the Agropark pond, UMK Jeli. In addition, risk assessment was conducted as part of this research to determine the potential health effects and provide information about the safety of fish consumption.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metal Pollutions

Heavy metal pollution in Malaysia has been a growing environmental concern, driven by rapid industrialization and urbanization. Several studies have documented the presence of elevated levels of heavy metals in various environmental compartments, including air, water, and soil. For instance, research conducted by Mustafa et al. (2018) highlighted the contamination of rivers with metals such as cadmium, lead, and copper, attributed to industrial and agricultural activities. Additionally, investigations by Mohamad et al. (2019) emphasized the accumulation of heavy metals in agricultural soils due to the use of contaminated water for irrigation and the application of metal- laden fertilizers. These findings underscore the urgent need for effective monitoring, regulation, and remediation strategies to mitigate the adverse impacts of metal pollution on ecosystems and human health in Malaysia. According to Navas-Acien et al. (2007) and Satarug et al. (2017), there is no acceptable level of exposure to non-essential metals like lead (Pb) and cadmium (Cd), which are harmful to human health. Since non-essential metals are not required for metabolic functions, these metals are toxic even in small concentrations (Patrick, 2006). According to Burger and Gochfeld, (2011), the concentration of heavy metals can harm human health if consumed by eating polluted aquatic species. Heavy metals can have an impact on aquatic life and are harmful even in very tiny amounts (Wang et al., 2016; Poynton & Nugegoda, 2018). Consequently, heavy metal pollution may influence the ecosystem's equilibrium and aquatic life (Borgmann & Norwood, 2004).

2.2 Profile Summaries of Heavy Metal

2.2.1 Lead (Pb)

Lead (Pb) is a very poisonous heavy metal that presents serious concerns to both human health and the environment. It is a dense, bluish-white metal with a low melting point (Patra et al, 2011). In the past, lead has been extensively employed in a variety of sectors, including construction, batteries, weapons, soldering supplies, and pigments (Koller et al, 2004). However, significant studies have shown that exposure to lead can have negative impacts. Pb is known to have negative effects on several bodily systems, including the neurological, cardiovascular, renal, and reproductive systems (Needleman et al., 2002). Particularly in children, it may result in neurodevelopmental difficulties, cognitive deficits, lower IQ, behavioral problems, anemia, and renal damage (Lanphear et al., 2005). Lead poisoning often occurs when individuals ingest food or water that has been contaminated with lead. Lead poisoning can also arise due to the unintentional ingestion of lead-based paint, dust, or contaminated soil (Wani et al., 2015).

2.2.2 Zinc (Zn)

Zinc (Zn), a crucial trace element, is involved in several physiological processes in the human body. It is a bluish-white metal with strong corrosion resistance and a low melting point (Prasad, 2013). According to Wessells and Brown (2012), zinc is required for the immune system to grow, expand, and function effectively. It affects the preservation of adequate taste and smell perception, DNA synthesis, cell division, wound healing, and enzymatic reactions (Haase & Rink, 2009). Zinc deficiency can cause reproductive issues, immune system weakness, delayed wound healing, and developmental retardation (Shankar & Prasad, 1998). However, excessive zinc consumption can have detrimental health consequences, such as digestive difficulties and decreased copper absorption (Vallee & Falchuk, 1993).

2.2.3 Copper (Cu)

Copper (Cu) is required for several physiological activities in the human body. Prohaska (2014) defines it as a reddish-brown metal with strong thermal and electrical conductivity. According to Leder (1991), copper is required for the formation of connective tissue, energy generation, enzyme function, and antioxidant activity. It is also necessary for neurotransmitter production, cardiovascular system maintenance, and iron metabolism (Crichton, 2014). According to Brewer (2015), a copper deficiency can result in skeletal deformities, immunological dysfunction, and hematological abnormalities. Excessive copper consumption, on the other hand, canbe hazardous and cause gastrointestinal difficulties, liver damage, and neurological disorders (Scheinberg & Sternlieb, 2006).

2.2.4 Cadmium (Cd)

The presence of metals in fish has garnered considerable research interest due to the potential risks they pose to human health. Mercury (Hg), lead (Pb), and cadmium (Cd) are among the key metals that have been extensively studied in relation to fish. Numerous investigations have highlighted the ability of these metals to accumulate in fish tissues through various pathways, including water, sediments, and the food they consume.

Cadmium (Cd) is a hazardous heavy metal that exists naturally in the environment but is also released into the environment via various industrial activities (Bernard, 2008). It is well-known for its carcinogenic qualities and negative effects on a variety of organ systems, including the kidneys, bones, and respiratory system (Nordberg et al., 2007). Consumption of contaminated food, particularly crops cultivated in cadmium-affected soil, as well as inhalation of cadmium-laden dust and fumes, are the primary routes of human exposure to cadmium (Nawrot et al., 2010). Chronic cadmium exposure has been linked to an increased risk of kidney dysfunction, osteoporosis, cardiovascular disease, and some malignancies, including lung and prostate cancer (International Agency for Research on Cancer, 2012; Järup et al., 1998). Strict limits on industrial emissions, monitoring of food and water sources, and the promotion of safer manufacturing practices are all efforts to reduce cadmium exposure (European Food Safety Authority, 2009).

2.3 Metals in Fish

The presence of metals in fish has garnered considerable research interest due to the potential risks they pose to human health. Mercury (Hg), lead (Pb), and cadmium (Cd) are among the key metals that have been extensively studied in relation to fish. Numerous investigations have highlighted the ability of these metals to accumulate in fish tissues through various pathways, including water, sediments, and the food they consume.

Cadmium (Cd), a hazardous metal, can accumulate in fish tissues and pose health risks to consumers. Storelli et al. (2013) conducted a study examining cadmium contamination in fish species from the Adriatic Sea and found elevated concentrations. The consumption of fish with high levels of cadmium can have adverse health effects, underscoring the importance of monitoring and regulating cadmium in seafood. Similarly, arsenic (As), a metalloid, can be present in fish due to environmental contamination. Queiroz et al. (2019) focused on heavy metals accumulation and speciation in fish from the Amazon Basin, emphasizing the potential health risks associated with exposure through fish consumption. Other studies conducted by Signes-Pastor et al. (2016) and López-Alonso et al. (2020) have also highlighted the presence and toxicological significance of cadmium in fish, further highlighting the need for ongoing monitoring and management of cadmium levels in seafood.

Nevertheless, it is important to consider the levels of heavy metals in fish muscle to determine safe consumption amounts. Excessive ingestion of heavy metals can lead to toxic reactions in the human body (Copat et al., 2012). Fish as Bioindicator Fish are highly susceptible to the presence of pollutants in their aquatic environments, as they cannot avoid them due to their reliance on food and habitat (Yarsan et al., 2013). They serve as important indicators of metal pollution levels in aquatic systems

(Authman, 2008). Heavy metals are widespread in various environments, both natural and human-made, and can enter aquatic habitats through sources such as wastewater treatment facilities, atmospheric deposition, and geological weathering (Maier et al., 2014).

Since fish are positioned at the end of the aquatic food chain, they can accumulate metals and transfer them to humans through the consumption of contaminated fish, potentially leading to chronic or acute diseases (Al-Yousof et al., 2000). The long-term persistence, toxicity, bioaccumulation, and biomagnification of heavy metal and metalloid pollution in water and sediment highlight the significance of this issue (Maier et al., 2014). Fish as bio monitors play a crucial role in aquatic systems by providing valuable information about the characteristics of natural aquatic systems and monitoring changes in their habitats (Authman et al., 2015).

2.4 Candidate Species

2.4.1 *Channa striatus*

According to Ab Wahab et al. (2015), *Channa striatus,* commonly known as the snakehead fish or Haruan, is a freshwater species found in Southeast Asia as shown in Figure 2.1. Exposure to heavy metals, including lead (Pb), cadmium (Cd), mercury (Hg), and chromium (Cr), can have detrimental effects on the physiology and overall health of *Channa striatus* (Mohanta et al., 2017; Hasan & Hashim, 2019). Aquatic environments can become contaminated with heavy metals through industrial discharges, agricultural runoff, and improper waste disposal, posing a risk of exposure for *Channa striatus* as a resident species in freshwater ecosystems (Mohanta et al., 2017). When *Channa striatus* is exposed to heavy metals, these contaminants have the capacity to accumulate in the fish's tissues, particularly in the liver, kidney, and gills, resulting in organ dysfunction and disruption of metabolic processes (Pandey et al., 2018). This accumulation leads to oxidative stress, as heavy metals generate reactive oxygen species within the fish's body, causing cellular damage, lipid peroxidation, DNA damage, and alterations in antioxidant defense mechanisms (Pandey et al., 2018; Chakraborty & Selvaraj, 2019).

Furthermore, heavy metal exposure can adversely affect the reproductive system of *Channa striatus*, including reproductive behavior, hormonal regulation, fertility, egg hatchability, and the development of offspring, potentially resulting in reduced reproductive success and deformities (Pandey et al., 2017; Chakraborty & Selvaraj, 2019). In addition to the direct effects on *Channa striatus*, heavy metal contamination in the aquatic environment can impact the food chain, leading to the bioaccumulation and biomagnification of heavy metals in *Channa striatus*. As a predatory fish, *Channa striatus* may consume smaller fish or organisms that have already accumulated heavy metals, resulting in higher concentrations of these 12 contaminants in its tissues (Mohanta et al., 2017). Heavy metal exposure poses a significant threat to the health and survival of *Channa striatus*, disrupting physiological processes, causing oxidative stress, impairing reproduction, and contributing to the accumulation of heavy metals in the fish's tissues. Proper management of industrial and agricultural activities, along with the monitoring and remediation of heavy metal pollution, are crucial to safeguard the populations of *Channa striatus* and maintain the ecological balance of freshwater ecosystems (Mohanta et al., 2017; Hasan & Hashim, 2019).

Figure 2.1*:* *Channa striatus*

2.4.2 *Oreochromis niloticus*

Nile tilapia (*Oreochromis niloticus*) is a widely distributed freshwater fish species found in various regions, including Africa, Asia, and the Middle East (Figure 2.2). Exposure to heavy metals such as lead, cadmium, mercury, and chromium can have significant detrimental effects on the physiology and overall well-being of *Oreochromis niloticus* (Sivaperumal et al., 2007; Nwani et al., 2010). Aquatic environments can become contaminated with heavy metals through activities such as industrial discharges, mining, agriculture, and improper waste disposal. *Oreochromis niloticus*, being a resilient species in freshwater ecosystems, can meet these heavy metals and accumulate them in their tissues (Sivaperumal et al., 2007; Oladimeji & Ogunlela, 2014).

Exposure to heavy metals can lead to various physiological and biochemical changes in *Oreochromis niloticus*. These metals can accumulate in organs such as the liver, kidney, gills, and muscles, resulting in impaired organ function, disruption of metabolic processes, and damage to cellular structures (Sivaperumal et al., 2007; Nwani et al., 2010). The presence of heavy metals triggers the generation of reactive oxygen species (ROS) within the fish's body, leading to oxidative damage to lipids, proteins, and DNA. This oxidative stress can disrupt cellular function, induce inflammation, and impair antioxidant defense mechanisms (Sivaperumal et al., 2007; Oladimeji & Ogunlela, 2014).

The immune system of *Oreochromis niloticus* is also affected by heavy metal exposure, resulting in suppressed immune responses and increased vulnerability to diseases and infections (Adeogun et al., 2016). These impacts can have negative implications for the overall health, growth, and survival of the fish (Nwani et al., 2010; Tung et al., 2018). Heavy metal contamination in the aquatic environment can further disrupt the reproductive system of *Oreochromis niloticus*, affecting reproductive behavior, hormonal regulation, and reproductive success. Reduced fertility, abnormal development of reproductive organs, and decreased hatchability of eggs are potential consequences of heavy metal exposure (Adeogun et al., 2016; Tung et al., 2018).

In terms of food safety, the accumulation of heavy metals in *Oreochromis niloticus* raises concerns for human health if consumed. As a popular aquaculture species, *Oreochromis niloticus* may be consumed by humans, and the presence of heavy metals in their tissues can pose risks to consumers (Sivaperumal et al., 2007; Adeogun et al., 2016). To mitigate the adverse effects of heavy metal exposure, it is crucial to implement effective management practices and regulations to minimize heavy metal pollution in aquatic environments. Monitoring programs, assessments of water quality, and remediation strategies play vital roles in safeguarding *Oreochromis niloticus* populations and ensuring the safety of both the fish and human (Nwani et al., 2010; Adeogun et al., 2016).

Figure 2.2: *Oreochromis niloticus*

2.5 Human Health Risk Assessment

Due to the rapid growth of industry and agriculture, heavy metal pollution poses a significant hazard to invertebrates, fish, and humans (Jones et al., 2001). Suspended sediments present in the water column have the capability to adsorb contaminants, and disturbing these sediments can release otherwise inert heavy metals into the water, posing a threat to fish species (Bryan et al., 1996; Campbell et al., 2006). Additionally, bottom sediments not only serve as a refuge but also a source of food for benthic organisms, making them vulnerable to the toxic effects of pollutants (Azim et al., 2008; Emery et al., 2017). The toxins present in the aquatic environment can harm species both directly and indirectly (Gilliom et al., 1995, Rocha et al., 2018). Moreover, due to the bioaccumulation and bioconcentration of pollutants in the food chain, these contaminants can also be detected on land, further amplifying the risk (Fisher et al., 2003; Luoma, 2008). To mitigate the risk to human health, it is crucial to control and regulate the levels of metals in fish, as this can help reduce the potential harm posed to individuals who consume them (Bose-O'Reilly et al., 2018).

CHAPTER 3

METHODOLOGY

3.1 Research Flowchart

This chapter outlines the methodology used in the research. The methodology can be found in figure 3.1. The method was confirmed using standard reference materials. Analyses were conducted using wet digestion. Safety and risk assessments were conducted on fish samples.

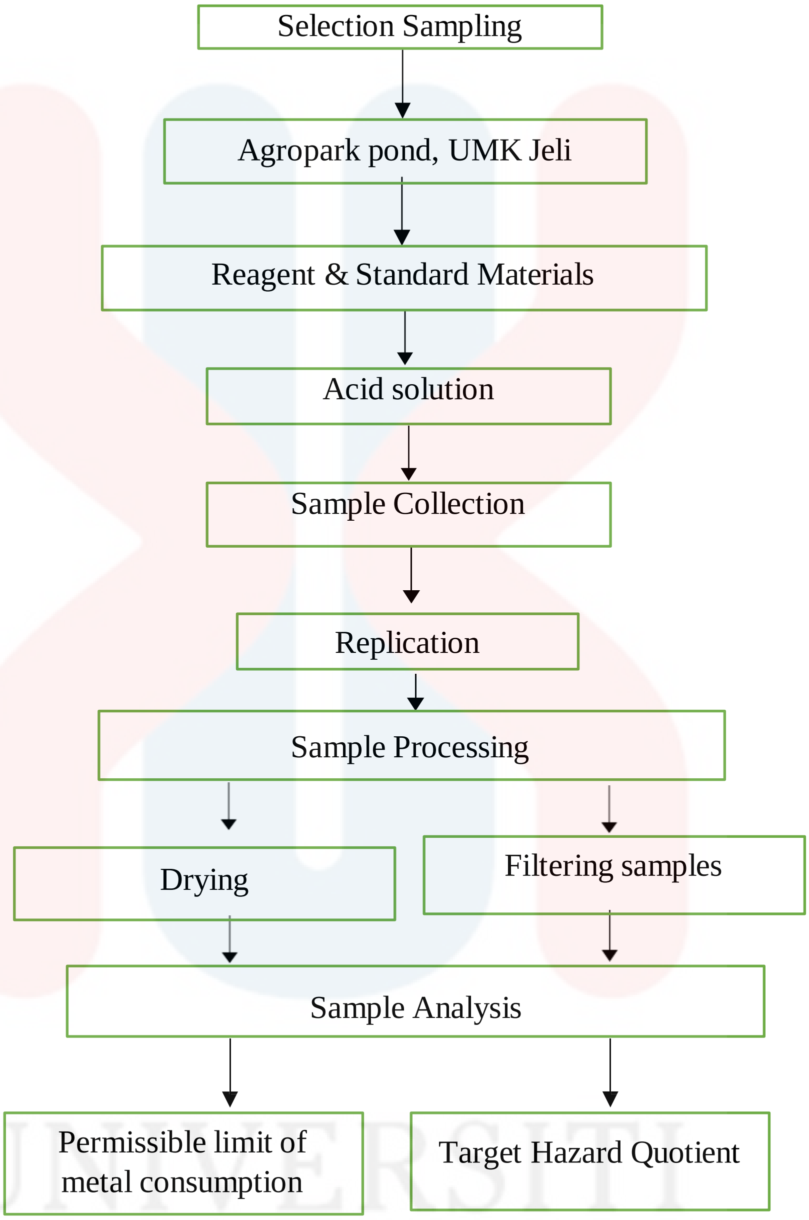

Figure 3.1: Research Flowchart

3.2 Study Area

The Agropark UMK Jeli Campus ponds, situated near the Faculty of Earth Sciences (FSB) building in UMK Jeli Campus, serve as the sampling location for this study. Managed by the Faculty of Agro Based Industry (FIAT), the Agropark ponds are a ground-type freshwater fishpond created by excavating the land for fish farming purposes. Unlike commercial fish farms that employ mechanical aeration systems, these ponds rely on natural aeration processes. An intriguing aspect to investigate regarding heavy metals in fish within the Agropark ponds is the proximity of animals such as cows that are kept near the pond area. This proximity introduces the potential for interactions between animal waste and the pond environment, which could influence heavy metal concentrations in the fish. The place can be found by using the coordinates of 5°44'49"N 101°52'04"E.

Figure 3.2: Maps of study area Agropark pond UMK Jeli(Source: Google Earth

3.3 Reagents and Apparatus

Nitric acid (HNO_3), dried fish samples, beaker, magnetic stirrer, and hot plates had been used in the wet digestion before moving on to the analysis process.

3.4 Sample Collection

Channa striatus and *Oreochromis niloticus* were captured from Agropark UMK pond. The samples were caught about 3 for each species using two methods, namely by using nets and fishing. Fish samples were individually packed in polyethylene sampling bags, stored in an icebox, and transported to the laboratory on the same day. The total length and weight of the fish were recorded before being stored below -20°C until surgery could be performed. Length was measured as the distance from the tip of the snout to the tip of the tail fin. The fish samples were then be analysed in the laboratory of the Faculty of Earth Sciences, Universiti Malaysia Kelantan.

3.5 Sample Pre-treatment

A dissecting kit and deionized water were used to extract muscle tissue from partially thawed fish samples. The purpose of taking the muscle tissue of the fish was because people ate muscle tissue rather than other parts. This research objective was to analyse the effects on human health if there are the existence of heavy metals in muscle tissue in both *Channa striatus* and *Oreochromis niloticus* in Agropark pond UMK Jeli. The samples were dried overnight at a temperature of 110 °C to ensure that the weight of each sample was the same. To increase the uniformity of the dissected samples, each sample was ground in a mortar and pestle. After that, the samples were filtered through a 35mm sieve and then stored in a desiccator in an amber jar until wet digestion was done.

3.6 Digestion of Fish

The wet digestion process was required by the analytical method for flame atomic absorption spectrophotometer (AAS). About 2.0 g of fish sample was put into the beaker. An analytical grade solution of HNO_3 (69 % v/v) was added to the beaker at this point. A magnetic stirrer and an observation glass were placed in a beaker, which was placed on a hot plate. For the first hour, a temperature of 40°c was maintained to avoid a rapid reaction. The temperature continued at 140 °C for the next three hours. All tissue samples were dissolved by acid at the end of digestion. After cooling to normal temperature, the mixture was ready for use. Approximately 250 ml volumetric flasks were half-filled with double- fluted flasks to facilitate the detection of heavy metals by Atomic Absorption Spectroscopy (AAS). The samples were injected into a 15 ml centrifuge tube with a 40 m filter used filter paper and a syringe. Samples were stored at 4°C until AAS metal analysis was completed (Plesswas, 2012). Even the second type of fish was also subject to the same treatment.

3.7 Target Hazard Quotient

The target hazard quotient (THQ) is an estimate of the level of (non-carcinogenic) risk from exposure to a pollutant. To estimate the human health risk from consuming metal-contaminated fish, the target hazard quotient (THQ) was calculated according to the USEPA Region III Risk-Based Concentration Table (Amirah et al., 2013).

$$THQ = [(Ef \times Ed \times QMC \times C) / (RfD \times BW \times Et)] \times 10{-3} \qquad \text{(Eq.3.1)}$$

The equation used for estimating THQ was as follows where, EF is the exposure frequency; 365 days/year, ED is the exposure duration; 70 years (Bortey-Sam et al., 2015), FIR is the fish ingestion rate; fish: 71 g/day per person (Łuczyńska et al., 2022), C is the metal concentration in muscle of studied fish; edible fish part (mg/kg; wet weight), RfD is the oral reference dose (Amirah et al., 2013) as stated in (Table 3.7), WAB is the average body weight; 64 kg, the reference weight were derived from numerous local Malaysia studies (Taweel, 2013), TA is the average time of exposure; 365 days/year multiplied by ED (Nędzarek et al., 2022). As a conclusion, there is no potential risk found related to studied metal if their hazard quotient less thanone (THQ <1).

Table 3.7 Oral Reference Dose of Heavy Metals (Amirah et al., 2013)

	Cadmium	**Lead**	**Copper**	**Zinc**
RfD (mg/kg-day)	1×10^{-3}	4×10^{-3}	4×10^{-2}	3×10^{-1}

3.8 Heavy Metals Analysis

Atomic Absorption Spectroscopy (AAS) was a commonly used method to analysed traces of heavy metals in fish samples. It is wrought by measuring the absorption of light by atoms in the vapor phase, allowing determination of the concentration of certain elements. When analyzing fish samples, the first step involved sample preparation. Typically, fish tissue undergoes acid digestion techniques such as wet digestion or microwave digestion. This process broke down the organic matrix and converted heavy metals into soluble forms suitable for analysis. Once the sample was prepared, it was inserted into the atomization source of the AAS instrument. Flame AAS and graphite furnace AAS were commonly used methods for the analysis of fish samples. In flame AAS, the sample solution was drawn into a flame, such as an air-acetylene or nitrous oxide-acetylene flame, which provided the heat necessary to vaporize and excite the metal atoms.

Graphite furnace AAS involved depositing a small amount of sample into a graphite tube, which was then heated in a furnace. The temperature was gradually raised to vaporize the solvent and further increased to atomize and excite the metal atoms. To measure light absorption, a hollow cathode lamp specific to the target metal was used as the light source in AAS. This lamp emitted light at a characteristic wavelength corresponding to the absorption line of the metal being analyzed. Light passing through the atomic sample was absorbed by the metal atoms, and this absorption was detected by the sensor.

Quantification of heavy metal concentrations in fish samples was achieved by constructing a calibration curve using standard solutions with known metal concentrations. The curve establishes a relationship between light absorption and metal concentration. By comparing the absorbance of the sample with the calibration curve, the metal concentration in the fish sample could be determined.

AAS offered several advantages for heavy metal analysis in fish, including its sensitivity and selectivity for certain elements. It was a relatively simple technique that could analyzed a wide variety of fish samples. However, AAS had limitations, such as limited multi-element capability and possible interference from matrix components or coexisting elements. Addressing these challenges may require additional sample preparation or matrix correction techniques. To ensure accurate and reliable results, quality control measures were essential in AAS analysis of heavy metals in fish samples.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Morphometric of *Channa striatus* and *Oreochromis niloticus*

In this study, an attempt was made to sample fish of the same or equal size. The morphometric data for each fish were summarized in Table 4.1. This was done to overcome the possible variability that may be linked to variations in size. To minimize the potential impacts of size and age variation on metal accumulation, collecting samples of comparable-sized fish is essential. On the other hand, size variation is seldom related to metal content (Rajeshkumar & Li, 2018). This is because physiological processes frequently regulate internal metal concentrations in fish.

Table 4.1 Biometric information related to of *Channa striatus* and *Oreochromis niloticus*

	Channa striatus	*Oreochromis niloticus*
Weight (kg)	0.63 ± 0.19	0.19 ± 0.03
Length (cm)	37.67 ± 6.43	12.67 ± 2.08

Mean±SD, *n* =3

4.2 Heavy metals distribution in *Channa striatus* and *Oreochromis niloticus*

The heavy metals concentrations in the muscle tissue of several species of *Channa striatus* and *Oreochromis niloticus* are listed in Table 4.2, with the units of mg/kg of dry weight. Even if the fish species is the same, the physiological function of the fish is the key factor determining the accumulation of metals in its tissues (Cordeli et al., 2023). According to Weichselbaum et al. (2013), there was a noticeable variability in the amounts of heavy metals identified in fish samples. This variability might be explained by differences in the fish's eating habits and overall behaviour.

Table 4.2: Heavy metals concentrations in dried *Channa striatus* and *Oreochromis niloticus*

Fish species.	Cd	Cu	Zn	Pb
Channa striatus (mg/kg)	nd	nd	0.26 ± 6.46	nd
Oreochromis niloticus (mg/kg)	nd	nd	0.18 ± 5.12	nd

Mean ± SD

nd = not detected

The concentrations measured varied over a wide range, where the levels of heavy metals in *Channa striatus* and *Oreochromis niloticus* that could be detected is Zn whereas the other four heavy metals such Cu, Cd, and Pb were identified as "not detected". The detection of variations in heavy metals within fish samples analyzed by Atomic Absorption Spectroscopy (AAS) can be attributed to several factors. The absence or low concentrations of Cu, Cd, and Pb in the fish sample may genuinely fall below the detection limits of the AAS method, potentially influenced by the fish's habitat, diet, and environmental conditions (Hashim et al., 2014). Heavy metals exhibit diverse bioavailability and bioaccumulation patterns in fish, with Zn potentially being more readily absorbed or accumulated compared to Cu, Cd, or Pb under specific environmental circumstances (Moiseenko & Gashkina, 2020).

In addition, the presence of *Hydrilla verticillata* in pond ecosystems contributes significantly to heavy metal dynamics, acting as a phytoremediator that absorbs and accumulates metals, particularly copper and zinc (Kameswaran & Vatsala,2015). This aquatic plant's intricate root system interacts with sediment, potentially stabilizing it and influencing the mobility of heavy metals (He et al., 2016). While reducing the immediate bioavailability of metals in the water column, Hydrilla introduces complexity to the ecosystem by potentially contributing to metal biomagnification in higher trophic levels (Kameswaran & Vatsala, 2015). Its impact on sediment-water interfaces alters the cycling and fate of heavy metals, emphasizing the importance of understanding Hydrilla's multifaceted role in shaping the environmental health of ponds (Bai et al., 2018).

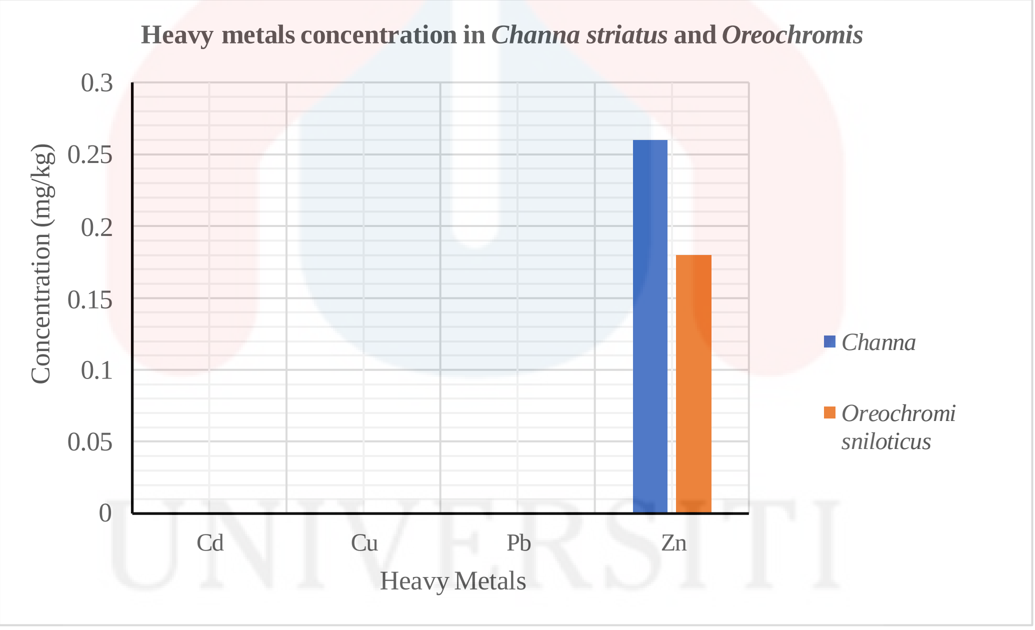

Figure 4.1: Heavy metals concentration in *Channa striatus* and *Oreochromis niloticus*

According to Francis et al. (2021), if the concentrations of cadmium (Cd), copper (Cu), and lead (Pb) in *Channa striatus* and *Oreochromis niloticus* samples are reported as "not detected" or "negative," it indicates that the concentrations were below the limit of detection (LOD) of the Atomic Absorption Spectroscopy (AAS) method.

Such occurrences are not uncommon, mainly when working with samples containing minute quantities of metals or when the analytical instrument lacks the necessary sensitivity to provide precise measurements at such low concentrations. The results are promising as they indicate that the levels of cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) in the fish samples do not exceed the acceptable limit (Hashim et al., 2014). Thus, it is widely accepted that the fish contains minimal or negligible amounts of this metal. The information provided is crucial for assessing the reliability of the tests and comprehending the potential health implications of the metal levels found in the samples (Ullah et al., 2017).

According to Figure 4.1, it is evident that the concentration of zinc in *Channa striatus* is higher compared to *Oreochromis niloticus*. Notably, zinc metal is commonly found in freshwater, even though zinc compounds are known for their relatively high solubility. Zinc plays a crucial role in supporting aquatic life (Li et al., 2021). The zinc concentration in the *Channa striatus* exhibits a higher level at 0.26 mg/kg. There may be a correlation between changes in metal concentration in aquatic systems and weather pattern fluctuations (Chan et al., 2021). According to a study by Hashim et al. (2014), heavy metal concentrations tend to be higher during the rainy season.

Predatory fish, like *Channa striatus*, tend to have higher heavy metal concentrations than non-predatory fish, such as *Oreochromis niloticus*. This phenomenon occurs due to biomagnification, where larger fish in Agropark ponds accumulate heavy metals by consuming smaller fish that have already accumulated these metals (Hossain et al., 2022). Multiple studies have supported this finding, suggesting that fish occupying higher trophic levels have a greater tendency to accumulate heavy metals than those at lower trophic levels (Moiseenko & Gashkina, 2021).

4.3 Estimation of potential health risk

To analyze the possible health risks associated with the consumption of fish that are not linked to cancer, the method of target hazard quotients (THQ) is used. THQ was often used to evaluate the possible dangers linked with long-term exposure (Antoine et al., 2017). When considering the premise of a level of exposure below one (THQ <1), it is very implausible that individuals will experience any adverse health impacts even though the heavy metal concentration of each metal served as the foundation for the computation of the EDI values, which were determined (Nyarko et al., 2023).

In Table 4.4, the THQ and EDI values are produced as results of this study. Aquatic food chain bioaccumulation can lead to elevated levels of Zn in fish. Water supplies zinc to zooplankton, phytoplankton, and small fish. Trophic transfer of zinc occurs when predatory fish consume these species. Additionally, precipitation can intensify the flow of water runoff. Zinc on the surface has the potential to be washed away and enter streams (Li et al., 2021). Human- polluted water may experience higher levels of zinc during wet seasons. Excessive zinc levels can lead to disorders affecting the gastrointestinal system, nutrient absorption, immune system, and neurological functions (Li et al., 2019). Additionally, it has the potential to cause acute poisoning, disrupt hormonal balance, and alter cholesterol metabolism. Chronic illness can result from prolonged exposure (Prasad, 2013).

Table 4.3: The Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) of metal ingestion in *Channa striatus* and *Oreochromis niloticus*

	EDI		THQ	
	Channa striatus	*Oreochromis niloticus.*	*Channa striatus.*	*Oreochromis niloticus*
Cd	-	-	-	-
Cu	-	-	-	-
Zn	1.3×10^{-3}	0.9×10^{-3}	6.6×10^{-3}	4.72×10^{-3}
Pb	-	-	-	-

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Heavy metals accumulation in the tissues of most consumers is uninformed regarding the consumption of freshwater fish, particularly *Channa striatus* and *Oreochromis niloticus*. Target Hazard Quotient (THQ) and Estimated Daily Intake (EDI) calculations will be used to assess the potential health risk associated with heavy metal accumulation through consumption and to determine the concentrations of Cd, Cu, Pb, and Zn in *Channa striatus* and *Oreochromis niloticus*. The obtained zinc concentration is found to be significantly higher than that of the other elements. The concentrations determined in the tissues of fish were otherwise illustrated by the results. *Oreochromis niloticus* was found to be preceding *Channa striatus* in terms of metal concentration distribution. Potentially attributable to *Channa striatus* feeding pattern, this variation exists. Since the THQ value of Zn is less than 1 (THQ<1), preliminary risk assessment results indicate that the ingestion of *Channa striatus* and *Oreochromis niloticus* from the examined sites does not likely pose a significant health hazard for metals. As a result, it is unnecessary to worry about the health effects associated with consuming *Channa striatus* and *Oreochromis niloticus* from the UMK Agropark pond.

5.1 Recommendation

According to this thesis's findings, several recommendations should be considered to tackle the issue of determining trace metals in fish from the Agropark UMK Jeli pond, Kelantan. Advocate for adopting optimal fish farming methods: Adopting proper fish farming practices involves ensuring optimal water quality and pH levels, providing sufficient feed, installing aerators to enhance dissolved oxygen levels and eliminate harmful gases, and improving the drainage system to prevent the accumulation of trace metals in fish. Conduct additional research: Additional research should be carried out to acquire a more comprehensive understanding of the sources of heavy metal pollution and to identify any other possible sources of pollution. This proposal aims to tackle the issue at hand.

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