Risk assessment of the possible contamination of trace metals in fish samples from Lake Kariba, Zambia

By

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Declaration

I, Phaphedi Maxwell Poopedi, student no: 16018034, declare that this dissertation titled "Risk assessment of the possible contamination of trace metals in fish samples from Lake Kariba, Zambia" is my own work entirely, submitted for the degree of Master of Science at the University of Venda, Thohoyandou, and has not been submitted before at any other university. All the sources used or quoted have been indicated and acknowledged by means of complete reference.

……………………………………

Date: 04 September 2023 at Thohoyandou

Dedication

My dear Grandmother,

For every support I have received from you all my life, I supplicate to dedicate this MSc degree in your name as a gratitude for taking care of me till today. No words could express how grateful I am to you.

Your indebted grandson,

Phaphedi M Poopedi.

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Abstract

Exposure to inorganic endocrine disrupting chemicals (EDCs) has been linked to cause carcinogenic and mutagenic effects on human health and aquatic species. Trace metals in food are widely acknowledged as a public health concern yet largely ignored in many African nations where legislation is not yet fully implemented. Thus, the study aimed to extract and determine the toxic levels of inorganic EDCs of interest at certain doses and possible health risk associated with metal ions in fish sold in the open markets from Siavonga, Lake Kariba (Zambia). Sampling was done for two consecutive years, 2021 and 2022. Two fish species, which constitute Lake Kariba, were sampled; the bream (*Oreochromis mortimeri)* and Kapenta (*Limnothrissa miodon*): Firstly, twenty-eight fresh Kariba bream (*Oreochromis mortimeri)*, five sun-dried Kariba bream (*Oreochromis mortimeri)* and five batches of sun-dried Kapenta (*Limnothrissa miodon*) were sampled from Lake Kariba open market. Secondly, six fresh Kariba bream (*Oreochromis mortimeri)*, five sun-dried Kariba bream (*Oreochromis mortimeri)* and five batches of sun-dried Kapenta (*Limnothrissa miodon*), were purchased in an open market in Siavonga. Fish muscle tissues were excised to analyze trace metal ions before being digested using a microwave digestion system and analyzed using ICP-OES and MS. Eleven trace elements: Aluminum (Al), Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb), Manganese (Mn), Mercury (Hg), Selenium (Se) and Zinc (Zn) were analyzed in fish muscle tissues (epaxial and hypaxial myomers). The results showed that the mean metal concentration for the first sampling was not significantly higher ($P > 0.05$) than the mean concentration for the second fish sampling, except for sun-dried Kapenta.

The first sampling collected in November (2021) revealed that the fish collected on a Zambian open market contained higher levels of Cr, Mn and Fe ranging from 6.50±3.30 to 101.40±22.69 mg kg^{-1} in sun-dried bream. At the same time, sun-dried Kapenta contained high levels of Cr, Mn, Fe, As and Al ranging from 0.49±0.05 to 160.72±132.60 mg kg^{-1}. In contrast, fresh bream contained high levels of Cr, As and Mn ranging from 13.99±7.96 and 22.30±12.20 mg kg^{-1}. Compared to the second sampling around August (2022) they revealed high levels of Cr, Mn, As, Se and Fe ranging from 0.15±0.07 to 110.6±63.82 mg kg^{-1} in Sun-dried bream. In contrast, sun-dried Kapenta contained high levels of Cr, Mn, As, Se, Fe and Zn levels ranging from 0.78±0.03 to 187.8±58.59 mg kg^{-1}. In comparison, fresh bream contained high Cr, Mn, As, Se and Fe ranging from 0.26±0.16 to 163.00±68.41 mg kg^{-1}.

The obtained results showed that the concentrations of metals exceeded the recommended maximum permissible limits proposed by the Joint Food and Agricultural Organization and

World Health Organization (FAO/WHO) Expert Committee on food for fish consumption. However, fish were safe from adverse health effects due to Hg not being detected in fish sampled. The pollution index was assessed to determine the extent of pollution. The estimated daily intake (EDI) for all metals were higher than the provisional tolerable daily intakes (PTDI) recommended by FDA for both adults and children. Target hazard quotients (THQ) and hazard indices (HI) was higher than 1, indicating health risks from a non-carcinogenic lifetime of fish consumption. The lifetime average daily dose (LADD) was used to estimate the incremental lifetime cancer risk (ILCR). All metals were lower than 1×10^{-4} except for fresh bream 2021 for both children and adults, indicating a carcinogenic risk of 1 in 10,000 from consumption of Kapenta and Kariba bream from Lake Kariba in a lifetime. This raises concern over an adverse health effect on the consumption of fish consisting of excess trace metals. Long-term exposure to trace metals through fish consumption poses potential non-carcinogenic and carcinogenic health risks to the residents and suggests possible adverse health effects.

Presentation of MSc work at conferences and Postgraduate Workshops

1. **Phaphedi M Poopedi**, Yannick Nuapia, Heidi Richards, Mokgaetji Monyai, Nikita Tavengwa, Imasiku Nyambe, Luke Chimuka. Health Risk assessment of trace metals using Total Microwave Acid Digestion followed by ICP-OES analysis of various fish samples from Siavonga, Lake Kariba, Zambia. Africa Food Safety Workshop 2022 (AFSW2022). Joining Hands to enable Food Safety in Africa, 27 June -1 July 2022, Emperor's Palace, Johannesburg (South Africa) - Poster presenter

2. **Phaphedi M Poopedi**, Heidi Richards, Mokgaetji Monyai, Luke Chimuka, Nikita Tavengwa. Presence and Human Health Assessment of Trace Elements in Fresh and Dry Fish from Lake Kariba, Siavonga and Zambia: Implications on Human Health. SACI NORTH SECTION, Youth Chemist Symposium. "CHEMISTRY FOR THE GOOD OF SOCIETY". Finding solutions for Africa. 13 October 2023. Hosted by University of Limpopo, Auditorium R40, Turfloop Campus, Mankweng, Sovenga (South Africa) - Poster presenter

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List of abbreviations

EDCs	: Endocrine disrupting compounds
MAE	: Microwave assisted extraction
FE	: Fusion extraction
EDI	: Estimated daily intake estimates
HI	: Hazard Indices
ILCR	: Incremental lifetime cancer risk
LADD	: Lifetime average daily dose
PTWI	: Provisional tolerable daily intakes
HQ	: Hazard quotients
FAO	: Food Agricultural Organization
US EPA	: United States Environmental Protection Agency
ICP-OES	: Inductively Coupled Plasma Optical Emission Spectroscopy
ICP-MS	: Inductively Coupled Plasma Mass Spectroscopy
BCF	: Bioconcentration Factor
BAF	: Bioaccumulation Factor
CF	: Contamination Factor
CD	: Contamination Degree
mCD	: Modified Contamination Degree
MSI	: Metal Selectivity Index
MES	: Multi elemental standard
MPI	: Metal Pollution Index
PTFE-TFM	: Modified Polytetrafluoroethylene
PTFE	: Polytetrafluoroethylene
PVDF	: Polyvinylidene Fluoride
ppm	: Parts per million
ppb	: Parts per billion
WHO	: World Health Organization
FAO	: Food Agricultural Organization

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CHAPTER 1: INTRODUCTION AND OBJECTIVES

This chapter entails background information on trace metal pollutants linked with aquatic environment. The sources and potential health risks are discussed. Trace metals are receiving attention as one of the most detected pollutants in foodstuff. The chapter furthermore, outlines the specific steps and objectives completed to achieve the main research aim of the study.

1.1 Background

Lakes and streams serve a key role in aquaculture, ecotourism, water transportation, aquatic habitat and environmental defense bearing to persons and aquatic environment (Haakonde et al., 2020; Islam, 2021; Rizk et al., 2022; Ali et al., 2022). However, aquatic environments are endlessly threatened at a breakneck, through the discharge of trace metals, as the main sinking point and a secondary source for persistent pollutants (Li et al., 2019; Raikar et al., 2020; Ehiemere et al., 2022; Hasimuna et al., 2022). Trace metals within waters and biota signify the presence of anthropogenic and/or natural sources (Hasimuna et al., 2022).

Water quality is affected by the release of trace metals into the water body (Timofeev et al., 2018; Ali et al., 2020; Karaouzas et al., 2021) and sediments which modify their natural water constituents under conditions such as pH, hardness, temperature and salinity. The metal concentration, time of exposure and metal uptake route determine water quality as metals are contributed deliberately or accidentally into an aquacultural environment (Alahabadi and Malvandi, 2018; Kinimo et al., 2018; Lee et al., 2019; Liu et al., 2018; Rinklebe et al., 2019; Varol et al., 2020; Cendrero et al., 2020; Hasimuna et al., 2022; Rizk et al., 2022; Ali et al, 2022). Deterioration of water quality renders serious environmental implications (Alahabadi and Malvandi, 2018; Kinimo et al., 2018; Lee et al., 2019; Liu et al., 2018; Rinklebe et al., 2019; Xiao et al., 2019; Varol et al., 2020). Metals continues to pose risks to human health and the aqueous ecosystem due to their ability to cause membrane DNA damage. Moreover, to perturb protein function and enzyme activity as metals are hardly eliminated or cast-off through physico-chemical and biological processes (Rinklebe et al., 2019; Ali et al., 2019; Kumar et al., 2020a, 2021; Simukoko et al., 2022).

Trace metals are one of the most hazardous contaminants in the world's rivers and aquatic ecosystems (Aghadadashi et al., 2019; Ali et al., 2020; Cai et al., 2019; He et al., 2019a, 2019b; Nour et al., 2019; Xiao et al., 2019; Hossain et al., 2020; Kahal et al., 2020; Liu et al., 2020a, 2020b; Hasimuna et al., 2022; Ehiemere et al., 2022). Trace metals pose a potential threat, because of their persistence, non-biodegradable, bio-accumulative, characteristics of environmental stability and biotoxicity not only to the aquatic environment but also to human beings (Khan et al., 2018, 2019, 2021; Shaheen et al., 2019; Ustaoglu and Islam, 2020; Zhang et al., 2019a, 2019b). Trace metals openly inhibit microbial activities after being released from their sources, by disturbing the physical and chemical characteristics of water and sediments (Omwene et al., 2018; Liu et al., 2018; He et al., 2019b; Lee et al., 2019; Rinklebe et al., 2019;

Varol et al., 2020; Cendrero et al., 2020; Hasimuna et al., 2022). Moreover, trace metals are detrimental, as trace metals bioaccumulate in multiple matrices in the aquatic ecosystem and biomagnify by escalating into the food chain. Trace metals have severe, long-lasting impacts on humans and cause numerous diseases and complications. The complications from trace metals includes a wide range of health issues, such as organ damage, neurological disorders, cardiovascular diseases, immune system suppression, and increased risk of cancer (He et al., 2019b; Idris et al., 2019; Kang et al., 2019; Nour et al., 2019; Weber et al., 2019, 2020; Kormoker et al., 2020a, 2022b). Trace metals remain in the form of solution, suspension, and precipitate in the ecological environment (Li et al, 2018a, 2018b; Huang et al., 2018; Qu et al., 2018; Lian et al., 2019; Li et al., 2019; Zhang et al., 2019a, 2019b; He et al., 2019b; Idris et al., 2019; Kang et al., 2019; Nour et al., 2019; Weber et al., 2019, 2020; Kormoker et al., 2020a, 2022b; Tusher et al., 2020; Wang et al., 2019; Ali et al, 2019, 2021).

Bioaccumulation and biomagnification of trace metals in biota represent the processes and routes of contaminants from one trophic level to the other (Rizk et al., 2020, 2022; Simukoko et al., 2022).

Trace metals are usually monitored by measuring the spread of contaminants through diverse environmental compartments (e.g., water, sediments and biota). Sediments and biota are critical in determining the effects of natural and human activity on lakes, rivers and estuaries (Kumari et al., 2018; El-Sorogy et al., 2018; Ali et al., 2019; Li et al., 2019; Proshad et al., 2018, 2019, 2020; Kabir et al., 2020; Amin et al., 2021; Chowdhury et al., 2021; Muhammad and Usman, 2021; Hasimuna et al., 2022).

Grab sampling of surface water provides evidence of the current condition of the streams and/or lakes, whereas measurements of contaminants in solid matrices indicate pollution for a longer period. Nonetheless, trace metals exist at low concentrations in water and accumulate extensive pollutants in biota and sediments (Imam et al., 2020; Rizk et al., 2020, 2022; Ali et al., 2022).

For the past 40 years, inorganic endocrine-disrupting metals have received some attention including Al, As, Cd, Cr, Cu, Fe, Hg, Mn and Pb. These metals tend to bioaccumulate within an organism (Nascimento et al., 2018; Paschoalini et al., 2019), resulting in long-term toxicity over time within a host (Elgawad et al., 2020). Trace metals have been found in fish and water samples (Gusso-Choueiri et al., 2018; Chaturvedi et al., 2018; Javed and Usmani, 2019; Keshavarzi et al., 2018; Korkmaz et al., 2019; Maurya et al., 2019; Ramalepe et al., 2022). This is due to modern human modern lives have causing the increase in quantities and toxicities of these EDCs and metals to increase over the years (Wooding et al., 2017; Horak et al., 2021). This is especially true in places with rapid economic and population growth such as Africa and globally (Ali et al., 2019; Amin et al., 2021; Chowdhury et al., 2021; Kumari et al., 2018; Muhammad and Usman, 2021).

Endocrine-linked diseases and complications have significantly increased due to countless releases of manufactured EDCs into the environment that end up in foodstuffs (Nilsen et al., 2019; Ramalepe et al., 2022). The toxicity of inorganic EDCs at a certain dosage causes health-related issues (Ramalepe et al., 2022). Fish-related intake of inorganic EDCs may result in decreased fertility and egg production in women. Also, it may lead to reduced size of gonads or shift in gender roles of fish male genetics (Langston, 2020; Olaniyan and Okoh, 2020). The brain and prostate glands in infants and children are target organs for manufactured EDCs (Bleak and Calaf, 2021; Ramalepe et al., 2022), as metals cause neurological and immune system defects (Matsushima, 2018; Benson et al., 2020; Wu et al., 2020). Endocrine-related health effects include infertility, diabetes, obesity, cancer and attention deficit hyperactivity disorder (Street et al., 2018; Rehman et al., 2018; Rahman et al., 2021; Ramalepe et al., 2022).

Some chemical contaminants interfere with crucial developmental processes, causing disruptions in the human hormonal system (Kaushik and Bhartiya, 2018; Kubincová et al., 2019; Kasonga et al., 2021; Ramalepe et al., 2022). Exposure of trace metals is thought to increase the risk of developing breast, testicular, and prostate cancer, mostly in industrialized countries like United States, Canada, Japan, United Kingdoms and South Africa (Perrot-Applanat et al., 2018; Rodgers et al., 2018; Kaushik and Bhartiya, 2018; Kubincová et al., 2019; Bouwman et al., 2019; Trasande et al., 2020; Ribeiro et al., 2021). In South Africa, there is a growing concern about the impact of trace metals on Cancer risk, although research specifically addressing these issues is limited (Bouwman et al., 2019; Aminot et al., 2023). The extent of the diseases varies depending on the level of exposure to trace metals (Parkkinen et al., 2018).

To prevent pollutants in water, it is necessary to comprehend their characteristics, generation, transportation, and side effects on fish and human health, as well as environmental analysis procedures and relevant techniques (Kumar et al., 2020b). Trace metals can be analyzed directly from foodstuff consumed, linked to everyday food intake (Ramalepe et al., 2022) using different extraction and analytical techniques.

Using relevant analytical methods is essential for monitoring and determining potential pollutants in a solid matrix. There are already various extraction techniques of endocrine disrupting chemicals, including microwave-assisted extraction (MAE), fusion extraction (FE), acid digestion and others (Rasul, 2018; Massimi et al., 2020; Ramalepe et al., 2022). Most extraction techniques have drawbacks, for instance, loss of volatile compounds, inconsistent results, contamination risk, evaporation of solutions and safety concerns (Zhao et al., 2019; Phong et al., 2022; Balaram et al., 2022). Using microwave-assisted digestion for metal analysis in fish, overcome the disadvantages of time consumption, large volume and sample usage. Microwave-assisted digestion has been used to extract trace metals (Massimi et al., 2020). It is ideal of the study because it limits the extraction labor intensities and uses less solvent to produce optimal recoveries for trace metals (Campêlo et al., 2021; Montemurro et al., 2021; Cloutier et al., 2017; Zhang et al., 2018a, 2018b, 2018c; Juhaimi et al., 2018; Hirondart et al., 2020).

It is imperative to establish water quality and fish metal content for environmental risk assessment and human health in particular, as fish is the last link in the aquatic food chain and serves as a bioindicator (Weber et al., 2020; Kormoker et al., 2020a; Tusher et al., 2020; Wang et al., 2019; Ali et al., 2019, 2021). Furthermore, non-essential metals are taken up by fish, which bioaccumulate in their tissues and biomagnify along their food chains (Rizk et al., 2022; Ali et al., 2022). Fish serves as a staple food in the Zambian population as most people live along the catchment area of Kariba, and the water supply is available to them for drinking, fishing, and other useful purposes (Gonkowski et al., 2018; Mulenga et al., 2021; Simukoko et al., 2022).

1.2 Problem statement

Zambia is under development with many problems, such as poverty and inequality, inadequate social services, poor healthcare, under developed infrastructure development, and an emerging economy (Arndt et al., 2019). Research on the extent of distribution of trace metals in freshwater in Lake Kariba is still in its infancy, and its environmental impacts have not been thoroughly evaluated.

Environmental risk assessment and monitoring of possible contamination of trace metals and other toxicants have focused on water, wastewater, and soil-related pollution. However, little attention has been given to trace metals in food such as fish.

The large Zambian population depend on fish sold in open markets in Siavonga, as fish are cheap, locally owned and healthier, precisely sun-dried Kapenta, as a major staple traditional food. According to Gonkowski et al, (2018), poor people prefer sun-dried Kapenta fish because it is shared fairly among family members than bigger fish or any other form of meat . Sun-dried Kapenta is usually bought in small amounts at local markets and the degree of consumption depends on many factors, including price, location and availability (Gonkowski et al., 2018; Carmona et al., 2020).

Many developed countries such as United State, Canada, Japan, United Kingdoms and South Africa, have sophisticated well-equipped laboratories to directly develop methods and use analytical methods to detect and quantify metals in fish, this is not the case in Zambia and Africa as a whole (Kubincová et al., 2019; Bouwman et al., 2019; Trasande et al., 2020; Ribeiro et al., 2021). Furthermore, as per WHO (2012) report, since the 1970s, diseases such as breast, prostate and testicular cancer have been increasing in both the developing and developed world. This can be attributed to the conditions in which the food people consume are subjected to, resulting in the presence of endocrine-disrupting inorganic compounds (Nuapia et al., 2016; Ramalepe et al., 2022). Endocrine-disrupting compounds are now detected almost everywhere in surface water and subsequently find their way into foodstuffs (Ramalepe et al., 2022).

Many open marketplaces in Zambia operate mainly on unhygienic and poorly maintained busy roads making it easier for vehicle pollutants and chemicals to contaminate food items sold in open markets (Nuapia et al., 2016, 2018). According to the Lake Kariba Fisheries Research Institute, fishing resources in Lake Kariba have been declining at an unprecedented rate since 2010, resulting in challenges and significant sustainability threats (Muringai et al., 2019). More importantly, polluted foodstuffs have been known to cause endocrine-linked diseases worldwide, ranging from minor and controlled effects to more prolonged ones (Ramalepe et al., 2022). The potential quantity and risks associated with trace metals in fish items sold in an open market are yet fully unknown. Therefore, there is a need to gather background data on inorganic EDCs data by using cheap analytical methods. This research is important as it may provide insights for the Zambian government to improve existing policies regarding food security and the fish industry. Zambia's fishing industry can potentially contribute to the country's economic growth.

1.3 Hypothesis and research questions

1.3.1 Hypothesis

- The sun-dried and fresh caught fish sold in Siavonga local open markets, are potential source for trace metals due to bioaccumulation and bioconcentration tendency in Lake Kariba. Furthermore, contaminated by trace metals originating from excessive operation on busy roads, poor road maintenance and industrial activities which makes it easier for chemicals from the vehicles and dust particles into the fish species sold in open markets within Siavonga town.

1.3.2 Research questions

As a secondary goal, the study aims to answer the following questions:

- What is the concentration of metals that in fish from Lake Kariba caught around Siavonga town?
- Do all fish samples sold in open markets in Siavonga contain some metals as endocrine disruptors?

Answers to these questions will assist in various guidelines and recommendations aimed at mitigating, reviving and implementing new policies on the levels and effect of endocrine disruption in Zambia.

1.4 Novelty

This research is novel because up to date there is no identical study that has been reported in this area. So far as the authors are aware, no comprehensive work was dedicated to analyzing the presence and quantity of trace metals wild Kariba bream, dry Kapenta and dry Kariba bream from Lake Kariba, Siavonga. Studies by Simukoko et al. (2022) and Hasimuna et al. (2022) explored trace metal extraction in Kariba bream from Zambian markets using open acid extraction, but with limited sample sizes. However, there is a gap in the research regarding trace metals in sun-dried Kapenta, though some studies have focused on sun-dried bream. This study aims to address this gap by examining both sun-dried Kapenta and bream, as well as fresh bream. Additionally, there are limited reports on the use of microwave digestion techniques for analyzing fish samples in Zambia.

1.5 Objectives of the study

1.5.1 General objective

This project aims to develop and optimize cost effective analytical extraction techniques to determine the presence and levels of trace metals in fish sold in Siavonga open street markets in Zambia.

1.5.2 Specific objectives

The general aim will be achieved on a completion of the following objectives, to:

- Develop a total microwave digestion procedure for determination of endocrine disrupting inorganic compounds in fish species (Kapenta and Kariba bream) sold on open markets in Zambia.
- To apply the developed methods to determine target chemicals such as metals like Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Pb, Se and Zn in fish items sold on open markets in Lake Kariba, Siavonga in Zambia.
- Assessment on the pollution index of trace metals in fish inclusive of bioaccumulation and bioconcentration factor.
- Perform a health risk assessment of the presence and quantity of trace metals in fish samples.
- Target samples in different fish, within the lake with various sizes to assess ecosystem health.

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CHAPTER 2: LITERATURE REVIEW

This chapter provides a literature review on inorganic EDCs within the Kariba catchment. It begins by discussing the significance of the catchment area as a source of water pollution concern. The chapter then explores the characteristics of these trace metals, their specific targets, and their potential to bioaccumulate in the aquatic environment, emphasizing their adverse effects on aquatic organisms and the ecosystem. It also investigates the regulatory standards of reputable organizations like the World Health Organization and Food and Agriculture Organization regarding acceptable concentrations of trace metals in water and biota. In addition, it addresses various extraction techniques used for solid samples and suitable analytical instruments for metals. Furthermore, the chapter concludes with a discussion on sample drying techniques for solid samples, which is crucial for preserving sample integrity during analysis. Overall, of this literature review serves as a foundational resource for understanding the presence and impact of inorganic EDCs in the surface water of the Kariba catchment.

2.1 Lake Kariba Catchment

2.1.1 Catchment area

Lake Kariba is situated in Siavonga district and is surrounded by Sinazongwe, Gweembe and Siavonga in Figure 2.1 with an estimated population of 98 246, 50 136 and 58 864 respectively (Hasimuna et al., 2019; Simukoko et al., 2022). Kariba is well known for commercial bream and Kapenta fishing.

The Kariba catchment area is shared by two independent nations, serving as a border (−17°S28°E) between the Southern Province of Zambia and Zimbabwe. The international river, nine provinces are involved in the Zambezi River catchment basins. The damming of Kariba was initiated for hydroelectricity generation between 1958 and 1963. Other beneficial industries associated with Kariba Dam include artisanal, commercial and subsistence fishing of Kapenta (Limnothrissa miodon), aquaculture, ecotourism, water supply, and lake transportation (Tran et al., 2018; Njiru et al., 2018; Aura et al., 2018; Hasimuna et al., 2019; Simukoko et al., 2022).

Lake Kariba was the largest artificial dam globally during construction in the late 1950s. It is Africa's second-largest reservoir by volume (Tran et al., 2018; Njiru et al., 2018; Aura et al., 2018; Simukoko et al., 2022). Kariba is about 320 km long with an area of 540 km^2. At a maximum height, the lake holds 157 million cubic metres with an average of 29 m depth. The shoreline is approximately 2164 km and 486 m above sea level (Simukoko et al., 2022). The Lakes cover 663 817 km^2 of catchment spreading to other parts of Zambia, Botswana, Zimbabwe, Angola and Namibia.

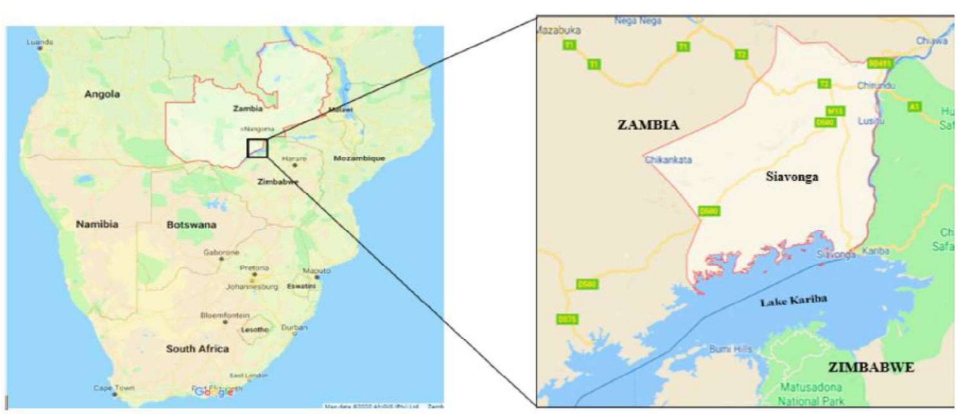

Figure 2.1: Lake Kariba, Siavonga Zambia (Hasimuna et al. 2019)

The lake controls 90% runoff of the Zambezi tributaries with a flow from the west-east with an annual rainfall of 400-700 mm, and a temperature of 13-40°C with subtropical climatic conditions. The wild and farmed fish flourish in subtropical and tropical conditions with a temperature of 9-42°C in shallow water. Whereas harsh climatic conditions create a unique environment that benefits and promotes fast aquacultural growth and blooming of Kapenta (Njiru et al., 2018; Aura et al., 2018; Tran et al., 2018; Maulu et al., 2019, 2020, 2021; Hasimuna et al., 2019; Simukoko et al., 2022).

2.1.2 Pollution issues in the lake

Pollution in Kariba may result in catastrophic consequences for the economy and environment downstream and upstream of the Zambian sides of the lake. Consequently, deteriorating and poisoning of fishes in the lake and possibly large human-life losses by inorganic EDCs (Mwakalapa et al., 2019; Hasimuna et al., 2022).

There is a saying by Shona people, "Mvura haina n`anga", translating to "water needs no witch doctor". Water is pure and harmless, so attributing a patient's illness to drinking water was considered senseless by the Shona people. This term and proximity issue led to dense human population growth by the lake and riverbanks (Marengo et al., 2018; Mavara et al., 2020; Winton et al., 2019, 2021). These communities draw water from the lake and send their waste back into the water system drew water from. This presents a potential health risk to the communities that consume the fish and water directly from the lake (Marengo et al. 2018; Mwakalapa et al., 2019; Sonone et al., 2020; Muhammad et al., 2021a, 2021b, Muhammad and Ali, 2022; Simukoko et al., 2022).

The massive growth of water hyacinth in Lake Kariba in the long term has affected water availability. Two foremost invasive weeds on Lake Kariba, the Kariba weed (Salvinia Molesta) and water hyacinth became a real nuisance from August 1994 to date. During the eutrophic fill-up level of the lake's nutrients, the weed was widely spread on Lake Kariba (Muhammad et al., 2021a, 2021b; Mwakalapa et al., 2019). Posing a significant challenge for the ecological integrity, economic activities, and water management practices of Lake Kariba and its surrounding areas

Zambezi Basin is widely populated, with many tributaries that spread relatively intact with Lake Kariba (Ndebele-Murisa et al., 2020). The region is changing rapidly due to developmental trends, and it is not clear how robust is the state of surface water quality is

(Winton et al., 2021). Massive aquaculture on the lake results in unpleasant odors around the lake, reducing the water quality (Kabir et al., 2020; Muhammad and Usman, 2021). Moreover, plentiful resorts, motels, and agricultural activities whose effluents directly or indirectly enter into and around the lake and also contribute to the reduction of water quality (Hasimuna et al., 2022).

Unfortunately, the status quo of metals and many other toxicants compounds in fish-related materials is unknown and has not been fully studied in Lake Kariba entirely (Muhammad et., 2021a, 2021b; Mwakalapa et al., 2019; Simukoko et al., 2022; Sonone et al., 2021; Winton et al., 2019, 2021a, 2021b). Nonetheless, fish stocks have declined in contrast to the end of the 20^{th} century (Mwakalapa et al., 2019; Simukoko et al., 2022) due to environmental pollution coupled with overexploitation of fish population (Gonkowski et al., 2018).

2.2 Sources of pollution on surface water

Point and non-point sources of threats concerning pollution and environmental degradation on Kariba outlined by FAO Fisheries Report No. 766 and 824 in Figure 2.2 includes industrial activities and urbanization, agro-chemicals, mining activities (copper, sulphide, coal, and manganese ores), waste and oils from boats on the lake, the discharge of domestic and animal waste, deforestation, artisanal and aquaculture (Mbewe et al., 2018; Tran et al., 2018; Njiru et al., 2018; Aura et al., 2018; Mwakalapa et al., 2019; Hasimuna et al., 2019, 2022; Muhammad et al., 2021a, 2021b; Simukoko et al., 2022).

Trace metals are primary pollutants and diffuse directly or indirectly into surface water (Kabir et al., 2020; Proshad et al., 2018, 2019, 2020). Pollution of surface water is caused by the release of industrial effluents containing trace metals above tolerable limits into the lakes, rivers or streams (Kumari et al., 2018; Naz et al., 2018; Proshad et al., 2018, 2019, 2020; Chowdhury et al., 2021; Kabir et al., 2020). This raises environmental problems and public health concerns (Ahmad et al., 2020; Amin et al., 2021; Awasthi et al., 2022). Figure 2.2 shows point and non-point sources of pollution on surface water

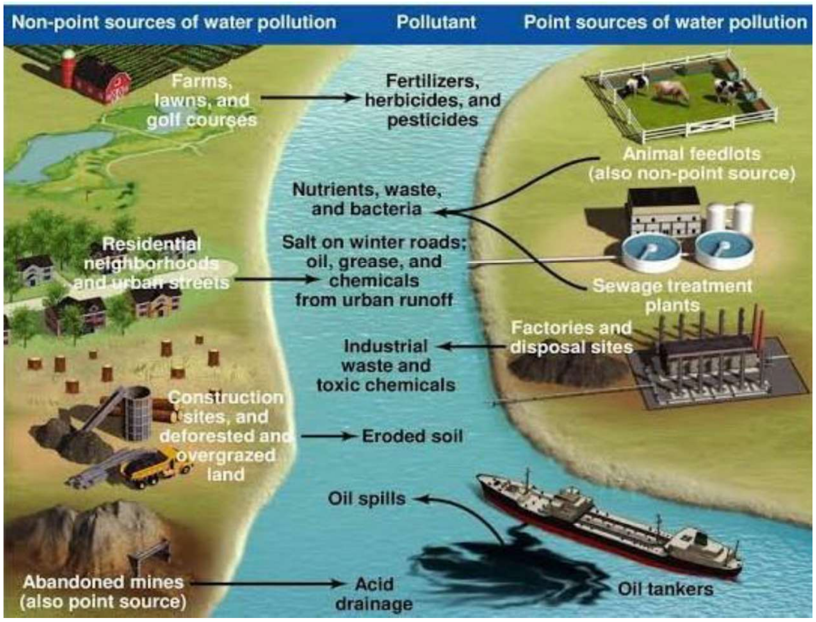

Figure 2.2: Point and non-point sources of pollution on surface water (Gheorghe et al., 2017)

The fast growth of urbanization coupled with heavyweight economic development has been recognized as the main driver for the discharge of trace metals in streams (Ahmad et al., 2020; Amin et al., 2021; Awasthi et al., 2022; Parihar et al., 2021). However, various large amounts of pollutants in surface water depend on what gets "thrown down the drain" (Kumari et al., 2018; Proshad et al., 2018, 2019, 2020; Chowdhury et al., 2021; Kabir et al., 2020; Muhammad and Usman, 2021).

Point sources are specific and identifiable locations where pollutants are attributed directly into the environment. In contrast, non-point sources are more diffused and results from runoff pollutants attributed by numerous sources, making their identification and controllability more complex as their entire contribution of pollutants is detrimental. However, individual sources may not reach harmful concentrations (Offiong et al., 2019; Maurya et al., 2019; Proshad et al., 2018, 2019, 2020 Kabir et al., 2020; Muhammad and Usman, 2021).

The pollution of trace metals attributed to point sources originates from anthropogenic activities, including agrochemicals, industrial activities, smelting, atmospheric deposition, mining and natural denudation of ore deposits of bedrock. Moreover, municipal wastewater treatment plants, hospitals and domestic waste, landfills and dumpsites, are other sources of pollution (Kumari et al., 2018; Ali et al., 2019; Offiong et al., 2019; Amin et al., 2021; Chowdhury et al., 2021; Proshad et al., 2018, 2019, 2020; Kabir et al., 2020; Muhammad and Usman, 2021).

Pollution from non-point sources includes cities, agricultural fields, metal-linked pollutants (pesticides and fertilizer, oil and road salt, animal waste, lawns, and antifreeze) and acid from toxic elements of abandoned mines and urban areas shown in Figure 2.3. The pollutants are transported by runoff from land into ground and surface water bodies, and aquatic ecosystems (Kumari et al., 2018; Ali et al., 2019; Offiong et al., 2019; Amin et al., 2021; Proshad et al., 2018, 2019, 2020; Kabir et al., 2020; Muhammad and Usman, 2021). Figure 2.3 shows sources of metal contamination affecting surface water and aquatic ecosystems.

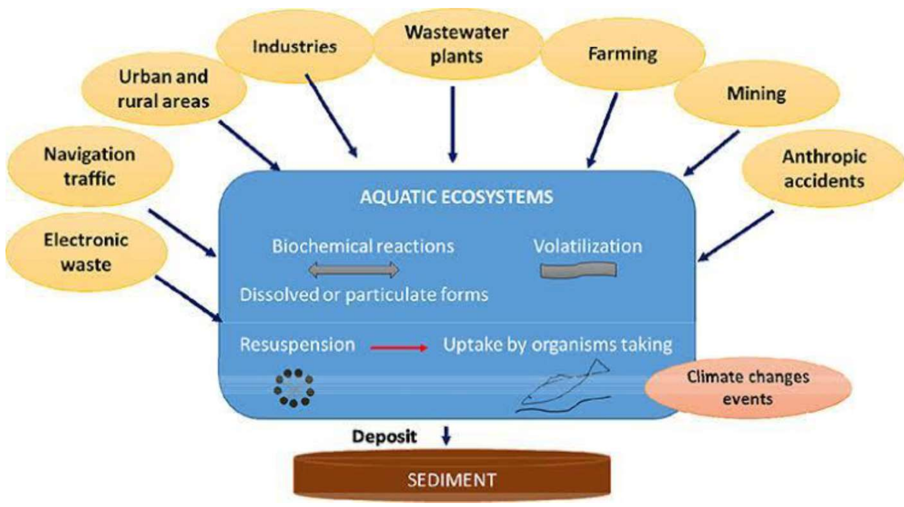

Figure 2.3: Anthropogenic and/natural sources of metal contamination affecting surface water and aquatic ecosystems (Gheorghe et al., 2017)

Non-point sources also include anthropic activities, such as emissions from traffic-related (oil/gasoline leakages, vehicle exhaust, asphalt wear, tyres and brake linings) incidents. Moreover, urban contributions by the water sewage influx include household's runoffs, water drainage and business effluents (e.g., dental uses, car washes, and other initiatives). Trace metal pollutants are transported by heavy rainfall in the sewerage system and eventually into the lake and riverine (Ukpaka et al., 2016; Offiong et al., 2019; Ali et al., 2019; Kabir et al., 2020; Muhammad and Usman, 2021; Ramalepe et al., 2022). This affects surface water and aquatic ecosystem, inclusive of pipes from factories, storage tanks, chemical spills and waste disposal sites (Offiong et al., 2019; Maurya et al., 2019; Ali et al., 2019; Amin et al., 2021; Chowdhury et al., 2021; Kumari et al., 2018; Proshad et al., 2018, 2019, 2020; Kabir et al., 2020; Muhammad and Usman, 2021).

Aquaculture is a massive source of pollution due to feeds spillage in streams focused relatively on small spaces. Drainage feedlot (from intensive cultivated poultry) produces extreme water pollution as feedlots are usually near surface water bodies (Hasimuna et al., 2022; Simukoko et al., 2022).

Some sources of pollution have been found to be prominent in developing countries. Most sources are appraised to be common in all nations (Offiong et al., 2019; Maurya et al., 2019; Ali et al., 2019, 2021; Kumari et al., 2018; Proshad et al., 2018, 2019, 2020; Ahmad et al., 2020; Parihar et al., 2021; Kabir et al., 2020; Muhammad and Usman, 2021; Amin et al., 2021; Awasthi et al., 2022).

2.3 Toxic effects of endocrine-disrupting chemicals

The United States Environmental Protection Agency (US-EPA) identified a group of heterogeneous chemicals as endocrine-disrupting chemicals (Ramalepe et al., 2022). Endocrine-disrupting chemicals have been linked as a source that causes cancer in human beings, as well as having undesirable adverse effects on aquatic environments (la Merrill et al., 2020; Lauretta et al., 2019). Endocrine-related disease and its consequences have increased significantly due to countless releases of man-made EDCs into the environment, which end up in foodstuffs (Nilsen et al., 2019; Ramalepe et al., 2022). Food is a key source of trace metals for human beings (Li et al., 2019; Sharma et al., 2021; Ramalepe et al., 2022). Toxicity of inorganic EDCs at certain doses causes health-related issues (Ramalepe et al., 2022) depending on the levels of trace metals exposure (Parkkinen et al., 2018; Perrot-Applanat et al., 2018; Rodgers et al., 2018; Kaushik and Bhartiya, 2018; Kubincová et al., 2019). At toxic levels, trace metals cause immune suppression, reproductive and behavioral effects and delays in immune responses in children (Pavlikova et al., 2020; Ramalepe et al., 2022).

Endocrine-disrupting chemicals openly inhibit microbial activities after being released from their sources by disturbing the physical and chemical characteristics of water, sediments and aquatic organisms (Omwene et al., 2018; Liu et al., 2018; He et al., 2019b; Lee et al., 2019; Rinklebe et al., 2019; Varol et al., 2020; Cendrero et al., 2020; Hasimuna et al., 2022). Trace metals are hardly eliminated nor cast off through physiochemical and biological processes, continuing to pose risk to water quality and human health by eating food contaminated with trace metals (Rinklebe et al., 2019; Ali et al., 2019; Kumar et al., 2020a, 2020b; Simukoko et al., 2022; Hasimuna et al., 2022). Trace metals can easily dissolve in water and bioaccumulate in fish (Timofeev et al., 2018; Ali et al., 2020; Karaouzas et al., 2021). Sediments modify their natural water constituents such as pH, hardness, temperature, and salinity, subsequently being absorbed in fish as the last link. Accumulation of trace metals is dependent on the time of exposure and metal uptake route (Alahabadi and Malvandi, 2018; Kinimo et al., 2018; Lee et al., 2019; Liu et al., 2018; Rinklebe et al., 2019; Varol et al., 2020; Cendrero et al., 2020; Hasimuna et al., 2022). Although the pollutants may not be in excess concentrations, their effects on human health are cumulative, effects manifesting in a long term (Mbewe et al., 2018; Mwakalapa et al., 2019; Muhammad et al., 2021a, 2021b; Hasimuna et al., 2022). Compared to other forms of water pollution in the lakes and streams, it is less obvious and direct, but its consequences on aquatic ecosystems and people are widespread and intense (Varol et al., 2020; Cendrero et al., 2020; Hasimuna et al., 2022).

Trace metals are defined as normal constituents of aquatic environments by Neiboer and Richardson (1980), functioning mostly in tandem with organic molecules, typically proteins. Metals naturally occur in low amounts, yet trace metals can exert considerable biological effects, at a quantity above the threshold limits: this includes all metals and metalloids. Trace metals, particular Pb, Hg, Cd, Cu and Ag are poisonous, causing complications in the aquatic environment and humans (Khan et al., 2018, 2019a, 2019b, 2021; Ustaoglu and Islam, 2020; Zhang et al., 2019).

Some trace metals are known to be lethal including of Cd, As, Pb and Hg among others which at trace levels do not play a role in the metabolism of living organisms (Das Sarkar et al., 2022; Mehana et al., 2020). According to the toxicity levels that metals can attain, Schroeder and Darrow (1973) have categorized metals into several groups which are essential metals, moderately toxic Metals and highly toxic metals. Huseen and Mohammed (2019) claim that trace metals impact fish through mutagenesis by interfering with their metabolic processes. There are significant public health concerns as a result of the bioaccumulation of trace metals in plants and fish, consequently having long-lasting effects on the human body (Li et al., 2018; Lian et al., 2019; Zhang et al., 2019; Weber et al., 2019; Ali et al., 2019, 2021; Awasthi et al., 2022; Hasimuna et al., 2022). Arsenic has been reported to have a widespread effect on human beings. Symptoms of early exposure to arsenic include abdominal pain, vomiting, muscle weakness, skin flushing and diarrhoea, whereas long-term exposure results in cancer and skin lesions (Engwa et al., 2019; Awuchi et al., 2020; Banasal, 2023; Kumar et al., 2023). It is fatal to humans above allowed limits in food and drinking water, just like other poisonous trace metals and metalloids (Das Sarkar et al., 2022). Cadmium has a long biological half-life in humans and is highly toxic, interfering with the removal of accumulated body load of trace metals, inhibiting the metal transporters, inducing oxidative stress, and binding to metal-regulating proteins, thus disrupting metal homeostasis (Samuel et al., 2021; Wang et al., 2021;

Vonnie et al., 2022). Fish intake is also a significant source of Pb exposure in some communities, whereas excessive exposure to lead causes cancer, hypertension, renal failure, neurological disorders, and hematological disorder/diseases. Furthermore, cardiovascular dysfunction, skeletal weakening, carcinogenic effects, and other abnormalities can significantly impact human health (Ali et al, 2019; Weber et al., 2019; Ahmad et al., 2020; Ramalepe et al., 2022). One to fifty g kg^{-1} of Cd in fish, meat, and fruit is primarily consumed by humans through diet (Li et al, 2018; Lian et al., 2019; Zhang et al., 2019).

Fish is thought to be the main source of Hg for humans and there have been multiple cases of ingesting of fish muscle containing Hg higher than the permitted maximum levels of ppm/ppb (Das Sarkar et al., 2022). Resulting in decreased fertility and egg production in women. It also led to reduced size of gonads or shift in gender roles of fish male genetics (Langston, 2020; Olaniyan and Okoh, 2020). Methyl mercury (MeHg) accumulates in the brain, resulting in cell loss in some parts of the brain, for instance the cerebellum, visual cortex, and other specialized areas. Mercury affects the neurological development of fetuses because methylmercury easily passes through the placental barrier (Bleak and Calaf, 2021; Ramalepe et al., 2022). It induces alterations in male and female fertility and disrupts the biosynthesis of steroid hormones, causing diabetes, obesity, cancer and attention deficit hyperactivity disorder (Street et al., 2018; Matsushima, 2018; Benson et al., 2020; Wu et al., 2020; Langston, 2020; Olaniyan and Okoh, 2020; Rahman et al., 2021; Ramalepe et al., 2022). Additionally, symptoms of MeHg poisoning in people include hearing loss, paraesthesia, blurred vision, headaches, loss of coordination and impaired mobility, ataxia, tremors, and weariness (Matsushima, 2018; Street et al., 2018; Rehman et al., 2018; Benson et al., 2020; Wu et al., 2020; Langston, 2020; Olaniyan and Okoh, 2020; Rahman et al., 2021; Ramalepe et al., 2022).

2.3.1 Definitions and overview

Trace metals are of a health concern due to their poisonous properties, however other metals are essential to humans and animals' well-being (Ore and Adeola, 2021). Toxicity caused by trace metals in food is lethal to patrons. Elements such as Hg, As, Pb and Cd are of interest for their bioaccumulation propensity in organisms (Ali et al., 2019; Hao et al., 2019). Mercury is toxic at trace amounts having a negative consequence on human health (Nuapia et al., 2018). For instance, MeHg is a potent organometallic form of Hg formed by microorganisms, releasing lethal Hg in the soil and aquatic environments posing a significant threat to living organisms (Zhao et al., 2019; Leister, 2019; Liu et al., 2020). Lead is an endocrine disruptive toxin, acting on the brain and delaying intellectual developments in children, by causing an injury to the brain cells (Leister, 2019; Zhao et al., 2019). Cadmium distresses kidneys, damaging cells and potentially foremost causing cancer (Genchi et al., 2020). It is imperative to evaluate the concentrations of trace elements in fish to establish a protective measure for humans' well-being.

Trace metals are natural parts of the Earth's crust, which neither can get damaged nor destroyed (Vijaya et al., 2020). Trace metals get into our systems through food, water and air (Ali et al., 2019). Chromium, copper, selenium, and zinc are trace elements that are crucial for preserving the body's metabolism. Though, acute and long-term consequences are possible at high concentrations. High ambient air concentrations close to emission sources, eating contaminated food through the food chain, drinking water contaminated with trace metals such as Pb pipes and others are common pathways through which humans are exposed to trace metals. Leading to significant health risks and increasing the likelihood of developing various diseases (Cannas et al., 2020; Huseen and Mohammed, 2019).

Trace metals are hazardous due to bioaccumulation. When compared to their chemical components in the environment. Bioaccumulation is the gradual increase in chemical concentration in a biological organism (Ali and Khan, 2019; Anițaș et al., 2020). Trace metals in living organisms build up quicker than digestion and excretion processes. Trace metals are also taken up and stored. Accumulation of trace metals and metalloids (e.g., Hg, Al, As, Pb, Cu, Fe, Cd, Mn and Zn) in humans causes adverse health effects, both on a short- and long-term exposure. This happens by affecting the metabolic activities, thus causing homeostatic changes within the body (Chakraborty, 2021; González-Casanova et al., 2020; Sabir, et al., 2019; Saris et al., 2019; Vieira et al., 2021). This is either by stimulating the body's normal hormonal binding process, or by complicating different body signals, while producing the same biological reaction from the host as a typical binding (Zhao et al., 2019). Some trace metal and metalloid are reported for discontinuing the production of the bodily hormones and thus preventing any cellular response (Swell et al., 2020).

Reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are produced more frequently as a result of prolonged contact to metals which eventually leads to oxidative stress of the cells resulting in the death of cells (Zhao et al., 2019; Leister, 2019; Liu et al., 2020). Due to their capacity to obstruct the action of estrogenic hormones, metals such as Cd, Al, Cu and Pb are categorized as metalloestrogens (Ramalepe et al., 2022).

Trace metals can find their way into water supply by acid rain which breaks down soils subsequently releasing a series of trace metals into streams and groundwater (Sirdar and Kunduz, 2018; Wei et al., 2019). The effect and toxicity mechanisms of trace metals in streams affect the algal growth leading to reduced growth and disrupting the photosynthesis, and causing cell damage. Trace metals are poisonous to Algae both cellular and molecular (Priyadarshini et al., 2019). It is generally known that the concentration of metal and the rate of microbiological development are inversely related (Priyadarshini et al., 2019; Wei et al., 2019). Ochari (1997) proposed three different mechanisms by which the toxic effect of metal ions on microorganisms is exerted, including (i) blocking the functional groups of biomolecules (enzymes and proteins), (ii) replacing the necessary metal ions with the toxic metal, and (iii) altering the conformation of the biomolecule to change its activity (Liu et al., 2020). Figure 2.4 shows the effect and toxicity mechanisms of trace metals into streams while Figure 2.5 shows the effects of oxidative stress of chronic exposure by trace metals.

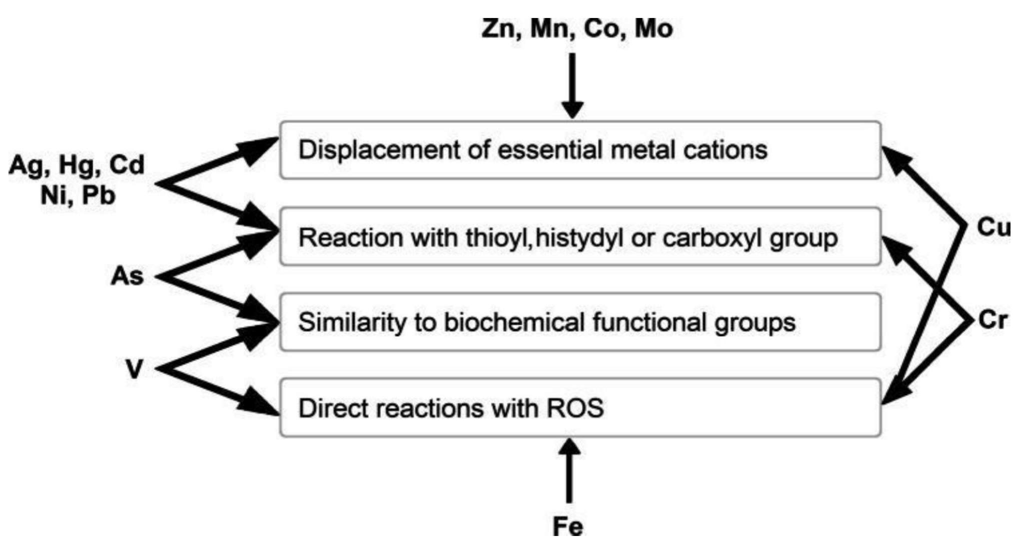

Figure 2.4: The effect and toxicity mechanisms of trace metals into streams (Jomova et al., 2023)

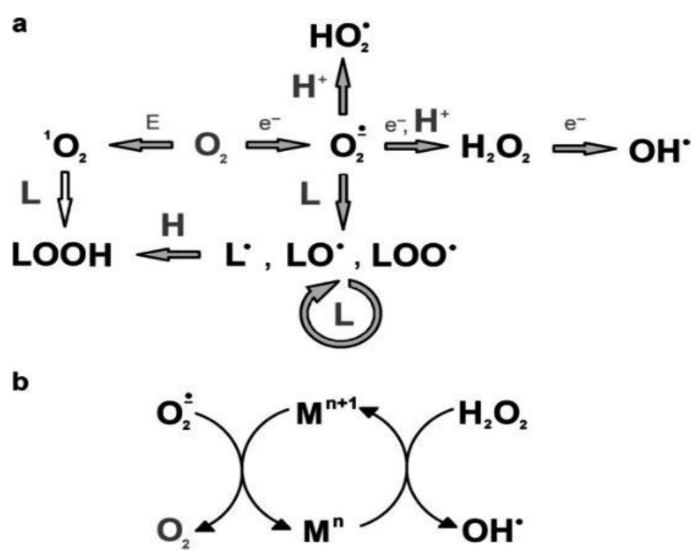

Figure 2.5: Trace metals effects of oxidative stress of chronic exposure (Jomova et al., 2023)

2.4 Target inorganic endocrine-disrupting chemicals

2.4.1 Aluminum

Aluminum (Al) is the third most prevalent and widespread metal on earth, following oxygen and silicon. It is toxic in its soluble ionic state. It is found in the atmosphere of large cities and industrialized places (Jaiswal et al., 2018). The physicochemical characteristics of water, specifically its pH, significantly impact on the toxicity of Al in fish. The poisoning mechanism in fish involves the disruptions of ionic and osmotic balance resulting in respiratory issues caused by the fish gills' coagulation mucus. In the gills, it has been discovered to severely fuse lamellae and filaments (d'Haese et al., 2019). In mature female bream (*Oreochromis niloticus*), Al is an endocrine-disrupting substance (d'Haese et al., 2019; Siblerud et al., 2019; Rashid et al., 2020). Fish development was reduced by Al concentrations as low as 0.52 mg L^{-1} in a study by Bonisławska et al. (2021). Most of the physiological changes caused by exposure of Al in fish are often connected to the cardiovascular system, reproductive, ion regulatory, haematologic, respiratory, metabolic and endocrine disturbances beyond structural gill damage (Jaiswal et al., 2018; Siblerud et al., 2019; Rashid et al., 2020).

2.4.2 Arsenic

Various sources expose aquatic environments to arsenic (As) (Raju, 2022; Khosravi-Darani et al., 2022). Arsenic can build up significantly in the sediments of reservoirs, waterbed courses, and aquatic animals (Liu et al., 2020). Fish rapidly absorbs As compounds in its third (III) oxidation state (arsenites), which is more poisonous than As compounds with (V) oxidation state (arsenates) (Liu et al., 2020; Raju, 2022). Acute exposures may cause asphyxia, increased mucus production brought on by the As, or direct damage to gill epithelium, all of which can cause instant death. Multiple disorder is caused by continuous exposure of metalloid build-up in hazardous amounts (Khosravi-Darani et al., 2022). Fish's gills expose them to As constantly by reducing antibody production, affecting the fish immune system (Liu et al., 2020; Raju, 2022). Fish are exposed to non-lethal levels of As which induces time-dependent and tissue-specific alterations that make them more vulnerable to infections. The induction of several important stress protein families is another effect of As and its compounds, which includes heat shock proteins (hsps) with a rapid dose-dependent response to acute exposure to As (III), both in vitro and in vivo in several organs and systems (Liu et al., 2020; Raju, 2022; Khosravi-Darani et al., 2022).

2.4.3 Cadmium

Cadmium (Cd) is the most hazardous trace metal, even at the trace levels, having both immediate and long-term negative impacts on the health of the aquatic environment. It is known for its oxidative stress and induces nutritional deficiencies in plants and freshwater fish (Jijie et al., 2020; Yang et al., 2022). Cadmium is a naturally occurring non-essential trace metal with a propensity to bioaccumulate in living organisms at hazardous amounts raising environmental concerns (Ali and Khan, 2019; Jijie et al., 2020; Rossi et al., 2020). Long exposure over an extended period causes many acute and chronic consequences in aquatic species. Kidneys are cadmium’s primary target organ (Ali and Khan, 2019; Jijie et al., 2020). Once ingested by humans, cadmium accumulates in the body over a lifetime, as it is a cumulative contaminant that is not readily excreted. Aquatic trophic levels are altered by Cd for centuries (Ali and Khan, 2019). It causes pathological changes of varying brutality in fish organs and tissues (Rossi et al., 2020). Moreover, Cd suppresses calcium uptake by gills, altering their metabolism of essential trace elements absorbed and interfering with their natural distribution of trace elements such as Cu and Zn in tissues (Ali and Khan, 2019; Jijie et al., 2020). Cadmium is an endocrine disruptor. It has been shown to alter hormone synthesis and interfere with the development of steroid hormones, eggs, and sperm in rainbow trout (Oncorhynchus mykiss) (Stojsavljević et al., 2019; Zheng et al., 2019).

2.4.4 Chromium

Numerous organisms require chromium (Cr) as it is essential for the metabolism of carbohydrates. It is an abundant trace element found in the earth's crust (Rinklebe et al., 2019; Ali et al., 2019; Kumar et al., 2020a, 2020b), toxic and mutagenic at higher levels (Rinklebe et al., 2019). Acute toxicity by Cr in humans causes disorders in the respiratory tract, cancer, and renal tubular necrosis. Cr (III) is an essential nutrient for glucose metabolism, whereas Cr (VI) is a potent carcinogen and causes a severe respiratory and skin damage upon the exposure. The toxicity of Cr is influenced by both biotic and abiotic variables, according to Velma et al. (2009). Age, developmental stage, and species type are examples of biotic influences. The abiotic factors in water are temperature, concentration, oxidation state, pH, alkalinity, salinity, and hardness (d'Haese et al., 2019). Chromium in the atmosphere is adsorbed by soil and water particles. The Cr with an oxidation state of trivalent Cr (III) and hexavalent Cr (VI) are more stable forms (Mushtaq et al., 2022; Wei et al., 2022; Solis-Ceballos et al., 2023). Because of its intense oxidative stages, the potential and capacity to cross cell membranes, particularly Cr (VI) are hazardous (i.e., carcinogenic) (Ali and Khan, 2019). Fish accumulate Cr through ingestion or gills uptake in tissues (Omwene et al., 2018; Liu et al., 2018; He et al., 2019b; Lee et al., 2019). Fish growth and behavior are negatively impacted by the overall toxic impact on organs, which has major consequences for metabolic and physiologic functions (Ali and Khan, 2019). Chronic exposure to Cr in Gambusia affinis (Mosquitofish) has been associated with decreased locomotor activity (Liu et al., 2018; He et al., 2019b; Ali and Khan, 2019).

2.4.5 Copper

Copper (Cu) is an abundant trace metal widely used and a crucial for cellular metabolism in aquatic species (Alahabadi and Malvandi, 2018; Kinimo et al., 2018; Lee et al., 2019). However, in higher quantities than required, it can harm aquatic species' intracellular processes when exceeding normal levels by affecting growth and reproduction (Kinimo et al., 2018). Through their diet or environmental exposure, fish can accumulate Cu (Ali and Khan, 2019). Copper-induced morphological and histological alterations of the mechanoreceptors, chemoreceptors, gills, kidney hematopoietic tissue, and other tissues, due to long-lasting damage (Khan et al., 2018). Copper is acutely hazardous at concentrations ranging from 10 to 20 $\mu g\ g^{-1}$ in freshwater according to the National Research Council of Washington, DC (Pace et al., 2019; Garnero et al., 2020). Copper`s bioavailability, also known as the capacity to transfer from water or food to receptors like gills and olfactory neurons, among others, determines how toxic Cu is to aquatic organisms (He et al., 2019b). Effects of Cu toxicity can be characterized as "acute" or "chronic," with deadly exposure known to impair fish tolerance to disease and reduce growth, immunological response, reproduction, or survival (Alahabadi and Malvandi, 2018).

2.4.6 Iron

Biologically, Iron (Fe), as a cofactor for numerous essential proteins and enzymes, is the most significant nutrient for the majority of organisms. Fish cannot metabolize Fe concentrations higher than those not dissolved in water, according to Khan et al. (2018). As a result, fish may eventually die from Fe poisoning due to the accumulation of Fe in their internal organs. Fish and aquatic plants with high Fe content are toxic to humans and other living organisms that consume them. Ferrous iron (Fe^{2+}) is more poisonous to fish than ferric iron (Fe^{3+}) (Ali and Khan, 2019). In a study conducted by Lee and others (2019), the liver and gonads of fish had the highest levels of Fe bioconcentration, whereas the brain, muscle, and heart had the lowest levels. Iron poisoning is responsible for the physical obstruction of the gills by interfering with respiration, harming the epithelium, and ultimately suffocating and killing the fish. Excessive Fe results in serious hazards and adversely affects the environment, according to Alahabadi and Malvandi (2018). Compounds containing Fe released into the environment persist and disrupt the eco-balance, causing various health issues (Ali and Khan, 2019).

2.4.7 Lead

Lead (Pb) is a naturally occurring persistent trace metal. Its environmental concentration has significantly increased by anthropogenic sources. Its widespread usage causes extensive environmental contamination and health problems globally. Lead is a hazardous substance that disturbs various living organisms and their physiological processes (Rossi et al., 2020). Its bioavailability and concentration are primarily influenced by how much of the water's natural organic matter is adsorbed into the sediments, as well as other factors including pH, alkalinity and hardness (Zheng et al., 2019). Lead deposits have been found in the liver, kidneys, spleen, digestive system, and gills of various fish (Ali and Khan, 2019). Accumulation of Pb has led to body disorders (Lee et al., 2019). Acute Pb toxicity is initially characterized by gill epithelial damage resulting in fish suffocation. However, it causes nausea, vomiting, dizziness, headaches, hypertension, stomach discomfort, kidney dysfunction, lethargy, and vertigo in human beings (Ali et al., 2022). Lead damages tissues and organs, interfering with fish species' embryonic and larval development (Zheng et al., 2019; Chyra-Jach et al., 2020). Juveniles with deformities had decreased mobility and foraging skills due to Pb which weakened the immune system by increasing infection vulnerability. Unlike other trace metals like Zn, Cu, and Mn, it has no essential function in biological processes (Chyra-Jach et al., 2020).

2.4.8 Manganese

Manganese (Mn) is one of the trace elements utilized mostly by industrial mining, fertilizer, and plants released as a by-product into the aquatic ecosystem. Manganese chloride and Manganese sulphate concentrations of 5.5 and 3–4 g L^{-1}, respectively, are lethal to fish, according to Agrawal et al. (2019). Manganese causes leukopenia and anemia in tilapia (Alam et al., 2021). Some trace elements, such as Pb, As, Al, and Cd, are recognized to be potentially harmful including Mn, but others, such as Zn and Cr, are needed in small quantities (Mehrandish et al., 2019).

2.4.9 Mercury

Unpolluted water with mercury does not exceed more than 0.1 µg L^{-1} (Zheng et al., 2019; Gikas et al., 2020). Methyl mercury ($MeHg^{+}$) is the most toxic chronically organic form of mercury compounds (Zheng et al., 2019). According to estimates, 70 to 100% of MeHg+ is present in fish (Ali et al., 2019). Methyl mercury, an organometallic compound that is extremely lipophilic and is produced by bacterial activity from inorganic mercury (Hg), readily penetrating the blood-brain barrier (Alahabadi and Malvandi, 2018). The binding, storage, and redistribution of Hg that enters peripheral circulation occurs mostly in the liver (Chyra-Jach et al., 2020). Fish tissues are sensitive indicators for aquatic contamination and have the capacity for both organic and inorganic forms of mercury to accumulate in the tissues (Ali and Khan, 2019). Long-term retention of Hg compounds in animal tissues causes irreparable injury, including neurological impairment and lesions, behavioral and cognitive abnormalities, ataxia, and convulsions. In addition to its detrimental influence on reproduction, mercury affects the viability of spermatozoa, egg formation and the survival rate of developing eggs even at a lower concentration (Alahabadi and Malvandi, 2018; Chyra-Jach et al., 2020; Gikas et al., 2020). Mercury affects the viability of spermatozoa, egg formation, and the survival rate of developing eggs and fry, even at a very low concentration (Kumar et al., 2020a; Gikas et al., 2020).

2.4.10 Selenium

Selenium (Se) is a vital dietary trace element (Zhang et al., 2019). It is required in trace amounts for fish and human beings to maintain physiological processes such as normal development, growth, and homeostasis (Chyra-Jach et al., 2020). It is widely dispersed in the environment and is present the majority of surface and ground waters at a concentration between 0.1 and 0.4 g L^{-1} (Li et al., 2021). Selenium is a potential teratogen and carcinogen, and it becomes extremely hazardous to fish at concentrations above a threshold (Zhang et al., 2019; Li et al., 2021). The range between dietary needs and hazardous levels is extremely narrow for Se. The range for most fish is 0.25 to 0.70 g g^{-1} in diet and as low as 3-8 µg L^{-1}, causing various alterations in feral freshwater fish that are life-threatening (Chyra-Jach et al., 2020; Li et al., 2021; Huang et al., 2021). The toxic levels might be as low as a 3 g g^{-1} diet with chronic exposure. The US Environmental Protection Agency (USEPA) proposed a chronic selenium threshold for fish at a concentration of 7.91 g g^{-1} dry weight (Li et al., 2021). However, the proposed selenium level for preserving fish populations is still up for debate (Hang et al., 2021).

2.4.11 Zinc

Zinc (Zn) is a crucial micronutrient and the second most prevalent trace element after Fe. It is vital for the production of nucleic acids and is found practically in all cells (Baltaci et al., 2018). Zinc bioaccumulates in fish, through the food chain, and biomagnifies in carnivorous fish species. Koca et al. (2005) claimed that smaller fish with high zinc concentrations accumulate in their muscular tissues and biomagnify through the food chain. Zinc has a role in more complex processes such as cell signaling, the immune system, and neurotransmission (Jin et al., 2019; Kim and Lee, 2021). At higher waterborne levels, Zn wastes are directly toxic to fish (Ali and Khan, 2019). Zinc impacts on fisheries either on its own or, more often, combined with Cu and other metals (Baltaci et al., 2018; Gikas et al., 2020). The gills are the primary site of waterborne Zn toxicity, where the disruption of Ca^{2+} uptake results in hypocalcemia and final mortality (Kim and Lee, 2021). Zinc exposure has been demonstrated to cause histopathological changes in fish ovarian and hepatic tissue (Jin et al., 2019). Causing death, growth retardation, alterations to the heart, lungs and inhibition of spawning. Some freshwater fish are substantially more sensitive to the effects of zinc on serum transaminases (d'Haese et al., 2019).

2.5 Sources of endocrine-disrupting chemicals in foodstuff

Aquatic organisms accumulate toxic chemicals from contaminated surface water. Fish is one of the most used bioindicator for pollution in aquatic environments (Simukoko et al., 2022). Trace metals are incorporated in fish either through gills from the water column or through diet (Li et al., 2018a, 2018b; Lian et al., 2019; Zhang et al., 2019; Weber et al., 2020; Ali et al., 2019, 2021; Hasimuna et al., 2019; Simukoko et al., 2022). Fish is a major priority for the public and aquatic environmental health (Maurya et al., 2019; Mwakalapa et al., 2019). Muscular tissue of polluted fish has a considerably smaller absolute rise in trace metals than any other organs (Hasimuna et al., 2022; Simukoko et al., 2022). To reduces the chances of direct chemical transfer into the human body, it is wise to wash the fish bought from local or open markets before consumption with clean water, as general knowledge. However, washing fish items does not remove 100% of the contaminants as some have penetrated the muscle tissues (Nuapia et al., 2018; Ramalepe et al., 2022). Organisms' capacity to accumulate trace metals is one of greater complexity of the physiological systems that control trace metal concentration and is not well understood (Simukoko et al., 2022).

Chemicals find their way into the human immune system through inhalation, ingestion, or skin contact (Gallo et al., 2018; Mostafa and Peters, 2017; Zeng et al., 2020). Open marketplaces influence sources of trace metal particulates operated mainly on unhygienic and poorly maintained busy roads, which makes it easier for pollutants and chemicals emanating from the vehicles into the food items sold in an open market (Nuapia et al., 2018; Ramalepe et al., 2022; Simukoko et al., 2022). These trace metals are of greater concern ranging from more hazardous such as Ag, As, Au, Cr, Cu, Cd, Hg, Sb, Pb and Zn to less hazardous Ga, La, Nb, Sr and Zr (Sobota et al., 2015; Streets et al., 2017; Borghesi et al., 2016; Kanduč et al., 2019; Lazareva et al., 2019; Nuapia et al., 2018; Ahmad et al., 2020; Awasthi et al., 2022; Masindi and Muedi, 2018; Naz et al., 2018).

2.6 Bioaccumulation of inorganic endocrine-disrupting chemicals

The possibility of trace metals to bioaccumulate is followed by their level of toxicity, which continues to be a vital environmental concern in Zambia and throughout the world (Liu et al., 2018; Ramalepe et al., 2022). Endocrine-disrupting chemicals have a tendency to spread and be distributed around the world by countless pathways, inclusive of the atmosphere, lakes, rivers, streams, oceans and many more water bodies (Hochella et al., 2019). Therefore, trace metals distributed on other sides of the countries of the world can travel far from their point source areas (Liu et al., 2018). Their potential long-range of trace metals has forced legislation bodies globally to take swift actions to eradicate their usages and discharge into the environment and surface water bodies.

Bioaccumulation is an important factor (Sattari et al., 2020) as a source of trace metals accumulated in fish. This is the most straightforward method for estimating the proportional abiotic medium contributor for the assessment of the efficiency of contaminants. A fish's bioaccumulation or biomagnification of metals is indicated by a bioaccumulation factor (BAF) of 1 or higher (Liu et al., 2018; Sattari et al., 2020). According to Abel's approach (1989), the BAF was determined as follows (Aladesanmi et al., 2019):

$$BAF = \frac{C_{\text{(fish muscles)}}}{C_{\text{(water)}}} \quad (1)$$

where $C_{\text{(fish muscles)}}$ is the concentration of trace metals in fish (mg kg^{-1} dry weight); $C_{\text{(water)}}$ is the concentration of trace metals in water (mg L^{-1} wet weight). It is used to assess an aquatic organism's capacity to acquire pollutants from the water. Where BAF > 1, indicates that the fish may be able to accumulate the metal but is typically not alarming till the BCF is 100 or above. The interpretation of BAF is shown in Table 2.3. Due to the constant availability of ambient media concentrations, ecological risk evaluations are streamlined. This data is necessary for site characterization and human health assessments, typically conducted in conjunction with ecological assessments (Aladesanmi et al., 2019; Sattari et al., 2020).

2.7 Toxicity of target inorganic endocrine-disrupting chemicals

Toxic elements adversely affect to human beings (Mahurpawar, 2015; Sun et al., 2014). Their causes range from cumulative to non-cumulative effects, which vary depending on dosage intake, duration of exposure, pathway route and the consumers' state of health (Varela et al., 2020). Cumulative effects arise after repetitive exposure to hazardous material, whereas non-cumulative effects may occur by hastily absorbance and elimination of lethal substances (Ambwani et al., 2018). Contamination by inorganic substances is a serious problem, as most are carcinogenic and mutagenic agents (Petrescu et al., 2018; Huang et al., 2021). Small exposure to trace metals is associated with pathogen resistance, chronic toxicity, and endocrine gland malfunction (Manisalidis et al., 2020). The Endocrine Society and the European Commission have shown evidence of adverse effects of the trace metals on reproduction, thyroid functioning, metabolism, obesity, and brain functioning (Manisalidis et al., 2020; Petrescu et al., 2018; Varela et al.,2020).

2.8 Regulations and limits of trace metals concentration by WHO/FAO

The joint WHO/FAO established maximum permissible limits in fish and tolerable daily intake of inorganic EDCs as non-toxic, as shown in Table 2.1 (USEPA, 2016; WHO/FAO, 2018). Tolerable daily intake is dependent on an individual's body mass index (BMI). The toxicity extent of each endocrine disruptor differs from one person to another and is based on transgenerational effects, non-traditional dose-response dynamics, age of exposure, and latency following exposure (Lymperi and Giwercman, 2018; Schug et al., 2011). Table 2.1 shows the maximum permissible levels in milligrams per kilograms recommended by FAO/WHO and the tolerable daily intake recommended by USEPA.

Table 2.1 Maximum permissible levels in parts per million recommended by FAO/WHO (2018) and tolerable daily intake recommended by USEPA (2016)

Trace element	Maximum permitted concentration (mg kg^{-1})	Tolerable daily intake (mg kg^{-1})
Aluminum	100	120
Arsenic	0.1	0.004
Cadmium	2	0.0012
Chromium	1	0.039
Copper	10	10
Iron	100	40
Lead	0.5	0.24
Manganese	0.5	11
Mercury	0.5	0.0016
Selenium	1	0.055
Zinc	100	40

2.9 Different Sample digestion techniques for solid samples

Several techniques have been developed over the years for the digestion of solid samples for metal and metalloid analysis. The digestion or dissolution of a sample remains a fundamental part of metals for a wide range of determination in various samples (Balaram et al., 2022). Environmental analytical chemists are continuously improving these techniques to miniaturize and make them feasible, simplify, and efficient, and to minimize reagent consumption. Moreover, shortening analysis time makes the techniques greener without compromising the accuracy and sensitivity (Maciel et al., 2019; Vázquez et al., 2019). Which is inherently capable of inspiring the development of microextraction methods and being applied in different samples (Safari et al., 2017; Tang et al., 2018).

Conventional techniques for sample preparation include open acid digestion and fusion extraction, requiring considerable time and effort, as they are expensive and use large amounts of reagents and the recovery is often compromised (Goh et al., 2019; Mwaurah et al., 2020; Pérez-Rodríguez et al., 2018). Therefore, modern sample preparation methods have been developed to overcome these drawbacks including microwave digestion. This section describes suitable modern extraction techniques, looking at their advantages, drawbacks and capacity to extract trace metals (Pérez-Rodríguez et al., 2018; Goh et al., 2019; Mwaurah et al., 2020).

2.9.1 Fusion extraction techniques

An alternative digestive technique is provided by the fusion decomposition of environmental samples For instance, trace metals can be found in fish, sediments, plants, wastewater, and municipal solid waste (Jin et al., 2020; Chen et al., 2020; He et al., 2020; Yadav et al., 2020; Rubalingeswari et al., 2021; Lin et al., 2021). This high-temperature method creates a residue that can be easily dissolved by heating powdered samples with the right flux. Fusion extraction digestion is one of the most thorough digestions, and when fused with the right flux, any silicate materials can be brought into a full solution. The main drawback of the fusion approach is the addition of salts from the flux into the final solution, increasing total dissolved solids. The ideal method for quantitative silicon analysis is still fusion breakdown, which may also be the only realistic way to completely decompose refractory minerals like zircon, rutile, and cassiterite. Many fluxes have been utilized, but lithium metaborate ($LiBO_3$) may be the most popular. This flux is applied at a relatively modest flux: sample ratio of 3:1, adding only Lithium and Boron to the final solution (Trueman, 2019).

2.9.2 Acid digestion

Open vessel acid digestion is a popular and straightforward technique for breaking down both inorganic and organic sample matrices, inclusive of sediments, soil, water, wastewater and biological tissues (Bellasi et al., 2020; Barbosa et al., 2020; Delgado-Gallardo et al., 2021; Hale et al., 2022). It is an acid attack in open containers or screw-top vials on a hot plate (at low pressure). This method is used for routine analysis because it offers flexible control over digestion parameters, such as temperature, duration, and reagent addition. It has drawbacks like poor volatile chemical recovery and airborne pollution. Moreover, it requires large amounts of reagents, long run times and lead to poor digestion quality. Because of this, open vessel acid digestion has not been regarded as a cutting-edge technique in trace and ultra-trace sample preparation (Goh et al., 2019; Mwaurah et al., 2020).

2.9.3 Microwave digestion

Microwave digestion is useful when preparing samples for trace metal analysis, and regarded as a cutting-edge technique for trace and ultra-trace sample preparation (Goh et al., 2019; Mwaurah et al., 2020). This technique dissolves solid matrices into liquids for analysis while heating up the sample in combination with concentrated acid (Mwaurah et al., 2020). Hudgins (2009) asserts that microwave digestion has a number of significant advantages over competing processes like open vessel hot block digestion, often known as acid digestion. In the field of trace metal analysis, microwave digestion has proven to be a very effective sample preparation technique (Goh et al., 2019). It provides quick, thorough digestion and prevents problems like contamination from outside sources and the loss of volatile components like Pb, Cd, As and Hg. The digestion method can be validated according to the food matrix in the lab (FSSAI, 2016). The technique is not foolproof, though, so it is crucial to examine the elements mentioned above to make sure the procedure is effective and safer (Goh et al., 2019; Mwaurah et al., 2020). Hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) can produce chlorides and sulfates of metal ions, hence nitric acid (HNO_3) is often utilized for acid digestion. Several researchers recommended that biological samples be digested using hydrogen peroxide as an oxidizer and either HCl and HNO_3 or HNO_3 and HF alone or in combination (Usman et al., 2019). The complete breakdown of silicon-rich plant material requires HF (Safari et al., 2017).

2.10 Trace metals: Inorganic EDCs

2.10.1 Trace metal pollution assessment for fish

2.10.1.1 Pollution indices used on the assessment of fish

The amount of pollution in aquatic habitats has been measured using a variety of metrics like bioaccumulation factor (BAF) and bioconcentration factor (BCF), contamination factor (CF), metal pollution index (MPI), contamination degree (CD), modified degree of contamination (mCD) and metal selectivity index (MSI) (Aguilera et al., 2021; Hasimuna et al., 2021).

Each of these indices has advantages and disadvantages. However, each of the indices are used depending on the user's preferences and the amount of information available to them. When using certain indices, it's crucial to have background knowledge on the levels of elements in a given area before significant anthropogenic activity occurred. If background information on elemental levels prior to significant anthropogenic activity is unavailable, other pollution indices that do not rely on this data may be used. The BAF and BCF, CF, CD, and MSI contamination indices were utilized in this investigation due to their descriptive behavior of chemicals in terms of their likelihood of concentrating in organisms and the environment (Buch et al., 2021; Koelmantset al., 2022).

2.10.1.1.1 Pollution contamination indices for CF and CD

The contamination factor (CF) is calculated for each trace metal against the background value of a metal, used when evaluating fish contamination pollution, while the CD provides a general data on the fish contamination caused by a particular element at a specific study site Using Eq. 2. The level/degree of contamination (CD) is determined by the difference between the pre-industrial "background or baseline value of metal" fish and the mean concentration of a certain pollutant in the contaminated fish. It is made up of all contamination elements added together resulting in an indication of an overall level of pollution from a specific site, Table 2.2 shows the interpretation level of contamination (Aguilera et al., 2021). The background mean concentration (mg kg^{-1}) of trace metals studied for 2021 and 2022 respectively in sun-dried bream were 37,05 and 24,33. Whereas sun-dried Kapenta were 54,42 and 52,11 where in fresh bream were 28,34 and 32,46. Whereas for WHO/FAO, 2018 and Simokoko et al., (2022) the background values was Al (100 and 4.28), As (0.1 and 0.042), Cd (2 and 0.0022), Cr (1 and 0,16), Cu (10 and 0.33), Fe (100 and 5.34), Pb (0.5 and 0.009), Mn (0.5 and nd), Hg (0.5 and 0.008), Se (1 and 0.18), Zn (100 and 4.75) respectively.

$$CF = \frac{C_{(element)}}{C_{(background)}} \quad (2)$$

where $C_{element}$ is the elements concentration in fish, and $C_{background}$ is the background concentration of the element. In this investigation, the lowest metal concentration was used as a baseline or background value. Generally, the background values found in healthy, non-degraded, or managed fish pond or farms are used as background values; when this information is not available, the global background values for biota can been used or even the mean value found in the data for the current study (Declercq et al., 2019; Aguilera et al., 2021). In this research, the maximum permissible limits recommended by WHO/FAO (2018) found in Table 2.1 was used because no background values have been established for fish ponds or biota. Moreover, we used the mean value of the current study and also the those determined by Simukoko et al. (2022) to allow some variation and tolerance to make comparisons between the three proposals (Aguilera et al., 2021). Table 2.2 shows the interpretation level of contamination (Aguilera et al., 2021).

Table 2.2: Interpretation for contamination degree (CD) and contamination factor (CF) (Tahity et al., 2022)

CD value	Interpretation	CF value
≥24	Very high degree of contamination	> 6
12 ≤Cd < 24	Considerable degree of contamination	3 < CF < 6
6 ≤Cd < 12	Moderate degree of contamination	1 < CF < 3
< 6	Low degree of contamination	< 1

2.10.1.1.2 Metal selectivity index

It is crucial to understand how the elements bioaccumulate in the fish muscle tissues for the sake of human health (Sattari et al., 2020). The metal selectivity index (MSI) for each tissue was used to express the total elements (TEs) concentrations in Eq. 3. Table 2.3 shows an interpretation for bioaccumulation factors.

$$\text{MSI} = \frac{A}{T} \times 100\% \quad (3)$$

Where A is the absolute concentration of a metal in a tissue and T is the total concentration of all TEs in that tissue.

Table 2.3: Bioaccumulation factor (BAF) interpretation

BAF	Interpretation
< 1	no accumulation
1-3	minor accumulation
3-5	moderate accumulation
5-10	moderately severe accumulation
10-25	severe accumulation
25-100	very severe accumulation

> 100	extremely severe accumulation

2.1 Human health risk by trace metal contamination in fish

For one to estimate the risk of trace metals, there are three main pathways that may occur for exposure of target analytes to human beings: (a) direct ingestion, (b) inhalation through the mouth and nose, and (c) dermal absorption. However, for trace metals in aquatic environments (fish), ingestion and dermal absorption play the most important role for estimated daily intakes for the study (Aguilera et al., 2021; Munyeshury et al., 2021; Hashempour-baltork et al., 2023). Considering the two pathways mentioned above, the exposure dose is calculated using Eq. 4 and 5 adapted from the US Environmental Protection Agency (Aguilera et al., 2021). The lifetime average daily dose (LADD) is used estimate the carcinogenic risk (Eq. 6). Anthropogenic pollution is determined by the bioaccumulation factor (BCF). Table 2.4 shows the exposure factors of reference populations for human health risk assessment.

$$EDI_{ing} = \frac{C \times IngR \times EF \times ED}{BW \times AT} \tag{4}$$

$$EDI_{dermal} = \frac{C \times SA \times AF \times ABS \times EF \times ED \times CF}{BW \times AT} \tag{5}$$

$$LADD = \left(\frac{C}{PEF \times ATcan}\right)\left(\frac{CR_{child} \times EF_{child} \times ED_{child}}{BW_{child}}\right) + \left(\frac{CR_{adult} \times EF_{adult} \times ED_{adult}}{BW_{adult}}\right) \tag{6}$$

The health risk calculations use assumptions by Ezemonye et al. (2019);

1. The amount of pollution ingested is equivalent to the amount absorbed.
2. The pollution is unaffected by cooking.

Table 2.4: Exposure factors of reference populations for human health risk assessment (Ali et al., 2019; Aguilera et al., 2021; Munyeshury et al., 2021)

Reference Factor	Definition and units	Value	
		Child	Adult
IngR	Ingestion rate (mg/day)	23.3	23.3
PEF	Particle emission factor	1.36×10^9	1.36×10^9
SA	Surface of exposed skin area (cm^2)	2800	5700
ABS	Dermal absorption factor	0.001	0.001
AF	Skin adherence factor (mg cm^{-2})	0.2	0.07

ED	Duration of exposure (years)	6	24
EF	Frequency of exposure (days/year)	156	156
AT	Average time non-carcinogens (days)	ED*156	ED*156
Atcan	Average time for carcinogens (days)	70*156	70*156
BW	Body weight (kg)	15	70
C	Trace metal concentration (mg kg^{-1})	This study	This study
CF	Conversion factor (kg/mg)	1×10^{-3}	1×10^{-3}

The study's exposure variables are all independent of the population references in Table 2.4. Although the exposure factors for Zambian City and its inhabitants have not yet been evaluated, the use of local factors could not only impact but also enhance the dependability of the model. The contact (or absorption) rate is known as carcinogenic risk (CR). For ingestion and dermal contact, CR_{ing} = IngR and CR_{dermal} = SA × AF × ABS respectively. Each carcinogenic metal has a different form of CR depending on the route of exposure where it can cause cancer (Table 2.4). As demonstrated in Eq. 7, risk ratios for dermal contact and ingestion (HQing/derm) is hazard quotient calculated by dividing the EDI by the reference dose (RfD), as shown in table 2.5.

$$HQ_{ing/dermal} = \frac{\mathrm{ED}_{\mathrm{ing/dermal}}}{\mathrm{RfD}} \quad (7)$$

The total of the HQs for the two exposure pathways is the hazard index (HI). An HI of greater than 1 could have non-carcinogenic consequences on population health; a lower HI would be predicted to have the opposite effect (Aguilera et al., 2021). Eq. 8 is frequently used to calculate the incremental lifetime cancer risk (ILCR) for carcinogenic substances:

$$\mathrm{ILCR} = \mathrm{LADD} \times \mathrm{CSF} \quad (8)$$

The range of 1×10^{-6} to 1×10^{-4} is an acceptable or tolerable risk for carcinogenic substances, and these values indicate that an additional case in a population of 1 in 1000000 and 10000 persons, respectively (Aguilera et al., 2021; Ikem et al., 2021).

Table 2.5: Reference dose (RfD) and cancer slope factor (CSF) for each route of exposure (Storelli et al., 2020; Aguilera et al., 2021)

Trace metal	Oral RfD	Dermal RfD	Oral CFS	Dermal CSF
Al	1.00×10^{0}	-	-	-
As	3.00×10^{-4}	1.23×10^{-1}	1.50×10^{0}	-
Cd	1.00×10^{-3}	5.00×10^{-3}	6.30×10^{0}	-
Cr	3.00×10^{-3}	15.00×10^{-3}	1.70×10^{-3}	-
Cu	4.02×10^{-2}	1.20×10^{-2}	-	-
Fe	8.40E + 00	7.00×10^{-2}	-	-
Hg	$3{,}00\times10^{-4}$	$2{,}10\times10^{-5}$	-	-
Mn	4.60×10^{-2}	1.85×10^{-3}	-	-
Pb	3.50×10^{-3}	5.25×10^{-4}	8.50×10^{-3}	-
Se	5.00×10^{-3}	2.2×10^{0}	-	-
Zn	3.00×10^{-3}	6.00×10^{-2}	-	-

2.11 Sample dying techniques for solid samples

Drying is one processing method, widely utilized for removal of moisture content from the sample. It has numerous advantages which include lowering storage volume, to maintain and extend the product shelf-life (Younis et al., 2018; Turkmen et al., 2020; Sridhar and Charles, 2022; Vu et al., 2022).

2.11.1 Freeze-drying technique

Vacuum freeze drying is scientifically known as lyophilization. The method uses both vacuum and freeze methods, allowing the moisture to sublimate under the vacuum state to form a dry solid product (Bhatta et al., 2020; Oyinloye and Yoon, 2020; Ramalepe et al., 2022). Freeze-drying involves using low temperatures to dehydrate a sample by freezing it, reducing the pressure, and removing moisture content (Bhatta et al., 2020; Oyinloye and Yoon, 2020; Silva-Espinoza et al., 2021; Ramalepe et al., 2022). The moisture is drained directly through the sublimation in a gaseous state (Bhatta et al., 2020; Oyinloye and Yoon, 2020; Ramalepe et al., 2022). The process allows for controlled freeze-drying samples without changing the characteristics and structure of the dried sample (Nowak and Jakubczyk, 2020). Sublimation refers to a direct phase change of a solid (ice) to a vapor without changing to a liquid state (Bhatta et al., 2020; Oyinloye and Yoon, 2020; Silva-Espinoza et al., 2021). The frozen sample is placed in a suitable container or glass vial, flask or tray. Then placed in deep vacuum, well below the triple point of water and dried by applying heat energy to the sample causing the ice to sublime (Hriberšek et al., 2018; Lenaerts et al., 2019; Taskin, 2020; Zhao et al., 2020; Ramalepe et al., 2022). Figure 2.6 and 2.7 shows the freeze-drying process while Figure 2.8 shows a freeze drier.

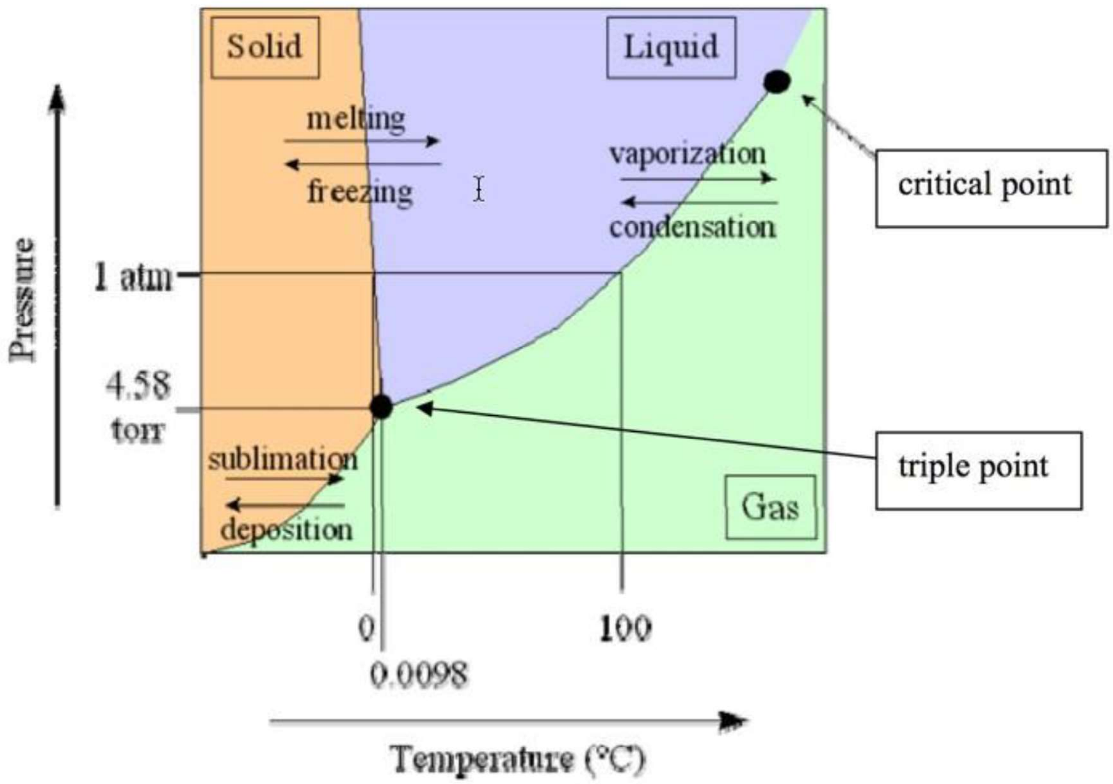

Figure 2.6: Basic principle of freeze-drying (Waghmare et al., 2022)

The steps required for the freeze-drying process include three stages that is freezing, primary drying and secondary drying stage summarized below (Figure 2.6-2.8).

i. Pre-treatment of the samples. Samples are pre-frozen before loading and the maximum cooling is achieved with a closed door. The sample temperature is monitored with probes.

ii. Loading of the sample into a suitable container (Bulk, Flask, Vials).

iii. Primary drying under vacuum (sublimation). The collector temperature is below −40°C

iv. Secondary drying (desorption) under vacuum.

v. Backfill and stoppering (for product in vials) under partial vacuum.

vi. Removal of dried product from freeze dryer.

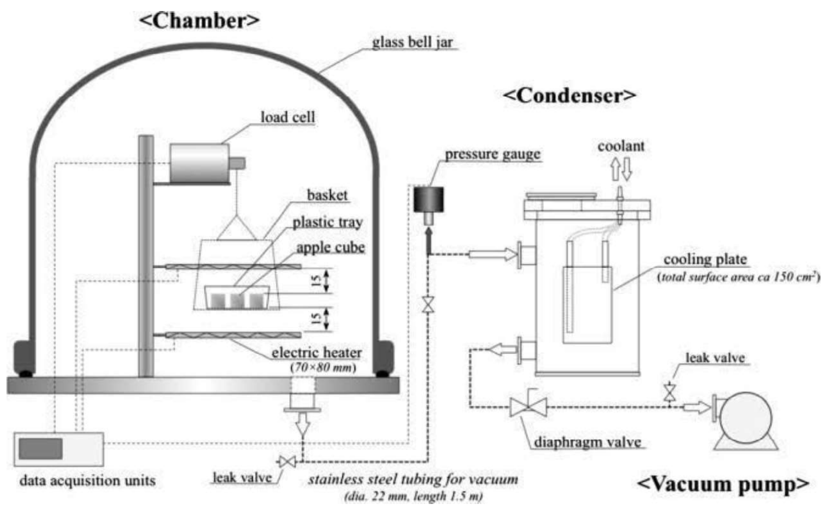

Figure 2.7: Schematic diagram of a freeze-drier (Nakagawa and Ochiai, 2015)

Figure 2.8: LABCONCO Freeze- drier (LABCONCO CORP. 811 Kansas City, Missouri)

From the perspective of the finished product's quality, freeze drying is regarded as the best method for drying food due to the absence of heating, sensory, while the nutrients properties of the materials are well retained (Fedchak et al., 2019; Ge et al., 2019). The process enables for maintaining the quality of dried thermolabile pharmaceuticals, food goods, and biological products (Shingisov and Alibekov, 2017; Taskin, 2020).

2.11.2 Oven-drying technique

Thermogravimetric technique which involves loss of moisture content by drying a sample for definite time at a constant temperature is known as an oven-drying technique (Figure 2.13). The sample is heated under different conditions, and the weight loss is utilized to determine the sample's moisture content (Fedchak et al., 2019; Ge et al., 2019; Vu et al., 2022). The type of oven used, the conditions inside the oven, as well as the drying time and temperature, all have a significant impact on the moisture (Sridhar and Charles, 2022; Vu et al., 2022). The moisture removed from the sample in the chamber loads at well-defined temperature, and atmospheric pressure. The way of nature or forcing convection and radiation, thermal energy entering the oven chamber (Figure 2.9a). The back of the interior walls aids in evenly distributing heat throughout the oven at high temperatures, allowing the dried air to circulate more quickly. The weighted sample is exposed for a definite drying time of a certain temperature resulting in loss of water contents (Figure 2.9b).

The oven-drying is useful for determining the humidity contents of fuel, grain and other food material. However, multiple studies indicated that high temperature drying procedures caused a number of changes in the product's qualitative characteristics and bioactive content, demonstrating that drying temperature and duration might impact the final product's quality (Sridhar and Charles, 2022; Vu et al., 2022). For instance, in 2022 Sridhar and Charles examined the impact of drying temperature on the physico-chemical characteristics of Kyoho seeds and concluded that drying temperature has an impact on the significant changes in physico-chemical characteristics of grape seeds (Fedchak et al., 2019; Ge et al., 2019). Additionally, a number of studies noted an increase or decrease in the bioactive potential of various fruit crops, which presents a problem for many food engineers who take the initiative to optimize drying temperatures and duration to maintain the product's quality (Vu et al., 2022). Figure 2.9 and 2.10 shows the oven drying process and the oven respectively.

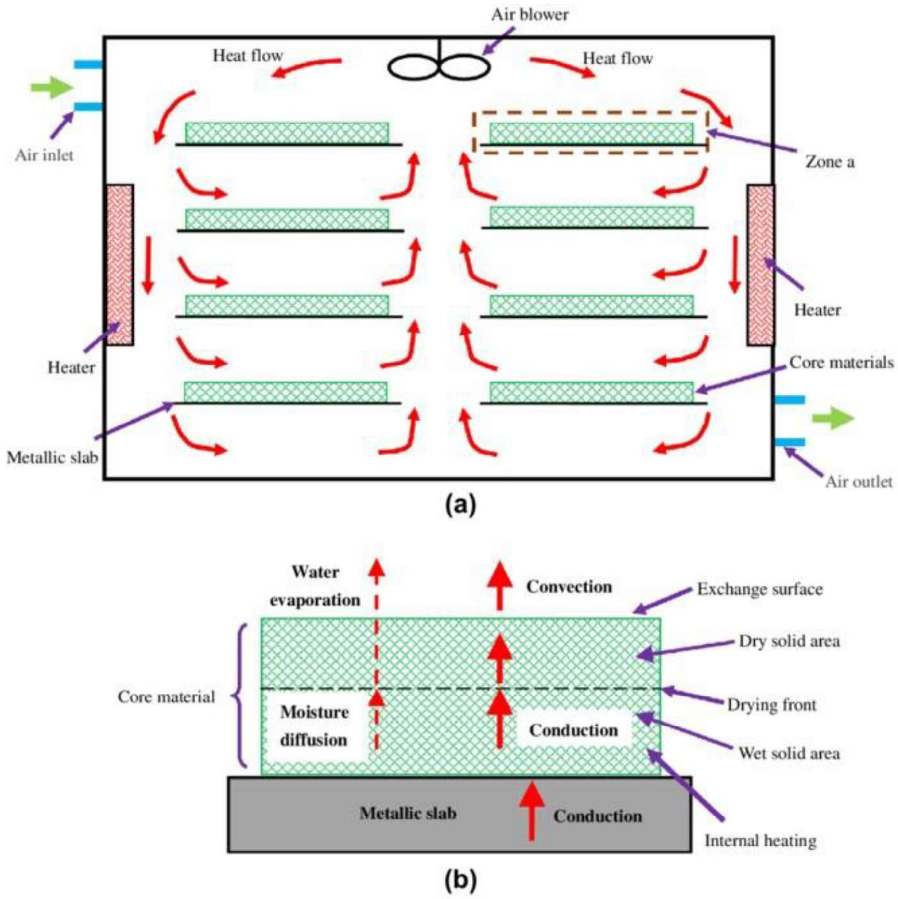

Figure 2.9: Outline of a drying model: (a) schematic diagram of vacuum oven drier; (b) Basic principle of vacuum drying process (enlarged from zone) (Li et al., 2013)

Figure 2.10: WiseCube Oven- drier (WiseCube WIS-10 Am bildacker, Deutschland)

2.12 Analytical instruments for metal analysis

2.12.1 Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES)

The technique has been utilized for analyze trace metals in agricultural samples, food matrix and aquatic environments, inclusive of soils, fertilizers, plant materials and aquatic samples (Borges et al., 2020; Khan et al., 2021). Analyzing metals with ICP-OES involves several challenges. The complexity of many metals' emission spectra can lead to spectral interferences, where different elements' emission lines overlap, complicating accurate analysis. Additionally, matrix effects occur when other components in the sample impact signal strength, affecting the accuracy and detection limits. These factors can reduce the dynamic range of the instrument, making it harder to detect and quantify specific metals (Nyika et al., 2019). However, these challenges can be overcome by selection of the careful calibration, method parameters, correct spectra or wavelength and correction techniques. Figure 2.11 and 2.12 shows the picture and the schematic diagram of the ICP-OES.

Figure 2.11: Spectro Genesis ICP-OES used for analysis (Kashani et al., 2010)

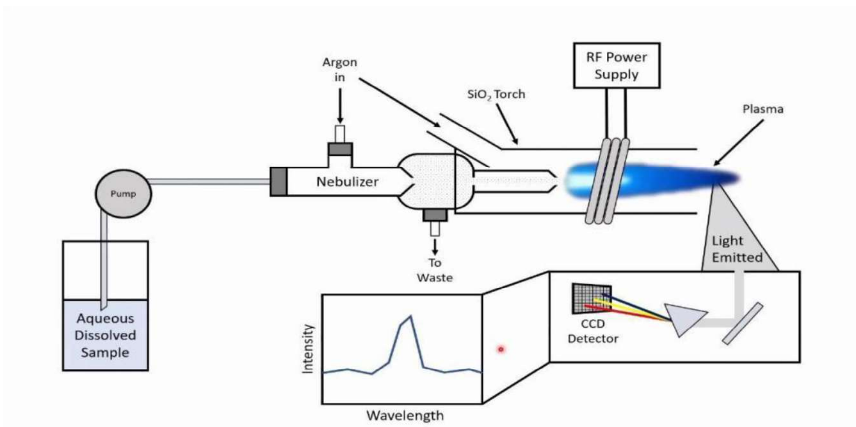

Figure 2.12: Cross-section schematic of an ICP-OES (Kashani et al., 2010)

Table 2.2 illustrate concentrations for 11 of thirty-four elements of interest in fish (Oreochromis niloticus) sampled in 2019 by Sattari and others. The trace elements were analyzed with ICP-OES and the rest with ICP-MS for ultra-trace elemental analysis. The illustration shows that the elemental levels in the sample are influenced by the environmental pollutants, with no record of exposure to other sources of pollution beyond the collection site. The study was done by Sattari et al. (2019).

Table 2.6: Concentrations (mg kg^{-1} dry wt.) of nine elements in selected fish samples

Element	Mean ± RSD (mg kg^{-1} dry wt.)	p value
Aluminum	4.94 ± 1.50	0.99
Arsenic	0.08 ± 0.04	0.30
Cadmium	0.01 ± 0.0	0.18
Chromium	0.04 ± 0.007	0.33
Copper	0.012 ± 0.05	0.92

Iron	1.46 ± 0.54	–
Lead	0.08 ± 0.02	0.19
Manganese	1.11 ± 0.95	0.15
Zinc	1.48 ± 0.32	0.60

2.13 The Inductively Coupled Plasma Mass Spectroscopy

The ICP-MS is used for ultra-trace elemental analysis and metalloids speciation (Williams and Marcus, 2020; Lu et al., 2020; Samanta et al., 2021). It is capable of detecting metal concentrations from 1 fg L^{-1} to 1 µg L^{-1}. For improved resolution and analyte-background intensity ratio, ICP-MS parameters like ion optics voltages, mass scan, duration scan, pump speed, and argon flow are tuned (Satyanarayanan et al., 2018; Williams and Marcus, 2020; Lu et al., 2020; Samanta et al., 2021). Figure 2.13 and 2.14 shows the picture and the schematic diagram of the ICP-MS.

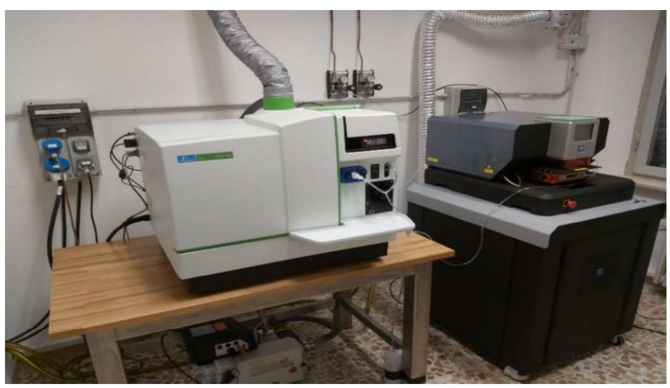

Figure 2.13: Laboratory ICP-MS used for analysis (Wilschefski et al., 2019)

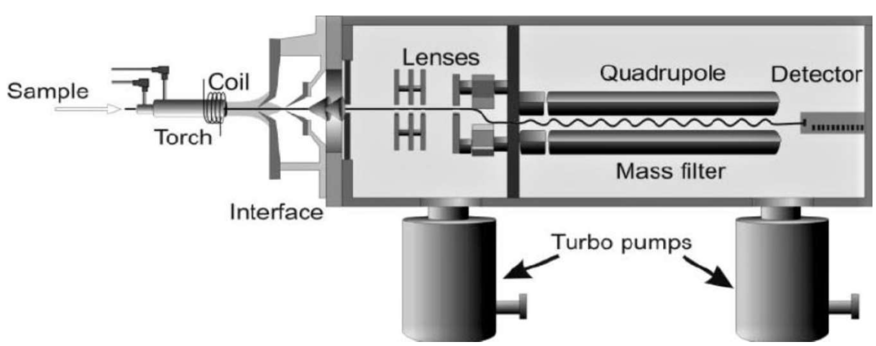

Figure 2.14: Cross section schematic of an ICP-MS (Wilschefski et al., 2019)

Atli et al. (2021) used ICP-MS to measure metalloid trace metals in fish, with Hg and Se being the primary target metals. Their study showed good sensitivity for Al, Cd, Cr, Cu, Fe, Mn, Pb, Se and Zn at low concentration levels from 1 fg L^{-1} to 1 µg L^{-1}. According to Georgescu et al. (2011), trace metals like Hg act as endocrine disruptors, with effects being the induction of alterations in male and female fertility and disrupt biosynthesis of steroid hormone.

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CHAPTER 3: MATERIALS AND METHODS

This chapter reviews the methodologies involved to complete the aims and objectives of the research. Use of analytical digestion technique and quantification of inorganic EDCs are discussed.

MATERIAL AND METHODS

3.1 Study area and sampling

The study was conducted in Lake Kariba fishery (Figure 2.1), situated in the middle of Zambezi River Basin in southern province of Zambia and shared with Zimbabwe (−17° S 28° E). It is currently the largest artificially man-made reservoirs in Africa by volume of 157 million cubic metres (Tran et al., 2018; Njiru et al., 2018; Aura et al., 2018; Simukoko et al., 2022; Mutasa et al., 2023).

Fish of interest, fresh and sun-dried Kariba bream and sun-dried Kapenta, were collected from open market in Siavonga in an appropriate container and transported to the lab with no sunlight exposure and stored in a cool dry place. In order to obtain a representative sample, twenty-eight fresh and five sun-dried Kariba bream fish species and sun-dried Kapenta fish species were obtained from five different stall-batch from open fish markets in Siavonga, Lake Kariba, Zambia in November 2021 on the first sampling. On the second sampling, four different stall-batch on sun-dried Kapenta, six fresh Kariba breams and five sun-dried Kariba breams were collected in August 2022. The sampling at these times served as a monitoring purpose, such as assessing the market dynamics which contribute to a broader study on the fish population and target open market sustainability (Tran et al., 2018; Njiru et al., 2018; Aura et al., 2018; Simukoko et al., 2022). Targeted open market sites were markets where most people purchase the foodstuff, mostly markets that operate in close proximity to Lake Kariba, heavy vehicles emissions and industrial activities.

3.2 Sample collection and preparation

Blank and recovery studies were conducted on commercially available fish samples bought from Johannesburg CBD. The fish were transported to the lab and descaled, washed thoroughly with deionized water, then filtered to remove muscle tissue, and freeze-dried over several days. Fresh fish species muscle tissues of real samples were thawed into pieces with the aid of a steam-cleaned stainless-steel knife and wrapped with aluminum foil and packed in polyethylene bags and placed in cooler boxes with iced-blocks. Fish samples were transported to the lab, rinsed with distilled water and freezed for 72 h in the laboratory between −18°C and 20°C. All fresh freeze-dried samples were homogenized into a fine powder using domestic blender and to a very fine particle sizes with a pestle mortar. While a mass of 5 g of the sun-dried fish sample were rinsed with distilled water and oven-dried in the oven at 40°C until a constant weight was obtained, which was the total weight of sample used for the analysis. The dry fine powder samples were then stored in the PTFE tubes lined with parafilm and kept frozen below −18℃ until further analysis. Figure 3.1 and 3.2 shows the sun-dried fish by oven and fresh dried fish by a freeze drier respectively.

Figure 3.1: Oven drier for sun-dried fish samples (WiseCube WIS-10 Am bildacker, Deustschland)

Figure 3.2: LABCONCO Freeze-drier (LABCONCO CORP. 811 Kansas City, Missouri) used, fresh dried bream samples

3.3 Chemicals and reagents

All reagents and chemicals used were of analytical grade or higher for the analysis of inorganic EDCs at a level of mg kg^{-1}. Purchased from Merck (Johannesburg, South Africa) were supra pure grade of 55% w/v nitric acid (HNO_3) and 30% w/v hydrogen peroxide (H_2O_2). Nitric acid (HNO_3) of 5% was used for instrument cleaning, prepared from 55% HNO_3 using distilled water.

3.4 Inorganic endocrine disrupting chemicals analysis

3.4.1 Preparation of the stock solution

A 1000 mg L^{-1} stock solution of multi-elemental EDCs used was prepared in a 25 mL volumetric flask by withdrawing 25 mg of individual salts into the flask and filling it to the mark with distilled water. A 10 mg L^{-1} standard solution of the eleven metals was prepared from the 1000 mg L^{-1} stock solution by withdrawing 100 µL of the prepared stock solution into a 10 mL volumetric flask and diluting to the mark with distilled water. It is from this 10 mg L^{-1} standard solution of that 0.1 mg L^{-1} to 10 mg L^{-1} standard solution was made using dilutions equation. The prepared multi-element standard solutions were then stored at 4℃ until analysis.

3.5 Instruments used for metal analysis

Inorganic endocrine disrupting chemicals for analysis of fish is described in this section.

3.5.1 Microwave digestion of foodstuff for trace metal analysis

Anton Paar Go microwave digestion system (Switzerland) was used for digestion of fish samples. The metal analysis was done using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Spectro, Kleve, Germany) and Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, PerkinElmer, Germany). Figure 3.3 shows the Anton Paar Go microwave used for closed digestion.

Figure 3.3: Anton Paar Go microwave used for closed digestion

The fish items were digested using the program (Table 4.1).

Table 3.1: Microwave digestion program

Ramp (mm:ss)	Temperature (°C)	Hold (mm:ss)
15 :00	180	15: 00

Parameter data for microwave digestion

Application type: Digestion, Vessel mode: multi-vessel, Temperature program mode: Average, Temperature limit (°C): 190

Microwave system of fish samples

A mass of 0.25 ± 0.018 g of sample powder was weighed using an analytical balance (Precisa 180A, Switzerland) and placed into acid washed digestion tubes (PTFE-TFM liners). A volume of 16 mL HNO_3 and 4 mL H_2O_2 was added to the tubes. The tubes were sealed and placed into a motor, which was subjected to Anton Paar microwave Go digestion. The digestion program conditions are displayed in Table 4.1. After digestion was completed, the digested solution was transferred into 50 mL PTFE tubes and diluted to the 50 mL mark with distilled water. The 10 mL diluted solution was then filtered using 0.22 µm PVDF syringe filters into acid washed PTFE tubes for ICP-OES/MS analysis. The concentrations of eleven trace elements (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Pb, Se and Zn) were analyzed in all fish samples.

3.5.2 Inductively Coupled Plasma-Optical Emission Spectroscopy analysis

The Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP - OES) analysis was calibrated first to get the best sensitivity for analyzing trace metals. Each trace metals were analyzed at different metal wavelengths (nm) for Al (396.153), As (193.696), Cd (228.802), Cr (267.716), Cu (324.752), Fe (238.204), Pb (220.353), Mn (257.610), Hg (253,652), Se (206,279) and Zn (213.856), to accommodate the relative concentrations of the trace metals with an increase in sensitivity. The instrument was calibrated with a blank and metal standards ranging between 0.1 mg L-1 to 10 mg L-1. The ICP-OES parameters on the day of analysis were provided by the instrument as shown in Table 3.2. Figure 3.4 shows the inductively coupled plasma-optical emission spectroscopy laboratory set-up for the analysis.

Table 3.2: Operation parameters for ICP-OES on the day of analysis

Parameter used	Optimal condition
Coolant flow	13 mL min^{-1}
Auxiliary flow	1 mL min^{-1}
Plasma power	1400 W
Type of Nebulizer	Cross flow
Nebulizer flow	1 mL min^{-1}

Figure 3.4: Inductively coupled plasma-optical emission spectroscopy (ICP - OES) laboratory set-up for the analysis

3.5.3 Inductively Coupled Plasma Mass Spectroscopy analysis

The Inductively Coupled Plasma-Mass Spectroscopy (ICP - MS) analysis was first calibrated for better sensitivity of analyzing trace metals. Each trace metal was analyzed at different metal isotopes to accommodate the relative concentrations of the trace metals with increase in sensitivity and matrix interferences. The instrument was calibrated with a blank and metal standards from the following concentrations 0.1, 5, 10, 20, 50, 100 µg L^{-1}. The ICP-MS parameters for the analysis were provided by the instrument shown in Table 3.3. Figure 3.5 shows the inductively coupled plasma-mass spectroscopy laboratory set-up for the analysis.

Table 3.3: Operation parameters for ICP-MS on the day of analysis

Operating conditions	Parameter
RF power	1.55 kw
Plasma gas flow rate	15 L min^{-1}
Makeup gas flow rate	0.8 L min^{-1}
Carrier gas flow rate	0.2 L min^{-1}
Sampling depth	10.0 mm
Date points	3 points peak^{-1}

Figure 3.5: Inductively coupled plasma-mass spectroscopy (ICP - MS) laboratory set-up for the analysis

3.5.4 Method limit of detection

The method limit of detection (LOD) for the ICP-OES/MS is the minimum measured concentration of each metal reported with 99% confidence distinguishable from method blank results. This was done using the formula in Eq. 9 (Alexander et al., 2019; Barros et al., 2019; Virgilio et al., 2020; Samanta et al., 2021; Ramalepe et al., 2022):

LOD = 3 x standard deviation of the blank samples (9)

3.5.5 Percentage recovery (accuracy)

Sample pre-concentrated experiment was designed for extraction of trace metal recovery (Virgilio et al., 2020; Samanta et al., 2021). Six fish samples were spiked in replicates of 1, 3 and 5 mg L^{-1} of multi elemental standard. For extraction method of trace elements in fish, the percentage recovery was calculated using Eq. 10.

$$\%\text{Recovery} = \frac{\text{concentration of spiked} - \text{concentration of } unspiked}{\text{concentration of spiked}} \times 100 \quad (10)$$

3.5.6 Statistical data analysis

Statistical analysis for the mean, standard deviation and P-values for comparison studies was done using Microsoft Excel with ANOVA plugin and Origin Pro software.

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CHAPTER 4: RESULTS AND DISCUSSION

This chapter presents the experimental results of the research methods used for trace metals as inorganic EDCs analysis

The chapter is made up of two parts:

Part 1: Metals as inorganic EDCs analysis for first sampling (November 2021) and second sampling (August 2022)

Part 3: Health risk assessment of inorganic endocrine disruptive compounds

RESULTS AND DISCUSSION

4.1 Concentration of metals in Zambian fish for the first and second sampling

4.1.1 Total metal concentration in the fish

The current study of trace metal analysis in musculature tissues of Kariba bream and Kapenta were particularly selected to evaluate the human potential health risk, because it is the only edible muscle tissue and serves as a staple food for Zambian population. Fish is deemed as one of the most considerable bio-indicators for metal pollution in aquaculture, thus concentration of toxicants in fish is of a greater concern (Bahgy et al., 2021). Selected fish samples were analyzed for eleven metals (Cr, Mn, Cu, As, Se, Cd, Al, Fe, Zn, Hg and Pb). Their concentrations are reported in Table 5.1 and 6.4 for sun-dried bream and Table 5.2 and 6.5 for Kapenta and fresh bream was reported in Table 5.3 and 6.6 for 2021 and 2022 samples respectively, together with their biometric data for fish and the maximum limits set by WHO/FA0 (2018) in appendix A. Figure 5.1 and 5.3 shows the concentration of metal ions detected in fish foodstuff from Siavonga open market for 2021 and 2022 respectively.

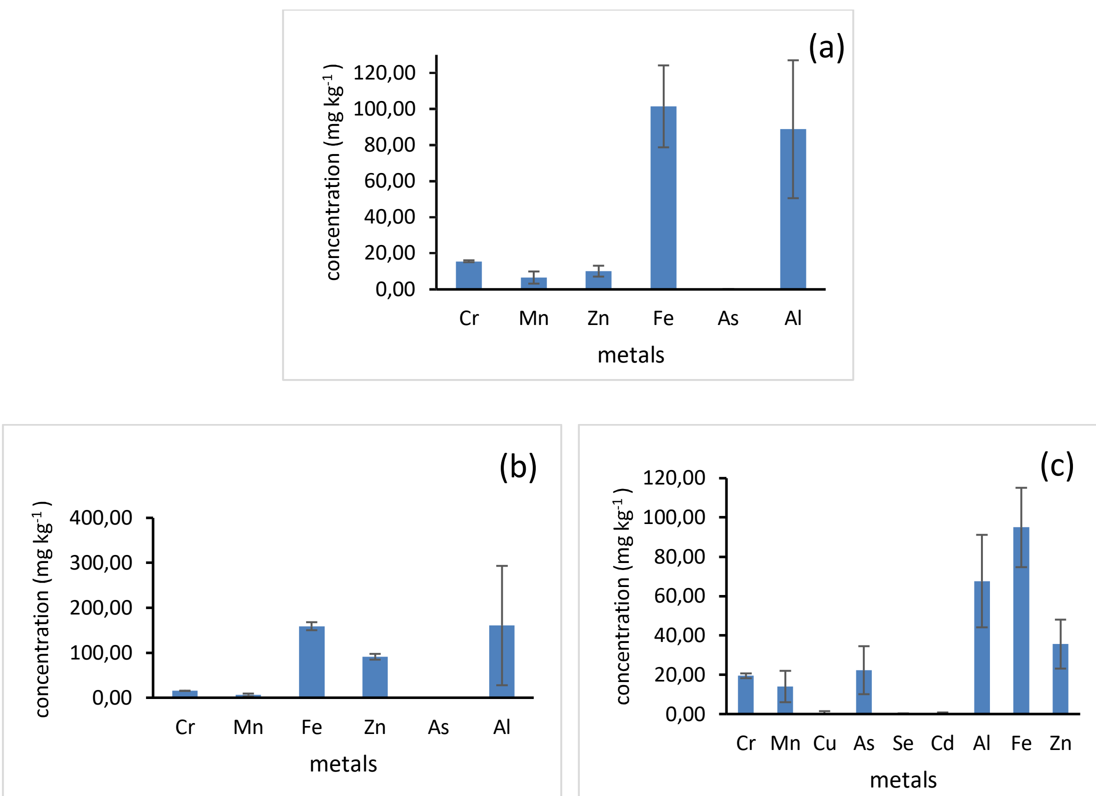

Figure 4.1: Concentration of metal ions detected in fish foodstuff from Siavonga open market (a) sun-dried bream (b) sun-dried Kapenta and (c) fresh bream for 2021

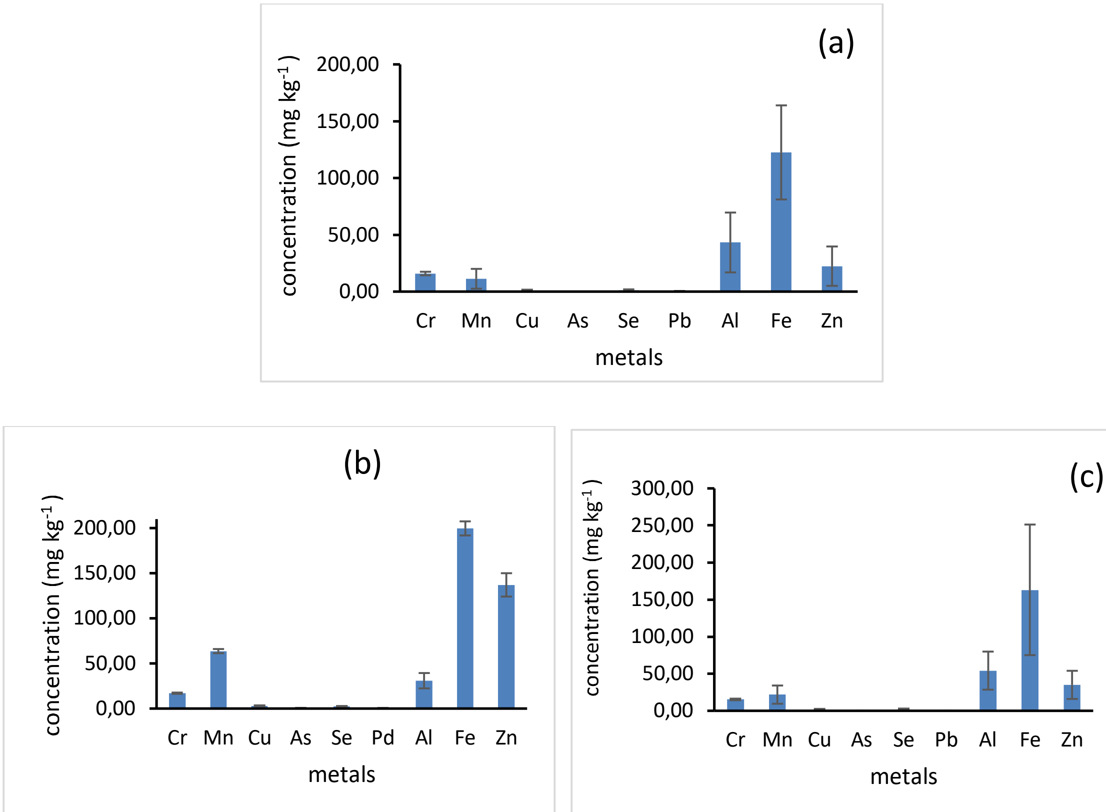

Figure 4.2: Concentration of metal ions detected in fish foodstuff from Siavonga open markets (a) sun-dried bream (b) sun-dried Kapenta and (c) fresh bream for 2022

There was a significant difference for all metal ion in fish musculature as shown in Figure 4.1 and 4.2. Concentration of trace metals in fish muscle were in descending order as follows: Fe > Al > Cr > Zn > Mn > As, however, Hg, Se, Cu, Cd and Pb were not detected in sun-dried bream; Al > Fe >Zn >Cr > Mn > As > Cu, except for Cd, Pb, Se and Hg were not detected in sun-dried Kapenta; Fe >Al > Zn > As > Mn > Cr > Cu > Cd > Se, except for Hg and Pb were not detected in fresh bream, respectively for 2021 samples. While for 2022 the descending order were as follows: Fe > Al > Zn > Cr > Mn > Se > Cu > Pb > As in sun-dried bream; Fe >Zn > Mn > Al > Cr > Se > Cu > As > Pb in sun-dried Kapenta; Fe >Al > Zn > Mn >Cr > Se >Cu >As >Pb in fresh bream. Cd and Hg were not detected in all fish samples, except for fresh bream in 2021. Trace metals were statistically higher in all fish samples ($p < 0.05$). Where, essential metals were higher in sun-dried bream and fresh bream, but, non-essential metals were lower in sun-dried Kapenta in 2021 samples. While in 2022 samples essential metals were higher in sun-dried bream and Kapenta, whereas non-essential metal ions were higher in fresh bream but lower in sun-dried Kapenta.

Essential metal ions include: Cr, Fe and Zn. Some metals are necessary for various biological functions and are therefore added as trace elements to fish feeds (Yildiz 2008; Fallah et al., 2011; Simukoko et al., 2022), which may explain the higher levels of trace elements in sun-dried bream and Kapenta. Due to feed spillages from the fish farms exposing wild bream and Kapenta nearby with higher levels of essential metals (Basaran et al., 2010; Ballester-Molto et al., 2017; Simukoko et al., 2022). The essential metals (Cu, Zn and Se) were below permissible limits recommended by WHO/FAO (2018) in all fish samples, whereas Cu was not detected in sun-dried bream. This explains the difference in essential metals in fish samples, as a result of fish feed composition of trace elements (Simukoko et al., 2022). Moreover, high level of Fe is as a result of fish feed spillages from fish farms in the Lake (Chali et al. 2014; Paulet 2014; Ge et al., 2019; Liu et al., 2014; Plessl et al., 2019; Sumukoko et al., 2022).

Non-essential metals such as Mn and As were higher in fresh bream. While Al was higher in sun-dried Kapenta than other fish samples as a result of Fishing Boats, households waste, wastewater, city runoff and industry (Chali et al. 2014; Paulet 2014; Ge et al., 2019; Liu et al., 2014; Plessl et al., 2019; Sumukoko et al., 2022). The high level of As in fresh bream could possibly be due to coal ash from coal mining area without proper management through heavy rainfall into waterways, groundwater and into drinking water. The fish could have accumulated the As during its life span because wild bream can live up to 9 years in Lake Kariba compared to farmed breams, which are harvested within 6 months of cage-rearing (Simukoko et al., 2022). Moreover, the method of fish drying and an unhygienic open market is another source of trace elements contaminants which explains higher levels than in fresh breams. Fishes are exposed to street dust and nearby busy roads through car exhaustion. This can lead to contamination of aquatic environments with pollutants such as trace metals, hydrocarbons, and particulates. These contaminants can accumulate in fish tissues, potentially impacting their health and posing risks to humans who consume them (Nuapia et al., 2018). Of which Cr, Al, Fe and Cu levels was contrary to expectations of high Cu, since the number of Kapenta fishing boats rose from 423 in 2009 to 962 in 2013. These boats are thought to be the sources of Cr, Al, Fe and Cu due to rusting of boats and paints on boats to prevent corrosion (Chali et al. 2014; Paulet 2014; Simukoko et al., 2022).

Baki et al. (2020) stated that fish musculature mostly retains the lowest metal concentrations as it is usually not considered an active site for metals to accumulate and biotransform. Chromium, Manganese and Iron were above acceptable limits in sun-dried bream. Whereas, Cr, Mn Fe, As and Al were above acceptable limits in sun-dried Kapenta. While, Cr, Mn and As were above permissible limits in fresh bream. Iron and Zinc tend to accumulate in liver tissue due to the presence of metallothionein proteins (Görür et al., 2012; Bahgy et al., 2021). On the other hand, Cr and Mn are more likely to accumulate in gills, following the pathway by which these trace metals move from water to gills, which serve as the primary exchange route for metal ions entering fish from their aquatic environment. (Qadir and Malik 2011; Jia et al. 2017; Bahgy et al., 2021).

Aluminum is known as the endocrine disruptor and a neurotoxic agent, as the metal tends to accumulate in the brain (Briffa et al., 2020; Gade et al., 2021). Several studies have correlated certain Al concentrations and different diseases such as Alzheimer's disease (Inan-Eroglu and Ayaz, 2018; Hardisson et al., 2017). In addition, Al can interfere with some essential metals. The mean Al concentration in fish samples from 2021 was of 88.80 ± 38.21 mg kg^{-1} for sun-dried bream, the highest level observed. This compared with an Al concentration of 67.62 ± 23.46 mg kg^{-1} in fresh bream, which was the lowest detected. However, Al was below permissible limits recommended by WHO/FAO (2018) compared in sun-dried Kapenta of 160.72 ± 132.60 mg kg^{-1} which was the highest detected in this study. According to FAO/WHO (2018), the permissible limits for Al content was set for 100 mg kg^{-1} in foodstuffs for 2021. While Al in 2022 fish samples was below permissible limits by FAO/WHO (2018) in a range of 100 mg kg^{-1}. The Al in fish sample was ranging from 55.80 ±23.23 mg kg^{-1} in sun-dried bream, to 33.60 ± 25.58 mg kg^{-1} in sun-dried Kapenta and to 54.00 ± 25.17 mg kg^{-1} in fresh bream. Their main source of accumulation in fish was due to the pathway routes from the water columns through gills and diet intake. Considering the two-exchange major route for metal ions passing from water to the fish (Qadir and Malik, 2011; Jia et al. 2017; Bahgy et al., 2021).

Arsenic was detected in all studied fish sampled with a mean concentration ranging from 0.03±0.04 to 0.15 ± 0.07 mg kg^{-1} in sun-dried bream, from 0.49 ± 0.05 to 0.78 ± 0.03 mg kg^{-1} in sun-dried Kapenta and from 22.30 ± 12.20 to 0.26 ± 0.16 mg kg^{-1} in fresh bream for 2021 and 2022 samples respectively. According to FAO/WHO (2018), the permissible limits for As in food is 0.1 mg kg^{-1}. All the studied fish samples in this study were above the permissible limit, except for 2021 sun-dried bream. The results found in this study for As were above those reported by Nuapia et al. (2018) at a concentration was ranging from 1.01-3.09 mg kg^{-1}. The reason for this may be due to accumulation of As during the lifespan of a fish. Moreover, from the open market as fishes are exposed to As from the environment when on display (Palm et al., 2011; Almroth et al., 2021; Huang et al., 2022).

The Cu was only found in fresh bream from Siavonga open market fish foodstuff in a range of 0.37 ± 0.53 mg kg^{-1} for 2021 fish sample as compared to 2022 fish samples. Where the Cu was below permissible limits recommended by FAO/WHO (2018) with a concentration of 10 mg kg^{-1}. The Cu in fish sampled was 1.12 ± 0.63 mg kg^{-1} in sun-dried bream, to 2.54 ± 0.56 mg kg^{-1} in sun-dried Kapenta and 1.25 ± 1.14 mg kg^{-1} in fresh bream for 2022 samples.

Cadmium and Selenium were only detected in 2021 fresh bream from Siavonga open market fish foodstuff in a range of 0.37 ± 0.53 mg kg^{-1} for Cd in 2021 as compared to 2022 fish samples, in a range of 0.22 ± 0.10 mg kg^{-1} for Se. The level of Cd, Cu and Se in the fresh bream were below the joint FAO/WHO (2018) permissible limits as follows; Cd (2 mg kg^{-1}), Se (1 mg kg^{-1}) and were not too concentrated to cause endocrine disruption related complications. Especially in larger amounts, it can lead to an increased risk of several diseases such as cancer, heart disease, viral diseases and other conditions that involve an increased levels of oxidative stress (Huag et al., 2009; Weber et al., 2019). Thus, the impacts of sample handling, transportation and treatment from the fisherman to the open market plays a huge role in the increased Cd concentration. Selenium is an essential metal in certain amounts for its health benefit, which can be calculated using health benefit values (HBVSe), a formula described by Ralston et al. (2016) and Simokoko et al. (2022). A positive HBVSe indicates protective effects of Se against Hg in the fish, while a negative value indicates that Hg poses a threat to human health (Ralston et al. 2019; Yabanli and Tay 2021; Simokoko et al., 2022). Nonetheless, fish was safe from Hg because was not detected/it was below detection limits in all fish samples. Whereas in 2022 Selenium was detected in all fish food samples with a concentration of 1.17 ± 0.90 mg kg^{-1} in sun-dried bream, 2.84 ± 0.46 mg kg^{-1} in sun-dried Kapenta and 0.26 ± 0.16 mg kg^{-1} in fresh bream. Selenium concentration was higher than the limits set by FAO/WHO (2018) in fish with a concentration of 1 mg kg^{-1}. The level of Se in first sampling was only found in fresh bream with a lower range of 0.22 ± 0.10 mg kg^{-1} below permissible limits by FAO/WHO (2018). Compared to the second sampling due to fish feed spillages as a results of essential elements composition (Simukoko et al., 2022).

There have been no reported cases of Cr toxicity due to food intake containing high levels of Cr. Chromium (III) helps to transport blood glucose from the bloodstream into the cells to be used as energy (Chang et al., 2020). Although, Cr (VI) has shown to be a potent occupational carcinogen (Nikfar et al., 2011). In the current study, Cr was detected in all fish samples with varying concentration ranging from 15.47 ± 0.53 mg kg^{-1} in sun-dried bream to 18.21 ± 1.07 mg kg^{-1} in sun-dried Kapenta and 19.53 ± 1.20 mg kg^{-1} in fresh bream. Whereas, the Cr in fish sampled 2022 was ranging from 15.94 ± 1.48 mg kg^{-1} in sun-dried bream to 17.67 ± 0.77 mg kg^{-1} in sun-dried bream and 15.39 ± 1.06 mg kg^{-1} in fresh bream. This is evident that Cr emerges from the paints and chromite mines as a source of Cr. The level of Cr in all the fish samples were above the permissible limit of 1 mg kg^{-1} in fish recommended by WHO/FAO (2018).

Manganese was found in a range of 6.50 ± 3.30 mg kg^{-1} in sun-dried bream, to 39.28 ± 4.27 mg kg^{-1} in sun-dried Kapenta and to 13.99 ± 7.96 mg kg^{-1} in fresh bream for 2021 samples. Whereas, 2022 fish samples was 11.40 ± 8.74 mg kg^{-1} in sun-dried bream, 68.11 ± 2.59 mg kg^{-1} in sun-dried bream and 21.79 ± 12.32 mg kg^{-1} in fresh bream. According to the WHO/FAO limits of 0.5 mg kg^{-1}, the level of Mn in the Kariba bream and sun-dried Kapenta for both sampling were above permissible limits. Which, were too concentrated (≥ 0.2 mg L^{-1}) to cause leukopenia and anemia in Kariba bream (tilapia) and endocrine disruption related complications (Alam et al., 2021). This could be due to nearby discharging of mining waste contaminates containing Mn which accumulates. Chromium and Manganese are accumulated in the gills from the water and it's considered the major exchange route of metal ions passing from water to fish (Qadir and Malik 2011; Jia et al. 2017; Bahgy et al., 2021).

Fish is considered a major priority on the health of the public and the aquatic environment (Maurya et al. 2019; Mwakalapa et al. 2019). Muscular tissue of polluted fish has a considerable smaller absolute rise in trace metals than any other organs (Hasimuna et al., 2022; Simukoko et al., 2022). Which, it is always wise for one to reduce chances of direct chemical transfer into human, by washing or rinsing the fish bought from local or open markets before consumption. The source of emerging contaminants of non-essential elements were detected in second sampling than in first sampling. It is evident that it could be due to the impacts of sample handling, treatment and transportation from the fisherman to the open market that plays a huge role in the increased metal concentration (Simokoko et al., 2022). Moreover, contamination of trace metals in fish due to sun-drying of fish at busy operating road dusty area and car exhaustion. Furthermore, sun-drying of fish at unhygienic place during dusty or wind seasons (Figure 6.3, appendix E), which can contaminate the fishes. Washing or rinsing may play a huge role in removing surface contaminants on the fish. However, washing fish items does not remove 100% of the contaminants as some would have penetrated within the sample tissues (Nuapia et al., 2018; Ramalepe et al., 2022). Organisms' capacity to accumulate trace metals is one of a greater complexity of the physiological systems that control trace metal concentration and little is understood (Simukoko et al., 2022).

Excess amounts of essential metals (Fe, Se, Cr and Zn) in fish are possibly due to the exposure of high spillage amounts of fish feeds composition from the fish farms nearby accessed by the wild bream and Kapenta (Basaran et al., 2010; Ballester-Molto et al., 2017; Simukoko et al., 2022). The essential metals were above permissible limits recommended by WHO/FAO (2018). Copper was below permissible limits by FAO/WHO in Kapenta, whereas Zn was below permissible limits recommended by FAO/WHO in both sun-dried bream and fresh bream which is evident enough due to fish feed composition and a manner in which bream accumulates trace metals (Basaran et al. 2010; Ballester-Molto et al. 2017; Simukoko et al., 2022). Kapenta is contaminated with essential metals such as Fe, Se, Cr and Zn during transportation and handling of Kapenta whereas, Kariba bream has accumulated Fe, Se and Cr due to spillage of fish feed composition (Simukoko et al., 2022). Essential elements are needed in minimum amounts, but trace elements can cause adverse health effects even if their intake is either too low (Zn and Fe deficiency) or too high (Zn and Fe toxicity) than anticipated (FAO/WHO, 2000).

There were non-essential metals that were not found in the first sampling that were found in the second sampling. Lead was in smaller quantities below permissible limits recommended by FAO/WHO (2018). Although it is in small quantities, it is alarming enough, due to unmonitored source of Pb which could accumulate in future and causes adverse health related issues (Simokoko et al., 2022). Lead occurs in foodstuff because of its presence in the environment that ends up in food. For example, municipal sewage, industrial wastes are enriched in Pb. Furthermore, Pb contamination can occur through paints containers nearby the lake which can settle into the soil or be adsorbed and washed off by heavy rainfall or storms into the lake. Lead can be absorbed into the fish musculature either through gills from the water column, or through diet (Zhang et al., 2019; Weber et al., 2020; Hasimuna et al., 2019; Simukoko et al., 2022). This Pb residues cannot be completely removed by simply washing or rinsing with water, as some Pb had penetrated into the muscles during its life-span in the lake. All the studied fish samples were found to contain Pb in a range of 0.34 ± 0.35 mg kg^{-1} in sun-dried bream to 0.38 ± 0.15 mg kg^{-1} in sun-dried Kapenta and 0.06 ± 0.12 mg kg^{-1} in fresh bream. The mean value of Pb found in the fresh bream from Siavonga open markets were similar to that reported by Simukoko et al. (2022) with a mean value of 0.004 to 0.009 mg kg^{-1} in Kariba bream. This is evident enough that there is an emerging source of Pb not closely monitored. Sun-dried Kapenta and sun-dried bream were similar to that reported by Nuapia et al. (2018) with a mean concentration value of 0.39 mg kg^{-1}.

4.1.2 Correlation among metals in fish

In 2021 Sun-dried bream had a positive correlation (Spearman's correlation) of Cr with Mn and Zn, suggesting a common source or accumulation pathway (Table 4.1). Furthermore, Fe had a significant positive correlation with Al, suggesting a similar contamination sources or physiological processes. Sun-dried Kapenta had a significant positive correlation of Mn with Cr and Al, potentially indicating a common environmental influence. Aluminum had a significant negative correlation with Zn in Table 6.12, appendix C. Fresh bream had a positive significant correlation for Zn with As in Table 6.13, appendix C. All trace metals concentrations were significantly different ($p < 0.05$). However, to determine which trace metal correlations had significance on each other, a Bonferroni correction was done on the p-value followed by a t-test between two sets of trace metals (Maiphetlho et al., 2020). The t-test results showed that two sets of Fe-Al had a statistically significant difference on sun-dried bream since $p < 0.003333$, where Cu-Cd in sun-dried Kapenta had a $p < 0.002381$, whereas Cu-Cd and Se-Cd in fresh bream had a $p < 0.0013889$ in appendix D, Tables A2, B2 and C2, respectively. Table 4.1 shows the correlation between trace metals in 2021 sun-dried bream.

Table 4.1: metal-metal correlation between trace metals in 2021 sun-dried bream

Metal	Cr	Mn	Zn	Fe	As	Al
Cr	1.00					
Mn	0.92	1.00				
Zn	0.83	0.71	1.00			
Fe	0.40	0.29	0.72	1.00		
As	0.25	0.43	-0.31	-0.46	1.00	
Al	0.46	0.25	0.76	0.96	-0.52	1.00

Whereas, in 2022 the correlations shifted. Sun-dried bream had a significant positive correlation for As with Se and Mn, and Al with Fe, and Se with Pb (Table 4.2). This could suggest differing pathways or contamination sources from the 2021 sample. In sun-dried Kapenta, Cr had a positive significant correlation with As and Se. Whereas, Mn had significant positive correlation with Zn and Fe, and Cu with Fe, and As with Se suggesting common environmental influences. On the other hand, negative correlations in the 2022 sample of sun-dried bream indicate divergent sources or metal behavior. A negative significant correlation was observed for Fe with Zn, and Pb with Al in Table 6.14, appendix C, suggest that these metals either do not share sources or have competing pathways for accumulation. Fresh bream had a significant positive correlation for As with Zn in Table 6.15, appendix C. All trace metals were significantly different ($p < 0.05$). The t-test results showed that two set of Cr-Mn, Cu-As, Cu-Se, Al-Zn, As-Al and Cu-Pb have a statistically significant difference from sun-dried bream since $p < 0.0013889$, where Cu-Se in sun-dried Kapenta had a $p < 0.001087$ and Cu-Pb, Cr-Zn and Al-Zn in fresh bream had a $p < 0.0013158$, in appendix D of Table A2*, B2* and C2*, respectively.

Understanding these correlations provides insight into environmental contamination sources and guides on the interventions to minimize health risk. Positive correlations indicates the shared sources of pollution, emphasizing the need for stricter environmental regulations to reduce trace metal contamination in fish, a vital food source. The observation of negative correlations could point toward a different environmental sources or pathways, suggesting that certain metals might not co-occur due to specific environmental factors. Table 4.2 below shows the correlation between trace metals in 2022 sun-dried bream.

Table 4.2: Correlation between trace metals in 2021 sun-dried bream

	Cr	Mn	Cu	As	Se	Pb	Al	Fe	Zn
Cr	1.00								
Mn	0.56	1.00							
Cu	0.09	-0.15	1.00						
As	0.50	0.85	-0.31	1.00					
Se	0.69	0.62	0.04	0.81	1.00				
Pb	0.73	0.35	0.19	0.49	0.86	1.00			
Al	0.01	-0.21	-0.01	-0.20	-0.40	-0.19	1.00		
Fe	0.33	-0.15	-0.10	-0.10	-0.09	0.19	0.81	1.00	
Zn	0.70	0.10	-0.04	0.08	0.43	0.65	-0.15	0.42	1.00

Positive correlation was observed between Cr with Zn and Mn, also Fe and Al in 2021 sun-dried bream, whereas in 2022 positive correlation was observed for Se with As and Mn, also with Mn with As. Lastly, it was observed in with Al and Fe. Whereas in Sun-dried Kapenta a positive and negative correlation was observed for both 2021 and 2022 sampling. A positive correlation was observed between Mn with Cr and Al, were in 2022 a negative correlation between Mn with Fe and Pb with Al. While a positive correlation was observed between Cr with As and Se. Where, it was also observed between Mn with Zn and As with Se. Whereas in both sampling of Fresh bream a positive correlation was observed only between As and Zn. The Developmental activities along Lake Kariba are the prime source of elevated levels of trace metals inclusive of industries, thermal plants, chemicals, fertilizers and fish feeds industries (Rajeshkumar and Li, 2018; Simukoko et al., 2022). Trace metals are also attributed to by municipal wastes, discharge of motel waste, mining wastes, aquaculture and agricultural discharges (Muringai et al., 2019; Hasimuna et al., 2019; Simukoko et al., 2022). Elevated levels of toxic trace metals have been reported from areas experiencing increasing settlement, traffic activities and agricultural activities. Moreover, population growth and fast growth of urbanization coupled with heavyweight economic development (Annabi et al., 2013; Egila and Daniel, 2011; Ahmad et a., 2016; Rajeshkumar and Li, 2018; Muringai et al., 2019; Hasimuna et al., 2019; Simukoko et al., 2022). Screening the levels of non-essential trace elements in fish is important because fish is an important source of food for the general human population. However, fish from freshwater bodies receiving industrial effluents have been reported to be unfit for human consumption due to its high tissue levels of some trace metals (Tyokumbur et al., 2014; Rajeshkumar and Li, 2018). These findings suggest that efforts to manage contamination must consider both common and distinct sources of metals and their pathways into fish populations. The information on positive and negative correlations informs public health policies and environmental regulations, ensuring the safety of fish products.

4.1.3 Metal selectivity index (MSI %)

Figure 4.3 shows a graph of metal selectivity index in fish representing the metal content (%) for both sampling. For one to understand how the elements bioaccumulate in fish tissues, as an important indicator for human health (Sattari et al., 2020). The MSI provides comprehensive information about the metal toxicity in a particular sample (Enuneku et al., 2018; Hasimuna et al., 2021). The results correlate with that of bioaccumulation graph in Figure 4.6 (Bahgy et al., 2021). Aluminum was in higher content, together with Cr, Fe, Mn and Zn which were present in all fish musculature samples (Bahgy et al., 2021). Except in sun-dried Kapenta sampled in

2022, whereas Zn was higher in fresh and sun-dried bream and Cr was higher in sun-dried bream as compared to sun-dried Kapenta in Figure 4.3, followed by fresh bream. However, Cu, Cd and Se were only present in 2021 fresh bream but in low quantity which could possibly be due to the coal mining in the area and release of coal waste in the environment reported in Lake Kariba (Liu et al., 2014; Plessl et al., 2019; Simukoko et al., 2022). Whereas in 2022 Cu was higher than Pb, As and Se in all fish samples. The elevated level is possibly due to open market sources as a result of operating at busy unhygienic and dusty roads in the area (Liu et al., 2014; Plessl et al. 2019; Simukoko et al., 2022). Kapenta's high elevated metal levels could be as a result of contamination during transportation and handling and whereas Cd was not detected (Plessl et al., 2019; Simukoko et al., 2022).

Arsenic was higher in fresh bream as compared to sun-dried bream and Kapenta, as volatile trace metal could have dissipated in the oven, during the drying process (Ge et al., 2019). Sun-dried bream contained high levels of trace metals as compared to fresh and sun-dried bream. All trace metals were found in fresh bream, except for Cd in 2022 sample in sun-dried Kapenta in, whereas Cu and Se were not present in sun-dried bream. Bahgy et al. (2021) did a study on metal selectivity of trace metals, showed a similar trend on essential metals (Zn, Fe and Mn) and non-essential metals (Al) in high contents in fish for 2022 compared to 2021 fish samples. Figure 5.2 shows metal selectivity index in fish samples.

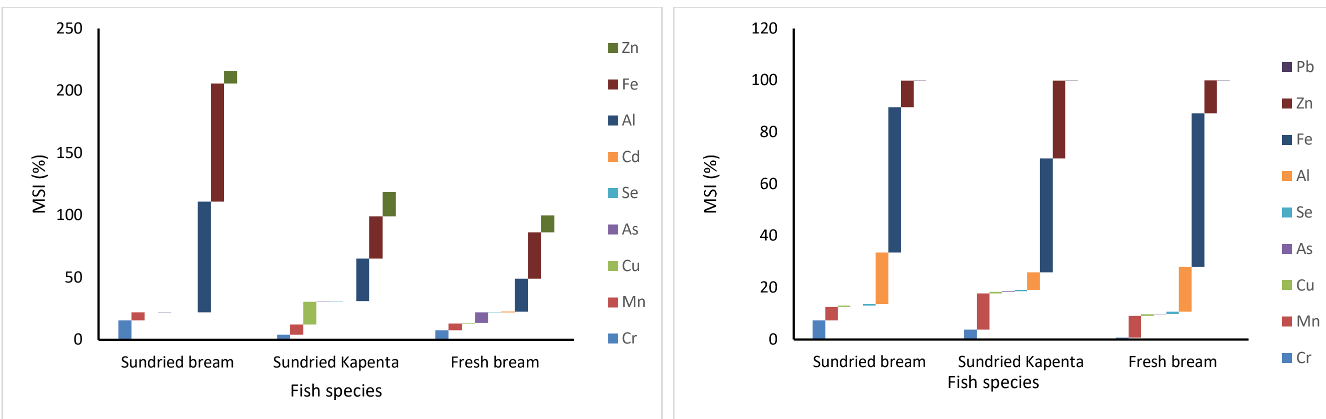

Figure 4.3: Metal selectivity index (MSI %) (a) for 2021 and (b) for 2022 in fish samples

4.1.4 Bioaccumulation factors

4.1.4.1 Correlation concentration in fish with its length

Figure 4.4 and Figure 4.5 shows the bioconcentration factor graph of (a) sun-dried bream and (b) fresh bream for both 2021 and 2022 sampling. There was no significant difference in 2021 fish samples between the metals and fish length as compared to 2022 samples. Manganese was bioconcentrated in 2021 sun-dried bream, whereas Al was bioconcentrated in fresh bream. Which explains the route of a source for Mn and Al as a result of non-point sources. For instance, contamination of trace metals from open market and during sun drying. Whereas in fresh bream the source is due to a point source as a result of coal mining area around Lake Kariba which was reported by Simukoko et al. (2022). Moreover, due to transportation and handling of Kariba bream to and from fisherman to an open market. Aladesanmi et al., (2019) observed a similar trend of bioconcentration of Al and Mn and also for Fe and Mn in fish sampled in 2021 and 2022. Bioconcentration and bioaccumulation factor are the most straightforward methods for accessing the state of trace metals in fish to acquire pollutants from the water and estimating the proportional abiotic medium contributor, as a source of trace metals accumulated in fish and to assess their efficiency (Aladesanmi et al., 2019; Sattari et al., 2020). The bioconcentration in fish samples showed that trace metals concentrated in both fresh and sun-dried bream specifically Al, Fe and Mn were statistically significant. While, other trace metals were observed in smaller quantities. Whereas, Simukoko et al. (2022) observed Fe as an accumulative metal in Kariba bream. Aluminum in sun-dried bream was accumulative as compared to fresh bream due to contamination during handling and transportation to open markets. Moreover, due to sun-drying process nearby busy roads (Nuapia et al., 2016, 2018). Figure 4.4 and Figure 4.5 shows the bioconcentration factor graph of Kariba bream sampled in 2021 and 2022 respectively.

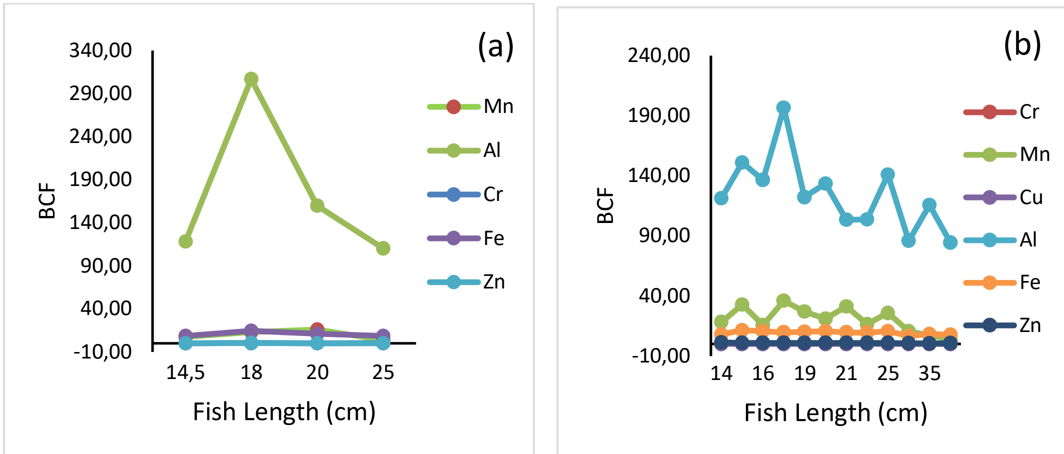

Figure 4.4: Bioconcentration factor graph of (a) sun-dried bream and (b) fresh bream for 2021 sampling

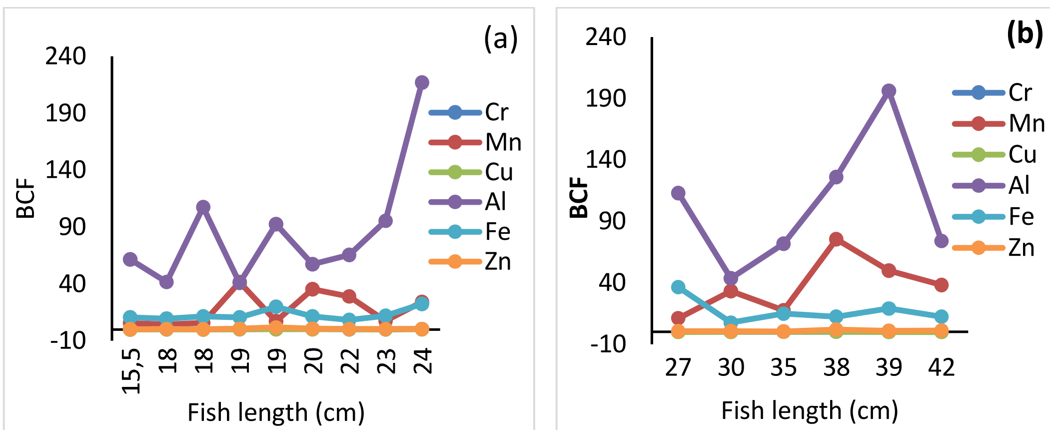

Figure 4.5: Bioconcentration factor graph of Kariba bream (a) sun-dried and (b) fresh for 2022 sampling

Bioconcentration uses the same concept as bioaccumulation (Sattari et al., 2020). However, it tells us how much quantity per fish length and as expected the high lifespan of a fish, the more concentrated the trace metals within a fish. However, it is not the case in Figure 4.4 and Figure 4.5 because trace metals are in different compositions. This means more studies need to be done to conclude on bioconcentration. Manganese and Aluminum are correlating with the bioaccumulation graph where Cr, Cu and Zn are significantly different in both samples because trace metals were not concentrated in all fish sampled (Maiphetlho et al., 2020).

4.1.4.2 Comparison of bioaccumulation

Figure 4.6 shows a bioaccumulation graph of trace metals of a sun-dried bream, fresh bream and sun-dried Kapenta. There was a significant difference in all fish sampled. Bioaccumulation and bioaccumulation factors are used to assess aquatic organisms' capacity to acquire pollutants from the water (Sattari et al., 2020). BAF > 1 suggests that the fish is able to accumulate the metals, however it is not alarming until the BAF is 100 or above (Aladesanmi et al., 2019; Sattari et al., 2020), as interpretation of BAF are shown in Table 2.3.

All metals in sampled fish were statistically significant ($P < 0.05$). It is evident enough that Al is bioaccumulative in fresh and sun-dried bream muscle. However, Al in sun-dried Kapenta is due to contamination during sun drying in an open space near busy roads from car emission. Moreover, during transportation and handling of Kapenta to and from fisherman to an open market as shown in appendix E (Simukoko et al., 2022). Aluminum and Manganese need to be closely monitored, specifically their sources, as metals are not extremely accumulative but in the long term, trace metals can be fatal to human health. Manganese in sun-dried Kapenta is as a result of contamination, whereas in sun-dried and fresh bream sampled in 2021 was ranging from severely to very severely accumulative, respectively. Whereas Mn in 2022 sun-dried Kapenta, was severely accumulative. Due to contamination during handling and transportation to open markets. Moreover, due to sun-drying process nearby busy roads (Mwakalapa et al., 2019; Simukoko et al., 2022). While in fresh bream accumulation was very severe. Chromium, Copper and Zinc for both sampling was not accumulative as their BAF was below 1, as shown in Table 2.3 except for Zn in sun-dried Kapenta which was minor accumulation and Fe was severely and moderately accumulative in fresh bream and sun-dried bream respectively (Aladesanmi et al., 2019; Sattari et al., 2020). Whereas, Zn was moderately accumulative in 2022 sun-dried fish and Fe was severely accumulative in all fish samples. Simukoko et al. (2022) observed a similar trend on bioaccumulation of Al in Kariba bream from Lake Kariba. Figure 4.6 shows the graph of bioaccumulation factor for both 2021 and 2022 sampling.

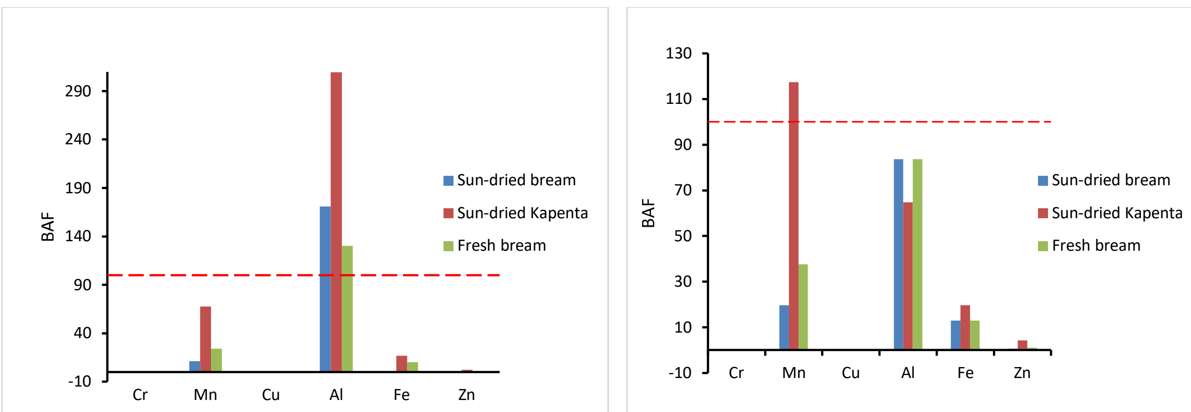

Figure 4.6: Bioaccumulation factor graph for (a) 2021 and (b) for 2022 sampling

4.2 Health risk assessment of inorganic EDCs

4.2.1 Human Health risk assessment

Table 5.7 – 5.8 of appendix B, gives a comparison of the maximum limits set by WHO/FAO and the tolerable daily intake (mg kg^{-1}). In sun-dried bream the measured metals Cr, Mn and Fe were above limits as compared to Zn, As and Al were below. Whereas Hg, Cu, Se, Cd and Pb were not detected. In sun-dried Kapenta Cr, Mn Fe, As and Al were above the set limits and Zn was below the limits, whereas Cu, Se, Cd, Hg and Pb were not detected, due to their sensitivity during sample drying in the oven. In fresh bream Cr, As and Mn were above as compared to Zn, Cu, Se, Cd, Fe and Al were below the limits recommended by FAO/WHO (2018), whereas Hg and Pb were not detected. The EDI from the consumption of all analyzed fish samples exceeded the PTDI for most trace metals. Exceptions included Se, Cd, Pb and Cu in sun-dried Kapenta. Lead was above the limits in fresh bream, however, Hg, Cu, Se, Cd and Pb in sun-dried bream as shown in Figure 4.7 were below the PTDI limits. It is worth noting that the FAO/WHO has not yet established an EDI dermal exposure to trace metals (Figure 4.10, appendix B). The calculated THQ and HI for all metals were above the threshold of 1 for sun-dried bream in Table 6.9, for sun-dried Kapenta in Table 6.10 and for fresh bream in Table 6.11, posing non-carcinogenic risk to human health. Consumption of As and Cr in sun-dried bream and Kapenta poses a carcinogenic risk whereas Cr, As, Cu and Cd in fresh bream were above 1×10^{-4} and below 1×10^{-6} (Table 5) showing a risk of cancer. The exposure factors of reference populations for human health risk assessment with the daily average fish ingestion per person, for both adults and children was 23.3 g/day (Ali et al., 2019; Aguilera et al., 2021; Kaminski et al., 2018; Tran et al., 2019; Munyeshury et al., 2021) with a life expectancy (exposure duration) of 70 years, the exposure frequency to trace metals of 156 days/ year, and average body weight of an adult 70 and 15 kg for children were used in the calculations (Table 2.4).

The incremental lifetime carcinogenic risk (ILCRing) due to lifetime exposure to Cr, As, Cd and Pb were calculated as described by Saha et al. (2016), Aguilera et al. (2021) and Simukoko et al. (2022), using Eq. 9, whereas non-carcinogenic risk was calculated using Eq.8. Hazard index for non-cancer risk due to fish consumption from the lake was calculated as the sum of the individual non-cancer risks (Bamuwamye et al. 2015; Aguilera et al., 2021). ILCRing is estimated as the incremental probability of an individual to develop cancer, over a lifetime, as a result of exposure to a potential carcinogen (Ahmed et al. 2016). According to USEPA (2018), acceptable lifetime cancer risk levels range from 1×10^{-4} (1 in 10 000) to 1×10^{-6} (1 in 1

000 000) chance of an individual developing cancer (Varol et al., 2017; Aguilera et al., 2021; Simukoko et al., 2022).

Ingestion of trace metals above permissible limits has its own implication on adverse health risk associated with them. The Zambian population is at risk for both carcinogenic and non-carcinogenic health effects, with a lifetime cancer risk of 1 in 10 000 and HI greater than 1, indicating potential health concerns (Aguilera et al., 2021; Simukoko et al., 2022). Cancer slope factors (CSF) (mg kg^{-1} day)$^{-1}$, 1.5 (As), 6.3 (Cd), 1.7 (Cr) and 0.0085 (Pb) from the Integrated Risk Information System USEPA (2018) database were used in line with the calculation of ILCRing (Aguilera et al., 2021). The LADD was used to calculate the ILCRing using the CSF of carcinogenic metals. Chromium is an essential metal at minimal levels, above the recommended limits pose complications on human and fish health (Nuapia et al., 2018; Simukoko et al., 2022). In long run, Cr could be devastating on the Zambian population and other African markets exporting Zambian fish (Mwakalapa et al., 2019; Simukoko et al., 2022; Hasimuna et al., 2022).

4.2.2 Fish health

WHO has set an Environmental Quality Standard (EQS) of 0.5 mg kg^{-1} of mercury in fish (WHO/FAO, 2018; Simukoko et al., 2022). The fish sampled is safer from adverse health effects related to Hg implications because Hg was not detected in all sampled fish. Simukoko et al. (2022) reported Hg concentration in fresh bream from Lake Kariba to have values of 0.021, 0.008 and 0.009 mg kg^{-1} due to coal mining in the area. Figure 4.7 shows the health risk assessment for ingestion of fish from Lake Kariba

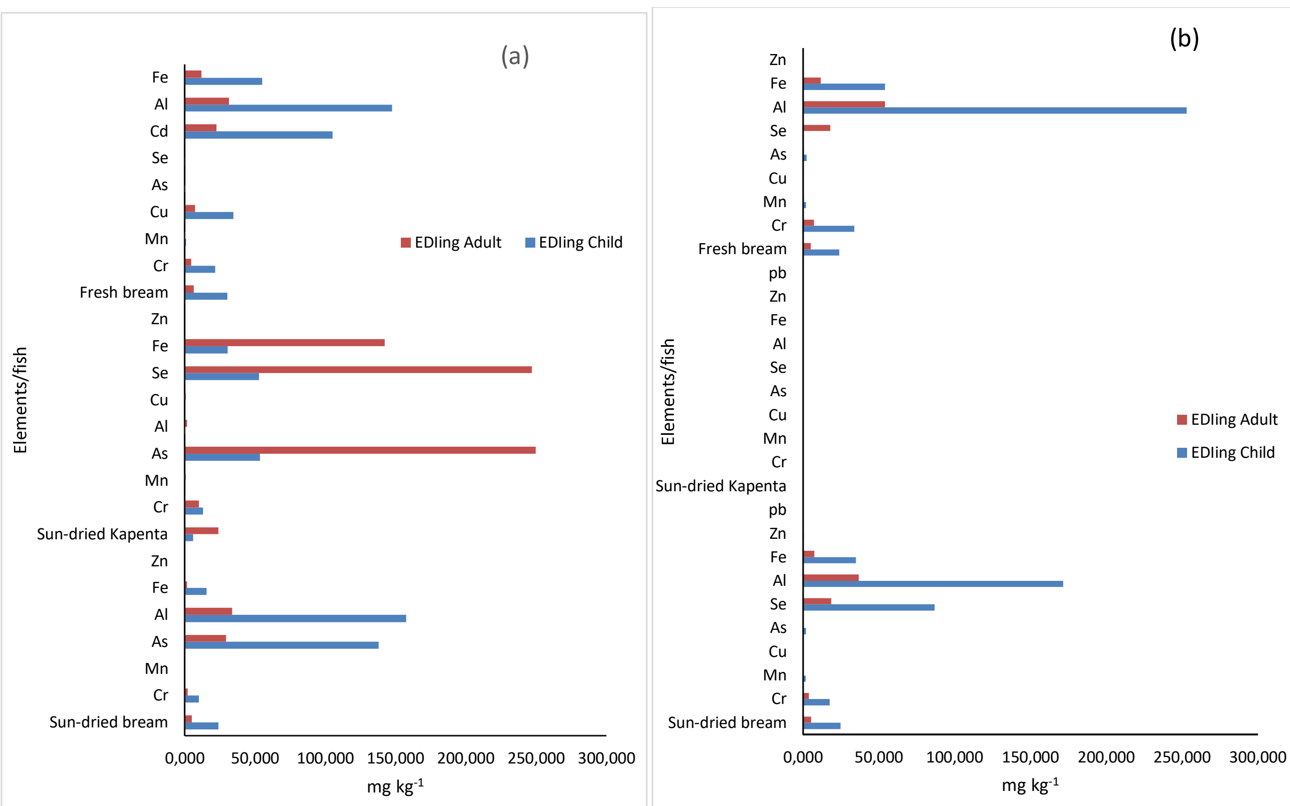

Figure 4.7: Health risk assessment for ingestion of fish from Lake Kariba (a) EDI ingestion for 2021 and (b) EDI ingestion for 2022

4.2.3 Contamination index

Pollution contamination in fish was assessed using a CF (Eq. 2) for each individual metal concentration of the study against the background value of the study by the mean value found in the current study of each fish sample, also the maximum permissible limits by WHO/FAO, and the mean value by Simukoko and other (2022) represented as CF 1, CF 2, and CF3, respectively. As a comparison to allow for some variation and tolerance between the three proposals (Aguilera et al., 2021), while the CD provides general data on the fish contamination of a certain element and at a specific study site. It is made up of all contamination elements added together. The results provide an indication of the level of the overall pollution from a specific site. Table 2 shows the interpretation level of contamination (Aguilera et al., 2021).

The average CD for elements in 2021 sampled fish followed the order: Fe >Al >Zn >As >Cr >Mn >Cu >Cd >Se in fresh bream; Al >Fe >Zn >Mn >Cr >Cu >As >Se in sun-dried Kapenta and Fe >Al >Cr >Zn >Mn >As in sun-dried bream. In 2022 the average CD followed the order: Fe >Al >Zn > Cr >Mn >As >Cu >Se in fresh bream; Al >Fe >Zn >Mn >Cr >As in sun-dried Kapenta and Fe >Al >Zn >Cr >Mn >Se >Cu >Pb >As in sun-dried bream. Where the 2021 CF in fresh bream in Figure 4.8 for CF 1 was extreme contamination with trace metals, except for Cu and Se was moderate contamination. The CF 3 and CF 2 was low contamination of trace metals, except for Al and Zn was moderate contamination where Fe was a major contamination in CF 3, whereas Cr, Mn and As was extreme contamination in CF 2 with their CD. Whereas the CF 1 in sun-dried Kapenta was moderate contamination for both CF and CD, where Se, Cu and As was low contamination. Where CF 2 and CF 3 was extreme contamination, except for Fe, Zn, Se and Cu were low contamination, whereas Fe and As; Se and Cu was moderate and major contamination respectively. Lastly the CF 2 and CF 3 in sun-dried bream was extreme contamination for trace metals in sun-dried bream with Cr and Mn in CF 2, where Zn, As and Al were low contamination in both CF 2 and CF 3, where Fe and also As and Zn was moderate contamination. Whereas CF 1 was low contamination for trace metals in sun-dried bream, except for Fe and Al were moderate contamination, where their CD was a major contamination of 6 in CF 1 of the sun-dried bream. However, in 2022 fresh bream the CF 1 in Figure 4.9 was low contamination, except for Al and Zn was moderate contamination and their CD was extreme contamination for CF 1, 2 and 3. Where CF 2 trace metals were extreme contamination with Cr and Mn, whereas Cu, Pb and Zn; As and Fe were low and moderate contamination in fresh bream respectively. The CF 3 and their CD was extreme contamination in fresh bream, except for Cu was major contamination. Whereas the sun-dried Kapenta was extreme contamination both the CF 3 and CF2, where CF 2 was extreme contamination with Cr, Mn and As; Cu, Pb and Al was low contamination; Se, Fe and Zn was moderate contamination. However, the CF 1 was low contamination for trace metals in sun-dried Kapenta, except for Zn and Fe where a major contamination was observed whereas Mn was moderate contamination. The CF 1 and CF 2 in sun-dried bream was low contamination, except for Al and Fe were moderate and major contamination respectively in CF 1. Whereas in Cr and Mn was extreme contamination in CF 2, where As, Se and Fe was moderate contamination and their CD of trace metals was extreme contamination and of 9 in CF 1. The CF 3 was extreme contamination of trace metals with their CD, except for Cu, As and Zn was a major contamination.

Pollution contamination is a very complicated concept, particularly in fish which could happen mostly due to sample handling, processing and treatment from the fisherman until its transportation to the open market. Contamination factor and Contamination degree plays a huge role in the increased metal concentration (Simokoko et al., 2022). The presence of non-essential elements was higher in the second sampling compared to the first. This suggests that contamination may have occurred during the transportation from the fisherman to the open market, leading to increased metal contamination (Simokoko et al., 2022). Contributing factors to this rise in metal concentrations include exposure to busy dusty roads, car exhausts and sun-drying of fish in unsanitary locations, especially during dusty or windy seasons. Washing or rinsing can help reduce surface contaminants, but these methods may not completely eliminate contamination from environmental sources. The study by Simukoko et al. (2022) assesses pollution contamination in fish using the CF and CD. The contamination factors are compared against the background values as the mean values of the study, and WHO/FAO limits for each metal. CFs and CDs indicate levels of pollution, with extreme contamination observed in some cases. The study finds that contamination levels vary between sampling years and fish types, with factors such as transportation processes impacting metal concentrations. Contaminants such as car exhaust and dust contribute to a significant role to the increased metal levels, especially during transportation and sun-drying of fish. The contamination index for fish samples in 2021 and 2022 shows varying degrees of contamination. Figure 4.8 and 4.9 shows the contamination index for 2021 and 2022 of fish samples respectively.

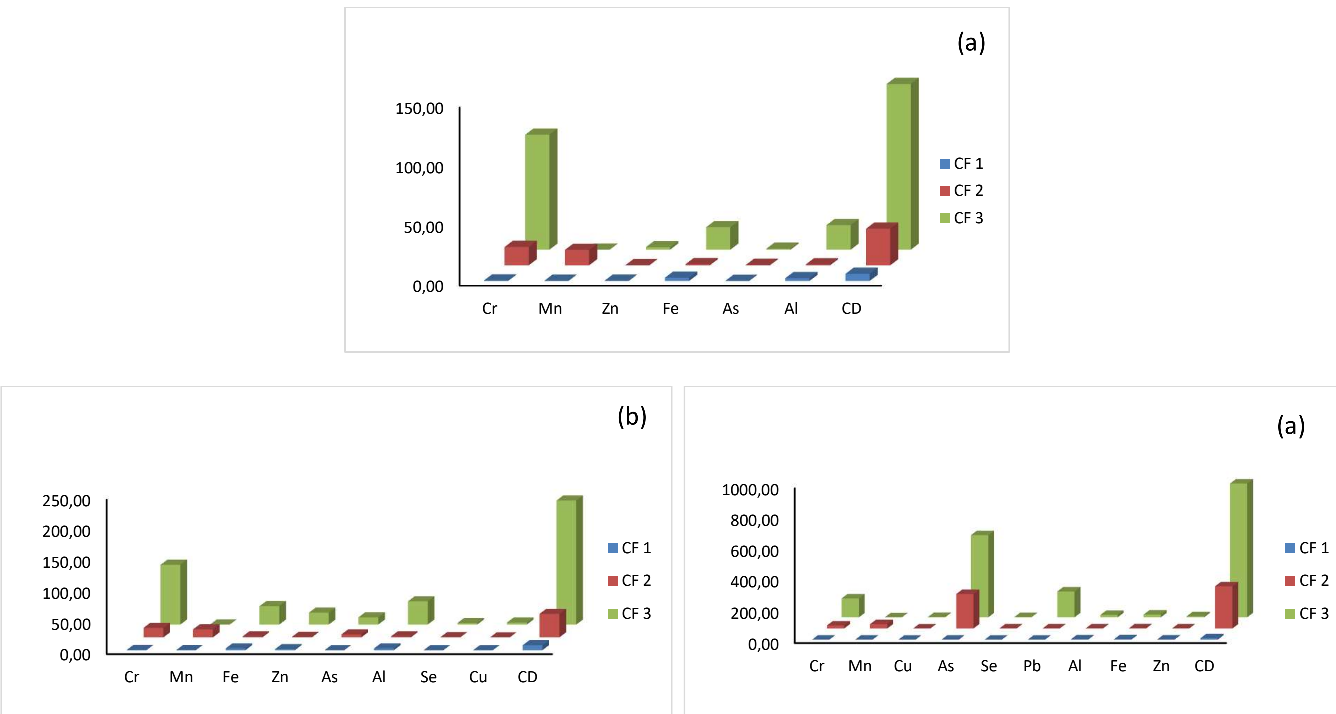

Figure 4.8: Contamination index of 2021 sampling (a) sun-dried bream (b) sun-dried Kapenta and (c) fresh bream

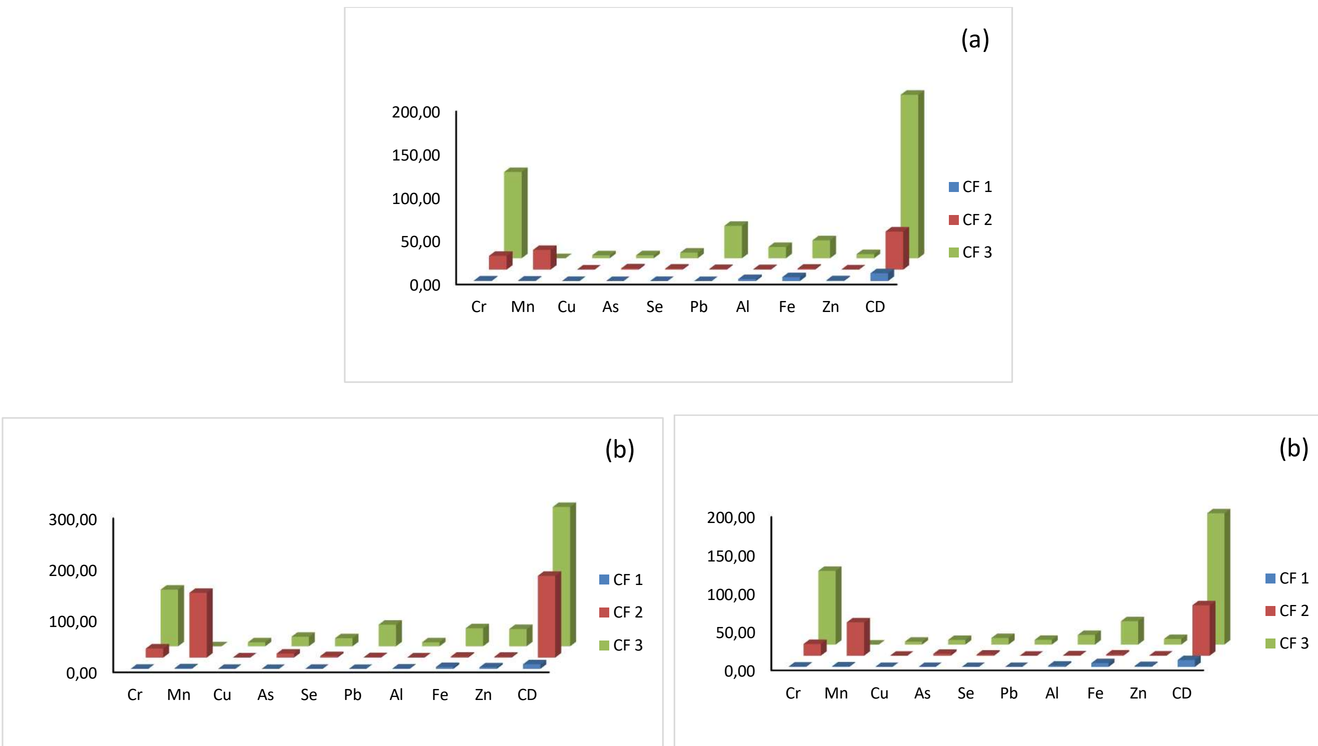

Figure 4.9: Contamination index of 2022 sampling (a) sun-dried bream (b) sun-dried Kapenta and (a) fresh bream

Aguilera et al. (2021) did a study on pollution of street dust in the largest city of Mexico and the same approach that was conducted in the current study. Their study was directed at the contamination index of a variety of street dust that was contaminated with inorganic EDCs. The current study had similar contamination factors in fish samples, as trace metals had a similar source of pollution (street dust). Sun-dried bream and Kapenta are often sold at open markets, where they can be exposed to various environmental contaminants. This exposure can increase the risk of metal contamination in these products. Moreover, fresh bream sold in similar open markets may also face similar contamination risks due to proximity to these conditions and handling practices, as trace metals were being discharged into the lake through heavy rain falls or storm water from urbanization, unmaintained busy roads and car exhaustion. This had insignificant contamination degree except for 2022 sampling of sun-dried Kapenta which was moderately contaminated. The contamination degree and contamination factor were calculated using. Eq. 2, and Table 2 shows the interpretation level of contamination (Aguilera et al., 2021).

The pollution index is key for assessing health risks. Figure 4.6 on bioaccumulation supports the observation that 2021 fish samples contained higher levels of metals, indicating more contamination compared to the 2022 samples from the current study. This aligns with the contamination index for inorganic Endocrine-Disrupting Chemicals (EDCs) of interest, which was high, indicating moderate contamination in both the 2021 and 2022 fish samples. However, contamination of metals such as Al, Fe, Zn and Pb in sun-dried Kapenta was as a result of sample handling and transportation to and from the fisherman during sun-drying process and includes the open markets. Moreover, from car exhaustion at busy roads near the lake and dusty unhygienic and unmaintained roads especially for adults' consumption shown in Figure 5.7 and 5.8 for both 2021 and 2022, respectively. Similarly, sun-dried bream contained high levels inorganic EDCs, such as Cr, Cu, Fe, Se, Zn, Al and Pb. Contamination in fresh bream is as a result of aquaculture due to fish feed spillages in the lake and mining area nearby the lake, which results in high level of contamination in both adults and children. Includes essential metals, such as Cr, Se, Fe, Zn and Cu whereas non-essential metals include Pb, Al and Mn (Simukoko et al., 2022). Degree of contamination was higher for all fish samples in 2022 than 2021 samples. Except for in fresh bream which was higher for 2021 fresh bream which could have been caused by changes in the level of water due to season variations at Lake Kariba (Mwakalapa et al., 2019; Simukoko et al., 2022).

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CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

This chapter finally, concludes the results discussed in chapter 5.

5.1 Conclusion and Recommendations

5.1.1 Conclusion

The comparison between the results from two sampling periods revealed a slightly significant difference in the concentration of trace metals in fish musculature. Across both periods, there is a consistent pattern of higher levels of essential metals (such as Fe, Cr, and Zn) in sun-dried bream and Kapenta compared to fresh bream. This suggests a potential influence of fish feed composition, with feed spillages from fish farms enriching wild bream and Kapenta with essential metals. Conversely, non-essential metal ions like Al, Mn and As show higher levels in fresh bream, possibly due to environmental factors such as coal ash contamination and unhygienic fish drying methods in open markets. Furthermore, the presence of Cr, Al, Fe, and Cu in sun-dried Kapenta, was possibly attributed to fishing boat activities, indicates additional sources of contamination affecting different fish species.

The Zambian fish sampled from the open market contained higher concentration of trace metals above permissible limits proposed by the Joint FAO/WHO Expert Committee for fish consumption. During first sampling, the sun-dried breams contained high levels of Cr, Mn and Fe while, sun-dried Kapenta contained high levels of Cr, Mn, Al and Fe. The fresh bream contained high levels of Cr, Mn and As. The following metals, Al, As and Zn were below detection limits in sun-dried Kapenta. Whereas in fresh and sun-dried bream Cu, As, Se, Al, Cd and Zn were below detection limits. Furthermore, in sun dried-bream, Zn, Cu and Se were below detection limits as recommended by the Joint FAO/WHO Expert Committee on Food. In the second sampling, sun-dried bream contained high levels of Cr, Mn, As, Se and Fe while sun-dried Kapenta contained high levels of Cr, Mn, As, Se, Zn and Fe. The fresh bream contained high levels of Cr, Mn, As, Se and Fe. However, Cu, Pb, Al and Zn were below detection limits in sun-dried bream whereas, Al, Cu and Pb were below detection limits in sun-dried Kapenta. The elements that were below detection in fresh bream includes Cu, Pb, Al and Zn. This can affect the health of the Zambian population at large, as it serves as a staple food for Zambian population who rely on fish as a local source of protein which is cheaper than any other meat source of protein. Fish from open markets in Zambia showed no evidence of health risks associated with Hg. Although some trace metals were in low quantities in fish, their EDI intake were higher than the tolerable daily intake recommended by FDA. Zambian fish in all open markets in Siavonga, Lake Kariba contained some of trace metals in fish sampled. In the long-run, this could cause non-carcinogenic and carcinogenic risk as a result of Cr, Cd, As and

Pb detected in sampled fish. Not only can this be devastating to human health but to the environment and causes implications on the Zambian economy, as metals bioaccumulate.

5.1.2 Recommendation

The key takeaway message from the above comparison of these sampling periods outlines the multifaceted nature of metal ion accumulated in fish, influenced by both natural processes and human activities. While essential metals remain within permissible limits in all fish samples, exceeding the permissible limits recommended by WHO/FAO (2018) of non-essential metals like As in fresh bream highlighting the potential health risks associated with certain fish sources. This not only emphasizes the importance of a comprehensive study for monitoring, but the managerial strategies to mitigate metal contamination in fish, considering not only the aquacultural practices but also the environmental factors contributing to contamination. Additionally, the findings outline the need for rigorous regulatory measures and public awareness campaigns to ensure the safety of fish consumption to protect the public health from the adverse effects of metal ion exposure. Where Suppliers and fisherman need to refrain from sun-drying of fish on top of the stones nearby the lakes as metal particulates contaminants the fish and become trapped onto the surface of the fish during sun-drying. It would be advised to rather make use of existing methods shown in Figure 6.1 of appendix E, such as using a net. Fish industries should implement measures to prohibit cheap practice of sun-drying, for safeguarding food safety. There should be a designated drying area with a high closed wall and well paved to prevent dust and car exhaust fumes and dust particles from poorly maintained roads. The Zambian government needs to invest in an enclosed well paved open market and prohibit cars from passing through. The type of cooking method used is crucial where by rinsing of sun-dried and fresh fish with clean running water is recommended. Washing plays a huge role in removing surface contaminants on the fish. Although, washing fish items does not remove 100% of the contaminants as metals would have already been ingested by the fish or penetrated the sample tissue, it remains the best method so far (Nuapia et al., 2018; Ramalepe et al., 2022). The policy maker should enforce the reduction of contamination of trace metals from the source into the lake or stream. The Zambian population or an individual should avoid consumption of two or more different fish types per day, which results in consumption of different trace metals composition as fish accumulates trace metals, rather consume twice per week based on the data from current study. Another recommendation would be to avoid usage of aluminum pots and utensils during cooking, as aluminum pots increase higher amounts of Al in food. This could be alleviated by using stainless steel pots. Lastly, cooking should always be done with lead free pots to minimize volatile trace metals from evaporating into the atmosphere during the steaming process.

APPENDIX

Appendix A

Part 1: Statistical data of trace metals in fish from Lake Kariba

Table 5.2: Concentrations of trace metals (mg kg^{-1}) in the muscles of the sun-dried Kariba bream (*Oreochromis mortimeri*) collected from Lake Kariba open market along Siavonga, Zambia

	Trace metals						
Sample no	**Cr**	**Mn**	**Zn**	**Fe**	**As**	**Al**	Dimensions*
1	14.79	2.07	4.77	81.8	0.06	57.2	12.5x25
2	16.2	11.91	11.37	89.6	0.1	67.2	7.5x20
3	15.22	4.41	9.68	79.8	0	61.4	6.5x14.5
4	15.99	7.58	14.24	137.4	0	159.4	7x18
5	15.18	6.53	10.23	118.4	0.02	98.8	8.5x20
Mean ± SD	15.47±0.53	6.50±3.30	10.06±3.08	101.40±22.69	0.03±0.04	88.8±38.21	
WHO/FAO	1	0.5	100	100	0.1	100	

*Dimensions represented by WxL: where W is width and L is Length. Elements that were not detected are no included in the table (Hg, Se, Cu, Cd and Pb).

Table 5.2: Concentrations of trace metals (mg kg^{-1}) in sun-dried Kapenta (*Limnothrissa miodon*) collected from Lake Kariba open market along Siavonga, Zambia

	Trace metals							
Sample no	Cr	Mn	Cu	Fe	Zn	As	Se	Al
1	17.19	34.02	0.89	154.2	92.72	0.49	0.25	78.4
2	16.89	40.51	0.82	164.4	79.72	0.49	0	425.2
3	19.83	46.75	1.97	169.4	97.59	0.45	0.54	109
4	18.51	37.15	0.66	144.4	96.58	0.43	1.11	95.2
5	18.64	37.95	1.13	162.6	91.52	0.58	0.34	95.8
Mean ± SD	18.21±1.07	39.28±4.27	1.09±0.46	159.00±8.79	91.62±6.37	0.49±0.05	0.45±0.37	160.72±132.60
WHO/FAO	1	0.5	10	100	100	0.1	1	100

Elements that were not detected are no included in the table (Cd and Pb).

Table 5.3: Concentrations of trace metals (mg kg^{-1}) in the muscles of the fresh Kariba bream (*Oreochromis mortimeri*) collected from Lake Kariba open market along Siavonga, Zambia

	Trace metals									
Sample no	Cr	Mn	Cu	As	Se	Cd	Al	Fe	Zn	Dimensions*
1	17.98	14.97	0.58	25.1	0.18	0.25	73.2	103	37.4	12.5x25
2	19.16	15.49	1.65	22.71	0.18	0	59.8	93.8	30.4	7x15
3	19.14	11.55	0	18.26	0.26	0	81.4	106.2	35.6	7x16
4	18.45	14.94	0	17.9	0.29	0.02	58.4	85.6	33	8x18
5	18.75	10.31	0	17.92	0.09	0	60	99	32.4	9x22
6	17.58	16.11	0	15.88	0.19	0	53	82.2	30.4	9x20
7	20.17	9.39	0	20.24	0.09	0	68.8	99.4	38.8	10x20
8	21.12	26.94	0.58	24.35	0.39	0.7	145.6	101	41.4	8x18
9	19.41	25.53	4.97	19.04	0.31	0	70	108	35.2	9 x21
10	19.11	16.1	0.82	19.54	0.13	0	76.6	117.8	38.4	8x19
11	19.23	13.17	0.44	25.81	0.15	0.91	42.8	86	43	9x21
12	18.06	12.21	0.07	18.76	0.49	0	80.4	116.4	33.2	6x15
13	19.13	16.92	0.44	18.95	0.36	0	69	104.6	34.6	7x15
14	19.06	15.87	0.18	15.52	0.24	1.43	48.4	85	25.8	9 x21
15	18.37	8.94	0	23.42	0.22	1.25	47.6	81.2	38.2	12x22
16	23.2	11.13	0.66	22.54	0.2	0.7	86	126.6	37.6	9x20
17	18.88	4.15	0	13.83	0.13	0	47.2	80.2	26.6	9x19

18	21.65	26.97	1.2	23.64	0.23	1.9	66.6	95.4	37.4	8x19
19	20.7	6.67	0.77	20.05	0.14	0	60.2	97.8	36	7x16
20	20.39	17.71	0.57	24.84	0.25	0.85	70.6	104.2	39.6	6.5x15
21	19.19	38.61	0.36	26.5	0.44	0.85	131.8	161.2	43	6.5x15
22	20.01	16.78	0.87	83.02	0.26	0.99	86	95.8	92.6	6x14
23	18.34	13.31	0.42	19.4	0.21	0	59	78.2	27.2	6x15
24	19.89	12.44	0.88	20.52	0.21	0.09	48.4	66.6	28.8	5.5x14
25	19.68	2.31	0.78	20.96	0.14	0	43.8	73.4	30	17x41
26	19.29	2.75	0.39	13.99	0.11	0	54.2	68.4	22.4	15x14
27	21.03	6.29	0.37	17.28	0.19	0.01	44.6	63.2	24.8	14.5x31
28	19.9	4.08	0.02	14.55	0.1	0.32	60	77.6	21.2	16x35
Mean ± SD	19.53±1.20	13.99±7.96	0.61±0.93	22.30±12.20	0.22±0.10	0.37±0.53	67.62±23.46	94.92±20.17	35.54±12.46	
WHO/FAO	1.00	0.5	10.00	0.1	1	2	100	100	100	

*Dimensions represented by WxL: where W is width and L is Length. Elements that were not detected are no included in the table (Hg and Pb).

Part 2: Statistical data of trace metals in fish from Lake Kariba

Table 5.4: Concentrations of trace metals (mg kg^{-1}) in the muscles of the sun-dried Kariba bream (*Oreochromis mortimeri*) collected from Lake Kariba open market along Siavonga, Zambia

	Trace metals									
Sample no	Cr	Mn	Cu	As	Se	Pb	Al	Fe	Zn	Dimension*
1	15.627	3.444	0.718	0.12	0.618	0	55.8	110.6	10.256	7x18
2	16.040	14.064	1.047	0.162	0.705	0.104	112.6	213.2	13.117	12x24
3	15.856	16.893	1.582	0.088	0.32	0	34	79.4	13.096	9x 20
4	13.040	3.342	0	0.115	0.52	0	32	102.4	8.991	8x15.5
5	14.316	2.593	1.981	0.082	0.835	0	21.6	91.6	16.716	10x18
6	15.645	4.332	2.252	0.119	1.768	0.589	49.6	115.6	14.578	10x23
7	18.123	24.151	0.969	0.271	2.846	0.537	21.2	102.6	27.253	10x19
8	18.097	4.277	1.091	0.085	1.442	0.567	48	189.6	69.414	9x19
9	17.033	27.258	0.79	0.273	2.572	0.519	16.2	97.4	34.238	8.5x20
10	15.596	13.676	0.766	0.135	0.086	1.054	43.44	122.49	16.620	8x20
Mean ± SD	15.94±1.48	11.40±8.74	1.12±0.63	0.15±0.07	1.17±0.90	0.34±0.35	55.8±23.23	110.60±63.82	22.43±17.32	
WHO/FAO	1	0.5	10	0.1	1	0.5	100	100	100	

*Dimensions represented by WxL: where W is width and L is Length. Elements that were not detected are no included in the table (Hg and Cd).

Table 5.5: Concentrations of trace metals (mg kg^{-1}) in sun-dried Kapenta (*Limnothrissa miodon*) collected from Lake Kariba open market along Siavonga, Zambia

	Trace metals								
Sample no	Cr	Mn	Cu	As	Se	Pb	Al	Fe	Zn
1	17.67	68.11	2.54	0.78	2.84	0.38	33.6	187.8	160.25
2	16.94	62.02	3.32	0.77	2.71	0.63	15.8	209.8	120.56
3	15.68	62.76	2.23	0.71	1.75	0.24	42.4	195.6	135.13
4	17.42	61.8	3.6	0.77	2.87	0.31	31.8	206	132.67
5	16.93	63.67	2.92	0.76	2.54	0.39	30.9	199.8	137.15
Mean ± SD	17.67±0.77	68.11±2.59	2.54±0.56	0.78±0.03	2.84±0.46	0.38±0.15	33.60±25.58	187.80±58.59	160.25±14.43
WHO/FAO	1	0.5	10	0.1	1	0.5	100	100	100

Elements that were not detected are no included in the table (Cd)

Table 5.6: Concentrations of trace metals (mg kg^{-1}) in the muscles of the fresh Kariba bream (*Oreochromis mortimeri*) collected from Lake Kariba open market along Siavonga, Zambia

	Trace metals									
Sample no	Cr	Mn	Cu	As	Se	Pb	Al	Fe	Zn	Dimensions*
1	14.374	6.489	0.506	0.124	0	0	58.6	346.4	20.188	12x27
2	13.861	19.298	0.099	0.113	0	0	22.6	72.6	21.958	12.5x30
3	16.945	22.077	0.152	0.307	2.172	0	38.4	117.6	38.065	15x42
4	16.326	28.999	1.254	0.471	2.672	0	101.8	181	36.955	16x39
5	15.499	43.752	3.033	0.436	3.070	0.333	65.4	119	73.244	16x38
6	15.322	10.139	2.466	0.083	1.386	0	37.2	141.4	18.914	14x35
Mean ± SD	15.39±1.06	21.79±12.32	1.25±1.14	0.26±0.16	1.55±1.21	0.06±0.12	54.00±25.17	163.00±68.41	34.89±18.82	
WHO/FAO	1.00	0.5	10.00	0.1	1	0.50	100	100	100	

*Dimensions represented by WxL: where W is width and L is Length. Elements that were not detected are no included in the table (Hg and Cd).

Appendix B

Health risk assessment of fish

Table 5.7: Health risk assessment on consumption of fish from Lake Kariba, 2021

	Child		Adult			
Elements	EDI_{Ing}	EDI_{dermal}	EDI_{Ing}	EDI_{dermal}	LADD	ILCR
Sun-dried bream						
Cr	24,0394	0,0006	5,15130	0,0004	21,3408	10,6704
Mn	10,0967	0,0002	2,16357	0,0002	21,3408	-
As	0,0559	0,0000	0,01198	0,0000	21,3408	32,0112
Al	137,9360	0,0033	29,55771	0,0024	21,3408	-
Fe	157,5080	0,0038	33,75171	0,0027	21,3408	-
Zn	15,6234	0,0004	3,34788	0,0003	21,3408	-
Sun-dried Kapenta						
Cr	24,0394	0,0006	6,0613	0,0005	21,3408	10,6704
Mn	10,0967	0,0002	13,0746	0,0010	21,3408	-
Cu	0,6990	0,0000	0,3628	0,0000	21,3408	-
As	0,7580	0,0000	0,1624	0,0000	21,3408	32,0112
Se	0,6990	0,0000	0,1498	0,0000	21,3408	-
Al	249,6517	0,0060	53,4968	0,0043	21,3408	-
Fe	246,9800	0,0059	52,9243	0,0042	21,3408	-
Zn	142,3257	0,0034	30,4984	0,0024	21,3408	-
Fresh bream						
Cr	30,3383	0,0007	6,5011	0,0005	21,3408	10,6704
Mn	21,7267	0,0005	4,6557	0,0004	21,3408	-
Cu	0,9442	0,0000	0,2023	0,0000	21,3408	-
As	34,6460	0,0008	7,4241	0,0006	21,3408	32,0112

Cd	0,5697	0,0000	0,1221	0,0000	21,3408	134,4470
Se	0,3428	0,0000	0,0735	0,0000	21,3408	-
Al	105,0386	0,0025	22,5083	0,0018	21,3408	-
Fe	147,4446	0,0035	31,5953	0,0025	21,3408	-
Zn	55,1988	6.10	11,8283	0,0009	21,3408	-

Tolerable daily intake recommended by FDA

Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Hg	Se	Zn
120	0.004	0.0012	0.039	10	40	0.24	11	0.0016	0.055	40

Table 5.8: Health risk assessment on consumption of fish from Lake Kariba, 2022

Elements	Child		Adult			
	EDI_{Ing}	EDI_{dermal}	EDI_{Ing}	EDI_{dermal}	LADD	ILCR
Sun-dried bream						
Cr	24,7559	0,0006	5,3048	0,0004	21,3453	10,6726
Mn	17,7127	0,0004	3,7956	0,0003	21,3440	-
Cu	1,7391	0,0000	0,3727	0,0000	21,3411	-
As	0,2252	0,0000	0,0483	0,0000	21,3408	32,0113
Se	1,8193	0,0000	0,3898	0,0000	21,3411	-
Al	86,6760	0,0021	18,5734	0,0015	21,3565	-
Fe	171,7987	0,0041	36,8140	0,0029	21,3718	-
Zn	34,8380	0,0008	7,4653	0,0006	21,3471	-
Pb	0,5235	0,0000	0,1122	0,0000	21,3409	0,1814
Sun-dried Kapenta						

Cr	0,0274	0,0007	0,0059	7.64	21,3458	10,6729
Mn	0,0980	0,0024	0,0210	0,0005	21,3585	-
Pb	0,0006	0,0000	0,0001	0,0000	21,3409	0,1814
Se	0,0044	0,0001	0,0009	0,0001	21,3416	-
As	0,0012	0,0000	0,0003	0,0000	21,3410	32,0115
Al	0,0522	0,0013	0,0112	0,0009	21,3502	-
Cu	0,0039	0,0001	0,0008	0,0001	21,3415	-
Fe	0,2917	0,0070	0,0625	0,0050	21,3935	-
Zn	0,2489	0,0060	0,0533	0,0043	21,3858	-
Fresh bream						
Cr	23,9024	0,0006	5,1220	0,0004	21,3451	10,6726
Mn	33,8508	0,0008	7,2537	0,0006	21,3469	-
Cu	1,9443	0,0000	0,4166	0,0000	21,3412	-
Pb	0,0862	0,0000	0,0185	0,0000	21,3408	0,1814
As	0,3971	0,0000	0,0851	0,0000	21,3409	32,0113
Se	2,4077	0,0001	0,5159	0,0000	21,3412	-
Al	83,8800	0,0020	17,9743	0,0014	21,3560	-
Fe	253,1933	0,0061	54,2557	0,0043	21,3865	-
Zn	54,1917	0,0013	11,6125	0,0009	21,3506	-

Tolerable daily intake recommended by FDA

Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Hg	Se	Zn
120	0.004	0.0012	0.039	10	40	0.24	11	0.0016	0.055	40

Estimated Daily Intake for dermal

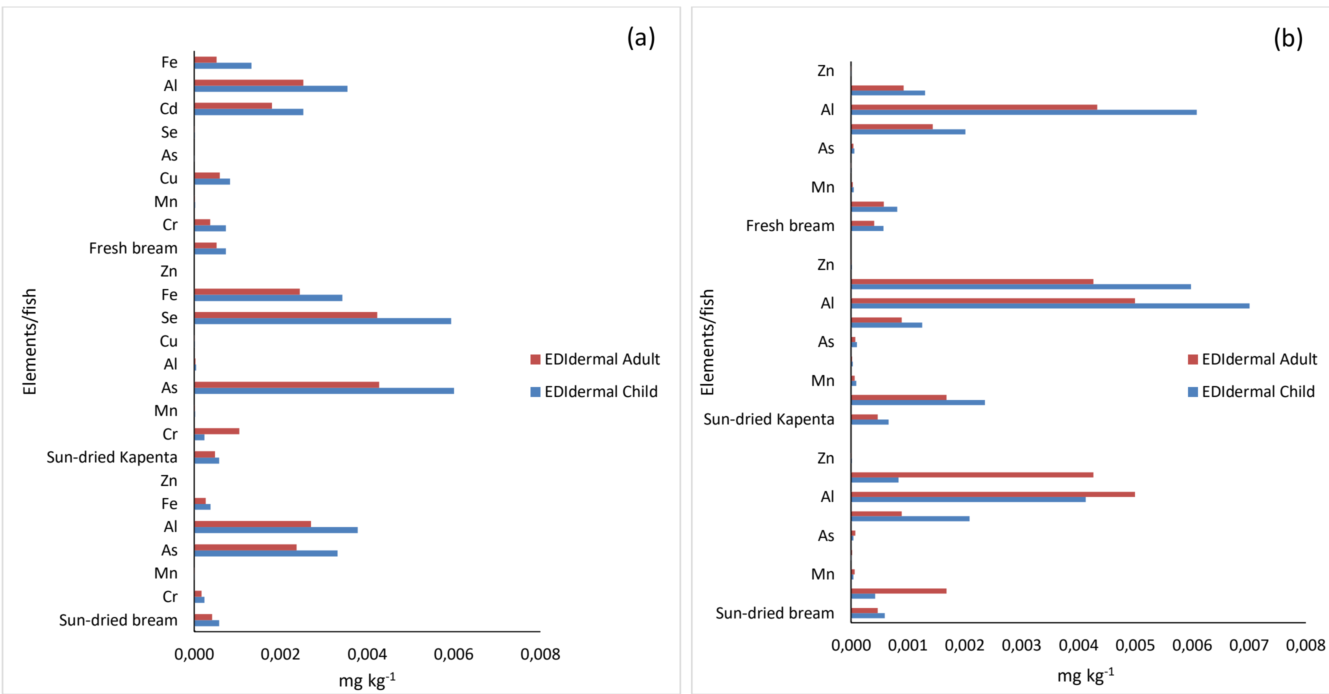


Figure 4.10: Health risk assessment for dermal on fish from Lake Kariba (a) EDI dermal for 2021 and (b) EDI dermal for 2022

Hazard Quotient and Hazard Index

Table 5.9: Health risk assessment on consumption of sun-dried bream from Lake Kariba

Child					Adult			
Metals	**HQ_{Ing}**	**HQ_{Dermal}**	**HQ_{Ing}^{*}**	**HQ_{Dermal}^{*}**	**HQ_{Ing}**	**HQ_{Dermal}**	**HQ_{Ing}^{*}**	**HQ_{Dermal}^{*}**
Cr	801,31	9,63	825,20	9,92	171,71	6,86	176,83	7,07
Mn	219,49	0,13	385,06	0,23	47,03	0,09	82,51	0,16
Cu	-	-	43,48	0,00	-	-	9,32	0,00
As	186,40	0,01	750,78	0,04	39,94	0,01	160,88	0,03
Se	-	-	363,85	0,01	-	-	77,97	0,01
Al	137,94	0,00	86,68	0.00	29,56	0,00	18,57	0.00
Fe	18,75	0,05	20,45	0,06	4,02	0,04	4,38	0,04
Zn	52,08	0,01	116,13	0,03	11,16	0,01	24,88	0,02
Pb	-	-	149,56	0,02	-	-	32,05	0,02
HI	1425,81		2751,50		310,43		594,75	

*2022 sampling

Table 5.10: Health risk assessment on consumption of sun-dried Kapenta from Lake Kariba

Child					Adult			
Metals	**HQ_{Ing}**	**HQ_{Dermal}**	**HQ_{Ing}^{*}**	**HQ_{Dermal}^{*}**	**HQ_{Ing}**	**HQ_{Dermal}**	**HQ_{Ing}^{*}**	**HQ_{Dermal}^{*}**
Cr	801,31	9,63	0,91	10,99	202,04	8,07	0,20	7,83
Mn	219,49	0,13	2,13	1,27	284,23	0,56	0,46	0,91

Cu	42,33	0.00	0,10	0,01	9,07	0.00	0,02	0,01
As	2526,76	0,15	4,04	0,24	541,45	0,11	0,87	0,17
Se	139,80	0.00	0,88	0,02	29,96	0.00	0,19	0,02
Al	249,65	0,01	0,05	0,00	53,50	0,00	0,01	0,00
Fe	29,40	0,08	0,03	0,10	6,30	0,06	0,01	0,07
Zn	474,42	0,11	0,83	0,20	101,66	0,08	0,18	0,14
pb	-	-	0,17	0,03	-	-	0,04	0,02
HI	4493,28		22,01		1237,10		11,13	

*2022 sampling

Table 5.11: Health risk assessment on consumption of fresh bream from Lake Kariba

Child					Adult			
Metals	**HQ_{Ing}**	**HQ_{Dermal}**	**HQ_{Ing}^{*}**	**HQ_{Dermal}^{*}**	**HQ_{Ing}**	**HQ_{Dermal}**	**HQ_{Ing}^{*}**	**HQ_{Dermal}^{*}**
Cr	1011,28	12,15	796,75	9,57	216,70	8,66	796,75	9,57
Mn	472,32	0,28	735,89	0,44	101,21	0,20	735,89	0,44
Cu	23,61	0,00	48,61	0,00	5,06	0,00	48,61	0,00
As	115486,63	2,78	1323,79	0,08	24747,14	1,98	1323,79	0,08
Se	68,57	0,00	481,53	0,01	14,69	0,00	481,53	0,01
Cd	1899,13	0,11	-	-	406,96	0,08	-	-
Al	105,04	0,00	83,88	0,00	22,51	0,00	83,88	0,00
Fe	17,55	0,05	30,14	0,09	3,76	0,04	30,14	0,09
Zn	184,00	0,04	180,64	0,04	39,43	0,03	180,64	0,04
Pb	-	-	24,63	0,00	-	-	24,63	0,00
HI	119283,55		3716,09		25568,44		3716,09	

*2022 sampling

Appendix C

Part 1. Correlation between trace metals in sun-dried Kapenta and fresh bream, 2021 sampling.

Table 5.12: Correlation between trace metals in sun-dried Kapenta

Metals	Cr	Mn	Fe	Zn	As	Hg	Al
Cr	1.00						
Mn	0.92	1.00					
Fe	-0.17	0.09	1.00				
Zn	-0.49	-0.73	-0.29	1.00			
As	-0.34	0.03	0.35	-0.33	1.00		
Hg	-0.48	-0.32	0.27	-0.41	0.27	1.00	
Al	0.70	0.84	0.34	-0.91	0.00	0.20	1.00

Table 5.13: Correlation between trace metals in fresh bream

Metals	Cr	Mn	Cu	As	Se	Cd	Al	Fe	Zn
Cr	1.00								
Mn	0.06	1.00							
Cu	0.16	0.36	1.00						
As	0.14	0.22	0.09	1.00					

Se	-0.08	0.66	0.14	0.16	1.00				
Cd	0.34	0.43	-0.04	0.35	0.19	1.00			
Al	0.25	0.69	0.06	0.27	0.62	0.18	1.00		
Fe	0.12	0.66	0.14	0.15	0.52	0.16	0.73	1.00	
Zn	0.15	0.34	0.10	0.96	0.23	0.36	0.39	0.33	1.00

Part 2. Correlation between trace metals in sun-dried Kapenta and fresh bream, 2022 sampling.

Table 5.14: Correlation between trace metals in sun-dried Kapenta

Metals	Cr	Mn	Cu	As	Se	Pb	Al	Fe	Zn
Cr	1.00								
Mn	0.44	1.00							
Cu	0.54	-0.52	1.00						
As	0.97	0.35	0.61	1.00					
Se	0.97	0.24	0.70	0.99	1.00				
Pb	0.32	-0.10	0.45	0.54	0.48	1.00			
Al	-0.41	0.25	-0.67	-0.62	-0.60	-0.96	1.00		
Fe	-0.02	-0.87	0.81	0.14	0.22	0.52	-0.68	1.00	
Zn	0.39	0.94	-0.53	0.22	0.16	-0.42	0.52	-0.92	1.00

Table 5.15: Correlation between trace metals in fresh bream

Metals	Cr	Mn	Cu	As	Se	Cd	Al	Fe	Zn
Cr	1.00								
Mn	0.06	1.00							
Cu	0.16	0.36	1.00						
As	0.14	0.22	0.09	1.00					
Se	-0.08	0.66	0.14	0.16	1.00				
Cd	0.34	0.43	-0.04	0.35	0.19	1.00			
Al	0.25	0.69	0.06	0.27	0.62	0.18	1.00		
Fe	0.12	0.66	0.14	0.15	0.52	0.16	0.73	1.00	
Zn	0.15	0.34	0.10	0.96	0.23	0.36	0.39	0.33	1.00

Appendix D – Statistical analysis of sampled fish

Part 1: Statistical analysis of fish – Anova and T-test analysis

Table A1: ANOVA analysis of trace metals in sun-dried bream

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Cr	5	77.38	15.476	0.35303		
Mn	5	32.5	6.5	13.6071		
Zn	5	50.29	10.058	11.83647		
Fe	5	507	101.4	643.73999		
As	5	0.18	0.036	0.00188		
Al	5	444	88.8	1825.06		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	51581.64543	5	10316.329086	24.8128005417529	9.35582861199773E-09	2.62065414786289
Within Groups	9978.39392	24	415.766413333333			
Total	61560.03935	29				

Table A2: t-Test analysis of trace metals in sun-dried bream Bonferroni's correction p = 0,0033333

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Mn*		*Cr*	*Zn*		*Cr*	*Fe*
Mean	15,476	6,5	Mean	15,476	10,058	Mean	15,476	101,4
Variance	0,35303	13,6071	Variance	0,35303	11,83647	Variance	0,35303	643,74
Observations	5	5	Observations	5	5	Observations	5	5
Pooled Variance	6,980065		Pearson Correlation	0,828314		Pearson Correlation	0,396415	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	8		df	4		df	4	
t Stat	5,371841		t Stat	4,083259		t Stat	-7,64174	
P(T<=t) one-tail	0,000334		P(T<=t) one-tail	0,007529		P(T<=t) one-tail	0,000788	
t Critical one-tail	1,859548		t Critical one-tail	2,131847		t Critical one-tail	2,131847	
P(T<=t) two-tail	0,000668		P(T<=t) two-tail	0,015058		P(T<=t) two-tail	0,001575	
t Critical two-tail	2,306004		t Critical two-tail	2,776445		t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*As*		*Cr*	*Al*		*Mn*	*Fe*
Mean	15,476	0,036	Mean	15,476	88,8	Mean	6,5	101,4
Variance	0,35303	0,00188	Variance	0,35303	1825,06	Variance	13,6071	643,74
Observations	5	5	Observations	5	5	Observations	5	5
Pearson Correlation	0,245708		Pearson Correlation	0,45682		Pearson Correlation	0,287215	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	4		df	4	
t Stat	59,01475		t Stat	-3,86213		t Stat	-8,63738	
P(T<=t) one-tail	2,47E-07		P(T<=t) one-tail	0,009057		P(T<=t) one-tail	0,000494	
t Critical one-tail	2,131847		t Critical one-tail	2,131847		t Critical one-tail	2,131847	
P(T<=t) two-tail	4,94E-07		P(T<=t) two-tail	0,018114		P(T<=t) two-tail	0,000988	

t Critical two-tail	2,776445	t Critical two-tail	2,776445	t Critical two-tail	2,776445

t-Test: Paired Two Sample for Means

	Mn	*Zn*
Mean	6,5	10,058
Variance	13,6071	11,83647
Observations	5	5
Pearson Correlation	0,705927	
Hypothesized Mean Difference	0	
df	4	
t Stat	-2,90011	
P(T<=t) one-tail	0,022057	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,044113	
t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means

	Mn	*As*
Mean	6,5	0,036
Variance	13,6071	0,00188
Observations	5	5
Pearson Correlation	0,431095	
Hypothesized Mean Difference	0	
df	4	
t Stat	3,938086	
P(T<=t) one-tail	0,008494	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,016987	
t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means

	Mn	*Al*
Mean	6,5	88,8
Variance	13,6071	1825,06
Observations	5	5
Pearson Correlation	0,248983	
Hypothesized Mean Difference	0	
df	4	
t Stat	-4,38636	
P(T<=t) one-tail	0,005908	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,011816	
t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means

	Zn	*Fe*
Mean	10,058	101,4
Variance	11,83647	643,74
Observations	5	5
Pearson Correlation	0,71544	
Hypothesized Mean Difference	0	
df	4	
t Stat	-8,86628	
P(T<=t) one-tail	0,000447	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,000894	
t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means

	Zn	*As*
Mean	10,058	0,036
Variance	11,83647	0,00188
Observations	5	5
Pearson Correlation	-0,30609	
Hypothesized Mean Difference	0	
df	4	
t Stat	6,488214	
P(T<=t) one-tail	0,001455	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,00291	
t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means

	Zn	*Al*
Mean	10,058	88,8
Variance	11,83647	1825,06
Observations	5	5
Pearson Correlation	0,758771	
Hypothesized Mean Difference	0	
df	4	
t Stat	-4,38288	
P(T<=t) one-tail	0,005924	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,011848	
t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Fe	*As*		*Fe*	*Al*		*As*	*Al*
Mean	101,4	0,036	Mean	101,4	88,8	Mean	0,036	88,8
Variance	643,74	0,00188	Variance	643,74	1825,06	Variance	0,00188	1825,06
Observations	5	5	Observations	5	5	Observations	5	5
Pearson Correlation	-0,45814		Pearson Correlation	0,963566		Pearson Correlation	-0,52043	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	4		df	4	
t Stat	8,926333		t Stat	1,445395		t Stat	-4,64359	
P(T<=t) one-tail	0,000435		P(T<=t) one-tail	0,110934		P(T<=t) one-tail	0,004854	
t Critical one-tail	2,131847		t Critical one-tail	2,131847		t Critical one-tail	2,131847	
P(T<=t) two-tail	0,000871		P(T<=t) two-tail	0,221867		P(T<=t) two-tail	0,009708	
t Critical two-tail	2,776445		t Critical two-tail	2,776445		t Critical two-tail	2,776445	

Table B1: ANOVA analysis of trace metals in sun-dried Kapenta

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Cr	5	77.38	15.476	0.35303		
Mn	5	32.5	6.5	13.6071		
Fe	5	795	159	96.62		
Zn	5	458.13	91.626	50.76808		
As	5	2.44	0.488	0.003320		
Al	5	803.6	160.72	21977.172		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	164489.639384971	5	27414.9398974952	8.66835460536209	2,25078335008045E-05	2.44525939508938
Within Groups	88554.0972972	28	3162.64633204286			
Total	253043.736682171	34				

Table B2: t-Test analysis of trace metals in sun-dried Kapenta Bonferroni's correction p = 0,002381

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Mn*		*Cr*	*Fe*		*Cr*	*Zn*
Mean	15,476	6,5	Mean	15,476	159	Mean	15,476	91,626
Variance	0,35303	13,6071	Variance	0,35303	96,62	Variance	0,35303	50,76808
Observations	5	5	Observations	5	5	Observations	5	5
Pearson Correlation	0,91675		Pearson Correlation	-0,17251		Pearson Correlation	-0,49129	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	S	Hypothesized Mean Difference	0	
df	4		Df	4		df	4	
t Stat	6,365612		t Stat	-32,2566		t Stat	-22,9017	
P(T<=t) one-tail	0,001561		P(T<=t) one-tail	2,75E-06		P(T<=t) one-tail	1,08E-05	
t Critical one-tail	2,131847		t Critical one-tail	2,131847		t Critical one-tail	2,131847	
P(T<=t) two-tail	0,003123		P(T<=t) two-tail	5,51E-06		P(T<=t) two-tail	2,15E-05	
t Critical two-tail	2,776445		t Critical two-tail	2,776445		t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Mn	*Fe*		*Mn*	*Zn*		*Mn*	*As*
Mean	6,5	159	Mean	6,5	91,626	Mean	6,5	0,488
Variance	13,6071	96,62	Variance	13,6071	50,76808	Variance	13,6071	0,00332
Observations	5	5	Observations	5	5	Observations	5	5
Pearson Correlation	0,090198		Pearson Correlation	-0,72647		Pearson Correlation	0,025289	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		Df	4		df	4	

t Stat	-33,4884		t Stat	-18,7954		t Stat	3,645355	
P(T<=t) one-tail	2,37E-06		P(T<=t) one-tail	2,36E-05		P(T<=t) one-tail	0,01093	
	2,13184							
t Critical one-tail	7		t Critical one-tail	2,131847		t Critical one-tail	2,131847	
P(T<=t) two-tail	4,74E-06		P(T<=t) two-tail	4,72E-05		P(T<=t) two-tail	0,021861	
	2,77644							
t Critical two-tail	5		t Critical two-tail	2,776445		t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Fe	*As*		Zn	Al		*Fe*	*Al*
Mean	159	0,488	Mean	91,626	160,72	Mean	159	160,72
			Variance	50,76808	21977,1			
Variance	96,62	0,00332			7	Variance	96,62	21977,17
Observations	5	5	Observations	5	5	Observations	5	5
	0,34606		Pearson Correlation	-0,91479				
Pearson Correlation	2					Pearson Correlation	0,344551	
Hypothesized Mean			Hypothesized Mean	0		Hypothesized Mean		
Difference	0		Difference			Difference	0	
df	4		Df	4		df	4	
	36,1317		t Stat	-0,99811				
t Stat	4					t Stat	-0,0265	
P(T<=t) one-tail	1,75E-06		P(T<=t) one-tail	0,187357		P(T<=t) one-tail	0,490065	
	2,13184		t Critical one-tail	2,131847				
t Critical one-tail	7					t Critical one-tail	2,131847	
P(T<=t) two-tail	3,5E-06		P(T<=t) two-tail	0,374714		P(T<=t) two-tail	0,980131	
	2,77644		t Critical two-tail	2,776445				
t Critical two-tail	5					t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Zn	As		Fe	Zn		As	Al
Mean	91,626	0,488	Mean	159	91,626	Mean	0,0554	160,72
Variance	50,7680	0,00332	Variance	96,62	50,7680			
	8				8	Observations	5	5

Observations	5	5
Pearson Correlation	-0,33208	
Hypothesized Mean Difference	0	
df	4	
t Stat	28,52409	
P(T<=t) one-tail	4,49E-06	
t Critical one-tail	2,131847	
P(T<=t) two-tail	8,99E-06	

Observations	5	5
Pearson Correlation	-0,28638	
Hypothesized Mean Difference	0	
Df	4	
t Stat	11,00207	
P(T<=t) one-tail	0,000194	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,000388	

Pearson Correlation	0,202578	
Hypothesized Mean Difference	0	
df	4	
t Stat	-2,42346	
P(T<=t) one-tail	0,036246	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,072491	
t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means

	Cr	*As*
Mean	15,476	0,488
Variance	0,35303	0,00332
Observations	5	5
Pearson Correlation	-0,34497	
Hypothesized Mean Difference	0	
df	4	
t Stat	54,36934	
P(T<=t) one-tail	3,43E-07	
t Critical one-tail	2,131847	
P(T<=t) two-tail	6,85E-07	
t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means

	Mn	Al
Mean	6,5	160,72
Variance	13,6071	21977,17
Observations	5	5
Pearson Correlation	0,83702	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-2,37541	
P(T<=t) one-tail	0,038185	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,07637	
t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means

	Cr	*Al*
Mean	15,476	160,72
Variance	0,35303	21977,17
Observations	5	5
Pearson Correlation	0,70029	
Hypothesized Mean Difference	0	
df	4	
t Stat	-2,19693	
P(T<=t) one-tail	0,046485	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,09297	
t Critical two-tail	2,776445	

t-Test: Paired Two Sample for Means

	Mn	*Hg*
Mean	6,5	0,0554
Variance	13,6071	0,000794
Observations	5	5
Pearson Correlation	-0,31588	
Hypothesized Mean Difference	0	
df	4	
t Stat	3,897086	
P(T<=t) one-tail	0,008792	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,017585	
t Critical two-tail	2,776445	

Table C1: ANOVA analysis of trace metals in fresh bream

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*		
Cr	10	190.87	19.087	1.048312		
Mn	10	161.33	16.133	34.29611		
Cu	10	8.6	0.86	2.374733		
As	10	200.94	20.094	9.1241605		
Se	10	2.11	0.211	0.009855		
Cd	10	0.97	0.097	0.050979		
Al	10	746.8	74.68	701.0151		
Fe	10	996	99.6	110.1867		
Zn	10	353	35.3	13.92222		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	99070.5893022222	8	12383.8236627778	127.81056760953	1.17908666378127E-42	2.05488162376201
Within Groups	7848.25336	81	96.8920167901234			
Total	106918.842662222	89				

Table C2: t-Test analysis of trace metals in fresh bream Bonferroni's correction p = 0,0013889

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Mn*		*Cr*	*Cu*		*Cr*	*As*
Mean	19,53107	13,98714	Mean	19,53107	0,607857	Mean	19,53107	22,30429
Variance	1,489914	65,70407	Variance	1,489914	0,90281	Variance	1,489914	154,4977
Observations	28	28	Observations	28	28	Observations	28	28
Pearson Correlation	0,058506		Pearson Correlation	0,160102		Pearson Correlation	0,144867	

Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	27		Df	27		df	27	
t Stat	3,609985		t Stat	70,42916		t Stat	-1,19186	
P(T<=t) one-tail	0,000615		P(T<=t) one-tail	1,93E-32		P(T<=t) one-tail	0,121844	
t Critical one-tail	1,703288		t Critical one-tail	1,703288		t Critical one-tail	1,703288	
P(T<=t) two-tail	0,00123		P(T<=t) two-tail	3,85E-32		P(T<=t) two-tail	0,243688	
t Critical two-tail	2,051831		t Critical two-tail	2,051831		t Critical two-tail	2,051831	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
Mean	13,98714	0,607857	Mean	13,98714	22,30429	Mean	13,98714	0,220714
Variance	65,70407	0,90281	Variance	65,70407	154,4977	Variance	65,70407	0,010637
Observations	28	28	Observations	28	28	Observations	28	28
Pearson Correlation	0,356343		Pearson Correlation	0,223266		Pearson Correlation	0,658729	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	27		Df	27		df	27	
t Stat	9,05582		t Stat	-3,32484		t Stat	9,062316	
P(T<=t) one-tail	5,7E-10		P(T<=t) one-tail	0,001277		P(T<=t) one-tail	5,62E-10	
t Critical one-tail	1,703288		t Critical one-tail	1,703288		t Critical one-tail	1,703288	
P(T<=t) two-tail	1,14E-09		P(T<=t) two-tail	0,002555		P(T<=t) two-tail	1,12E-09	
t Critical two-tail	2,051831		t Critical two-tail	2,051831		t Critical two-tail	2,051831	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cu	*Se*		*Cu*	*Cd*		*Cu*	*Al*
Mean	0,607857	0,220714	Mean	0,607857	0,366786	Mean	0,607857	67,62143
Variance	0,90281	0,010637	Variance	0,90281	0,291008	Variance	0,90281	570,9225
Observations	28	28	Observations	28	28	Observations	28	28
Pearson Correlation	0,136268		Pearson Correlation	-0,0398		Pearson Correlation	0,056806	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	27		Df	27		df	27	

t Stat	2,175465		t Stat	1,148044		t Stat	-14,8625	
P(T<=t) one-tail	0,019261		P(T<=t) one-tail	0,13051		P(T<=t) one-tail	8,04E-15	
t Critical one-tail	1,703288		t Critical one-tail	1,703288		t Critical one-tail	1,703288	
P(T<=t) two-tail	0,038522		P(T<=t) two-tail	0,261021		P(T<=t) two-tail	1,61E-14	
t Critical two-tail	2,051831		t Critical two-tail	2,051831		t Critical two-tail	2,051831	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Al	*Fe*		*Al*	*Zn*		*Fe*	*Zn*
Mean	67,62143	94,92143	Mean	67,62143	35,53571	Mean	94,92143	35,53571
Variance	570,9225	422,0336	Variance	570,9225	160,9246	Variance	422,0336	160,9246
Observations	28	28	Observations	28	28	Observations	28	28
Pearson Correlation	0,733285		Pearson Correlation	0,390784		Pearson Correlation	0,328524	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	27		Df	27		df	27	
t Stat	-8,74191		t Stat	7,63153		t Stat	15,48661	
P(T<=t) one-tail	1,17E-09		P(T<=t) one-tail	1,65E-08		P(T<=t) one-tail	2,96E-15	
t Critical one-tail	1,703288		t Critical one-tail	1,703288		t Critical one-tail	1,703288	
P(T<=t) two-tail	2,34E-09		P(T<=t) two-tail	3,3E-08		P(T<=t) two-tail	5,93E-15	
t Critical two-tail	2,051831		t Critical two-tail	2,051831		t Critical two-tail	2,051831	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Fe*		*Cr*	*Zn*		*Mn*	*Zn*
Mean	19,53107	94,92143	Mean	19,53107	35,53571	Mean	13,98714	35,53571
Variance	1,489914	422,0336	Variance	1,489914	160,9246	Variance	65,70407	160,9246
Observations	28	28	Observations	28	28	Observations	28	28
Pearson Correlation	0,117095		Pearson Correlation	0,1486		Pearson Correlation	0,336022	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	27		Df	27		df	27	
t Stat	-19,5204		t Stat	-6,74146		t Stat	-9,08497	

P(T<=t) one-tail	9,42E-18		P(T<=t) one-tail	1,54E-07		P(T<=t) one-tail	5,34E-10	
t Critical one-tail	1,703288		t Critical one-tail	1,703288		t Critical one-tail	1,703288	
P(T<=t) two-tail	1,88E-17		P(T<=t) two-tail	3,08E-07		P(T<=t) two-tail	1,07E-09	
t Critical two-tail	2,051831		t Critical two-tail	2,051831		t Critical two-tail	2,051831	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cu	*As*		*As*	*Al*		*As*	*Cd*
Mean	0,607857	22,30429	Mean	22,30429	67,62143	Mean	22,30429	0,366786
Variance	0,90281	154,4977	Variance	154,4977	570,9225	Variance	154,4977	0,291008
Observations	28	28	Observations	28	28	Observations	28	28
Pearson Correlation	0,094991		Pearson Correlation	0,27229		Pearson Correlation	0,346701	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	27		Df	27		df	27	
t Stat	-9,27682		t Stat	-10,1001		t Stat	9,473686	
P(T<=t) one-tail	3,46E-10		P(T<=t) one-tail	5,73E-11		P(T<=t) one-tail	2,24E-10	
t Critical one-tail	1,703288		t Critical one-tail	1,703288		t Critical one-tail	1,703288	
P(T<=t) two-tail	6,93E-10		P(T<=t) two-tail	1,15E-10		P(T<=t) two-tail	4,47E-10	
t Critical two-tail	2,051831		t Critical two-tail	2,051831		t Critical two-tail	2,051831	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cd	*Fe*		*Cd*	*Zn*		*Al*	*Fe*
Mean	0,366786	94,92143	Mean	0,366786	35,53571	Mean	67,62143	94,92143
Variance	0,291008	422,0336	Variance	0,291008	160,9246	Variance	570,9225	422,0336
Observations	28	28	Observations	28	28	Observations	28	28
Pearson Correlation	0,155067		Pearson Correlation	0,360994		Pearson Correlation	0,733285	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	27		Df	27		df	27	
t Stat	-24,4463		t Stat	-14,8865		t Stat	-8,74191	
P(T<=t) one-tail	2,97E-20		P(T<=t) one-tail	7,73E-15		P(T<=t) one-tail	1,17E-09	

t Critical one-tail	1,703288		t Critical one-tail	1,703288		t Critical one-tail	1,703288
P(T<=t) two-tail	5,94E-20		P(T<=t) two-tail	1,55E-14		P(T<=t) two-tail	2,34E-09
t Critical two-tail	2,051831		t Critical two-tail	2,051831		t Critical two-tail	2,051831

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Al	*Zn*		*Fe*	*Zn*		*Se*	*Fe*
Mean	67,62143	35,53571	Mean	94,92143	35,53571	Mean	0,220714	94,92143
Variance	570,9225	160,9246	Variance	422,0336	160,9246	Variance	0,010637	422,0336
Observations	28	28	Observations	28	28	Observations	28	28
Pearson Correlation	0,390784		Pearson Correlation	0,328524		Pearson Correlation	0,524454	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	27		Df	27		df	27	
t Stat	7,63153		t Stat	15,48661		t Stat	-24,4568	
P(T<=t) one-tail	1,65E-08		P(T<=t) one-tail	2,96E-15		P(T<=t) one-tail	2,94E-20	
t Critical one-tail	1,703288		t Critical one-tail	1,703288		t Critical one-tail	1,703288	
P(T<=t) two-tail	3,3E-08		P(T<=t) two-tail	5,93E-15		P(T<=t) two-tail	5,87E-20	
t Critical two-tail	2,051831		t Critical two-tail	2,051831		t Critical two-tail	2,051831	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Se	*Zn*		*Cd*	*Al*
Mean	0,220714	35,53571	Mean	0,366786	67,62143
Variance	0,010637	160,9246	Variance	0,291008	570,9225
Observations	28	28	Observations	28	28
Pearson Correlation	0,232001		Pearson Correlation	0,179581	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	27		Df	27	
t Stat	-14,7582		t Stat	-14,951	
P(T<=t) one-tail	9,53E-15		P(T<=t) one-tail	6,97E-15	
t Critical one-tail	1,703288		t Critical one-tail	1,703288	

P(T<=t) two-tail	1,91E-14	P(T<=t) two-tail	1,39E-14
t Critical two-tail	2,051831	t Critical two-tail	2,051831

Part 2: Statistical analysis of fish –Anova and T-test analysis

Table A1*: ANOVA analysis of trace metals in sun-dried bream

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Cr	10	159373	15937,3	2442275
Mn	10	114030	11403	84777833
Cu	10	7956,243	795,6243	828322,4
As	10	1,45	0,145	0,005074
Se	10	8631,084	863,1084	1385614
Pb	10	1056,316	105,6316	111037,4
Al	10	434,44	43,444	769,8025
Fe	10	1224,89	122,489	1906,73
Zn	10	224279	22427,9	3,33E+08

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5,91E+09	8	7,39E+08	15,73124	9,76E-14	2,054882
Within Groups	3,81E+09	81	46998081			
Total	9,72E+09	89				

Table A2*: t-Test analysis of trace metals in sun-dried bream Bonferroni's correction p = 0,0013514

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Mn*		*Cr*	*Cu*		*Cr*	*As*
Mean	15,9373	11,403	Mean	15,9373	1,1196	Mean	15,9373	0,145
Variance	2,442275	84,77783	Variance	2,442275	0,435042	Variance	2,442275	0,005074
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	0,548805		Pearson Correlation	0,103957		Pearson Correlation	0,499328	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	1,696607		t Stat	28,71407		t Stat	32,67327	
P(T<=t) one-tail	0,062002		P(T<=t) one-tail	1,84E-10		P(T<=t) one-tail	5,8E-11	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,124004		P(T<=t) two-tail	3,67E-10		P(T<=t) two-tail	1,16E-10	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Se*		*Cr*	*Pb*		*Cr*	*Al*
Mean	15,9373	1,1712	Mean	15,9373	0,337	Mean	15,9373	43,444
Variance	2,442275	0,906596	Variance	2,442275	0,134687	Variance	2,442275	769,8025
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	0,64225		Pearson Correlation	0,473811		Pearson Correlation	0,005586	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	38,9455		t Stat	34,59508		t Stat	-3,1311	
P(T<=t) one-tail	1,21E-11		P(T<=t) one-tail	3,48E-11		P(T<=t) one-tail	0,00605	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	2,41E-11		P(T<=t) two-tail	6,96E-11		P(T<=t) two-tail	0,0121	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Fe*		*Cr*	*Zn*		*Mn*	*Cu*
Mean	15,9373	122,489	Mean	15,9373	22,4279	Mean	11,403	1,1196
Variance	2,442275	1906,73	Variance	2,442275	333,435	Variance	84,77783	0,435042
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	0,326922		Pearson Correlation	0,704786		Pearson Correlation	-0,16054	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	-7,8032		t Stat	-1,1937		t Stat	3,483141	
P(T<=t) one-tail	1,35E-05		P(T<=t) one-tail	0,131555		P(T<=t) one-tail	0,003452	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	2,7E-05		P(T<=t) two-tail	0,263111		P(T<=t) two-tail	0,006904	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Mn	*As*		*Mn*	*Se*		*Mn*	*Pb*
Mean	11,403	0,145	Mean	11,403	1,1712	Mean	11,403	0,337
Variance	84,77783	0,005074	Variance	84,77783	0,906596	Variance	84,77783	0,134687
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	0,83899		Pearson Correlation	0,527793		Pearson Correlation	0,313745	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	3,891744		t Stat	3,701005		t Stat	3,845883	
P(T<=t) one-tail	0,001833		P(T<=t) one-tail	0,002456		P(T<=t) one-tail	0,001965	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,003665		P(T<=t) two-tail	0,004913		P(T<=t) two-tail	0,003931	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		

	Mn	*Al*		*Mn*	*Fe*		*Mn*	*Zn*
Mean	11,403	43,444	Mean	11,403	122,489	Mean	11,403	22,4279
Variance	84,77783	769,8025	Variance	84,77783	1906,73	Variance	84,77783	333,435
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	-0,20572		Pearson Correlation	-0,15126		Pearson Correlation	0,08971	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	-3,2707		t Stat	-7,6418		t Stat	-1,76983	
P(T<=t) one-tail	0,004836		P(T<=t) one-tail	1,59E-05		P(T<=t) one-tail	0,055268	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,009672		P(T<=t) two-tail	3,19E-05		P(T<=t) two-tail	0,110535	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cu	*As*		*Cu*	*Se*		*Cu*	*Pb*
Mean	1,1196	0,145	Mean	1,1196	1,1712	Mean	1,1196	0,337
Variance	0,435042	0,005074	Variance	0,435042	0,906596	Variance	0,435042	0,134687
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	-0,29226		Pearson Correlation	0,11319		Pearson Correlation	0,008532	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	4,507114		t Stat	-0,14899		t Stat	3,290681	
P(T<=t) one-tail	0,000737		P(T<=t) one-tail	0,442424		P(T<=t) one-tail	0,004684	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,001474		P(T<=t) two-tail	0,884847		P(T<=t) two-tail	0,009368	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cu	*Al*		*Cu*	*Fe*		*Cu*	*Zn*

Mean	1,1196	43,444	Mean	1,1196	122,489	Mean	1,1196	22,4279
Variance	0,435042	769,8025	Variance	0,435042	1906,73	Variance	0,435042	333,435
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	-0,00718		Pearson Correlation	-0,09378		Pearson Correlation	-0,01315	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	-4,82175		t Stat	-8,77609		t Stat	-3,68599	
P(T<=t) one-tail	0,000472		P(T<=t) one-tail	5,24E-06		P(T<=t) one-tail	0,002514	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,000945		P(T<=t) two-tail	1,05E-05		P(T<=t) two-tail	0,005029	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	As	*Se*		*As*	*Pb*		*As*	*Al*
Mean	0,145	1,1712	Mean	0,145	0,337	Mean	0,145	43,444
Variance	0,005074	0,906596	Variance	0,005074	0,134687	Variance	0,005074	769,8025
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	0,761031		Pearson Correlation	0,322965		Pearson Correlation	-0,20232	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	-3,60919		t Stat	-1,73209		t Stat	-4,93243	
P(T<=t) one-tail	0,002833		P(T<=t) one-tail	0,05865		P(T<=t) one-tail	0,000405	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,005667		P(T<=t) two-tail	0,1173		P(T<=t) two-tail	0,000811	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	As	*Fe*		*As*	*Zn*		*Se*	*Pb*

Mean	0,145	122,489	Mean	0,145	22,4279	Mean	1,1712	0,337
Variance	0,005074	1906,73	Variance	0,005074	333,435	Variance	0,906596	0,134687
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	-0,09719		Pearson Correlation	0,085725		Pearson Correlation	0,298625	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	-8,85867		t Stat	-3,86018		t Stat	2,891058	
P(T<=t) one-tail	4,86E-06		P(T<=t) one-tail	0,001923		P(T<=t) one-tail	0,008927	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	9,72E-06		P(T<=t) two-tail	0,003846		P(T<=t) two-tail	0,017853	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Se	*Al*		*Se*	*Fe*		*Se*	*Zn*
Mean	1,1712	43,444	Mean	1,1712	122,489	Mean	1,1712	22,4279
Variance	0,906596	769,8025	Variance	0,906596	1906,73	Variance	0,906596	333,435
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	-0,36485		Pearson Correlation	-0,08007		Pearson Correlation	0,438746	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	-4,7561		t Stat	-8,7684		t Stat	-3,76307	
P(T<=t) one-tail	0,000518		P(T<=t) one-tail	5,28E-06		P(T<=t) one-tail	0,002232	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,001035		P(T<=t) two-tail	1,06E-05		P(T<=t) two-tail	0,004464	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Pb	*Al*		*Pb*	*Fe*		*Pb*	*Zn*
Mean	0,337	43,444	Mean	0,337	122,489	Mean	0,337	22,4279

Variance	0,134687	769,8025	Variance	0,134687	1906,73	Variance	0,134687	333,435
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	-0,13845		Pearson Correlation	0,136781		Pearson Correlation	0,392091	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	-4,90373		t Stat	-8,85606		t Stat	-3,85539	
P(T<=t) one-tail	0,000422		P(T<=t) one-tail	4,87E-06		P(T<=t) one-tail	0,001937	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,000843		P(T<=t) two-tail	9,74E-06		P(T<=t) two-tail	0,003874	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Al	*Fe*		*Al*	*Zn*		*Fe*	*Zn*
Mean	43,444	122,489	Mean	43,444	22,4279	Mean	122,489	22,4279
Variance	769,8025	1906,73	Variance	769,8025	333,435	Variance	1906,73	333,435
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	0,809551		Pearson Correlation	-0,15153		Pearson Correlation	0,412987	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	9		df	9		df	9	
t Stat	-9,34845		t Stat	1,87466		t Stat	7,956477	
P(T<=t) one-tail	3,13E-06		P(T<=t) one-tail	0,0468		P(T<=t) one-tail	1,16E-05	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	6,25E-06		P(T<=t) two-tail	0,0936		P(T<=t) two-tail	2,31E-05	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	

Table B1*: ANOVA analysis of trace metals in sun-dried Kapenta

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Cr	5	84,64	16,928	0,58757
Mn	5	318,36	63,672	6,69027
Cu	5	14,61	2,922	0,31072
As	5	3,79	0,758	0,00077
Se	5	12,71	2,542	0,21297
Pb	5	1,95	0,39	0,02165
Al	5	154,5	30,9	92,09
Fe	5	999	199,8	75,02
Zn	5	685,76	137,152	208,2472

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	216638,4	8	24070,93	628,1865	3,73E-40	2,124029
Within Groups	1532,725	40	38,31812			
Total	218171,1	49				

Table B2*: t-Test analysis of trace metals in sun-dried Kapenta Bonferroni's correction p = 0,001087

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Mn*		*Cr*	*Cu*		*Cr*	*As*
Mean	15937,3	11403	Mean	15937,3	795,6243	Mean	15937,3	0,145
Variance	2442275	84777833	Variance	2442275	828322,4	Variance	2442275	0,005074
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	0,548805		Pearson Correlation	-0,11971		Pearson Correlation	0,499328	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	9		df	9		df	9	
t Stat	1,696607		t Stat	25,19719		t Stat	32,24953	
P(T<=t) one-tail	0,062002		P(T<=t) one-tail	5,87E-10		P(T<=t) one-tail	6,51E-11	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,124004		P(T<=t) two-tail	1,17E-09		P(T<=t) two-tail	1,3E-10	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Mn	*Cu*		*Mn*	*As*		*Mn*	*Se*
Mean	11403	795,6243	Mean	11403	0,145	Mean	11403	863,1084
Variance	84777833	828322,4	Variance	84777833	0,005074	Variance	84777833	1385614
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	-0,39325		Pearson Correlation	0,83899		Pearson Correlation	0,556359	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	9		df	9		df	9	
t Stat	3,49341		t Stat	3,916294		t Stat	3,871836	
P(T<=t) one-tail	0,003397		P(T<=t) one-tail	0,001765		P(T<=t) one-tail	0,001889	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,006794		P(T<=t) two-tail	0,003531		P(T<=t) two-tail	0,003778	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
	Cu	*Se*		*Cu*	*Pb*		*Cu*	*Al*

Mean	795,6243	863,1084	Mean	795,6243	105,6316	Mean	795,6243	43,444
Variance	828322,4	1385614	Variance	828322,4	111037,4	Variance	828322,4	769,8025
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	-0,13531		Pearson Correlation	-0,30686		Pearson Correlation	0,145173	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	9		df	9		df	9	
t Stat	-0,13486		t Stat	2,056716		t Stat	2,623912	
P(T<=t) one-tail	0,447844		P(T<=t) one-tail	0,034925		P(T<=t) one-tail	0,013817	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,895687		P(T<=t) two-tail	0,06985		P(T<=t) two-tail	0,027633	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	As	*Fe*		*As*	*Zn*		*Se*	*Pb*
Mean	0,145	122,489	Mean	0,145	22427,9	Mean	863,1084	105,6316
Variance	0,005074	1906,73	Variance	0,005074	3,33E+08	Variance	1385614	111037,4
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	-0,09719		Pearson Correlation	0,085725		Pearson Correlation	-0,2569	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	9		df	9		df	9	
t Stat	-8,85867		t Stat	-3,88401		t Stat	1,838132	
P(T<=t) one-tail	4,86E-06		P(T<=t) one-tail	0,001854		P(T<=t) one-tail	0,049603	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	9,72E-06		P(T<=t) two-tail	0,003709		P(T<=t) two-tail	0,099206	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Pb	*Al*		*Pb*	*Fe*		*Pb*	*Zn*
Mean	105,6316	43,444	Mean	105,6316	122,489	Mean	105,6316	22427,9
Variance	111037,4	769,8025	Variance	111037,4	1906,73	Variance	111037,4	3,33E+08

Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	-0,0002		Pearson Correlation	0,000159		Pearson Correlation	-0,11124	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	9		df	9		df	9	
t Stat	0,588114		t Stat	-0,15862		t Stat	-3,85728	
P(T<=t) one-tail	0,285458		P(T<=t) one-tail	0,438734		P(T<=t) one-tail	0,001932	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,570917		P(T<=t) two-tail	0,877468		P(T<=t) two-tail	0,003863	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Al	*Fe*		*Al*	*Zn*		*Fe*	*Zn*
Mean	43,444	122,489	Mean	43,444	22427,9	Mean	122,489	22427,9
Variance	769,8025	1906,73	Variance	769,8025	3,33E+08	Variance	1906,73	3,33E+08
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	0,809551		Pearson Correlation	-0,15153		Pearson Correlation	0,412987	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	9		df	9		df	9	
t Stat	-9,34845		t Stat	-3,87561		t Stat	-3,86663	
P(T<=t) one-tail	3,13E-06		P(T<=t) one-tail	0,001878		P(T<=t) one-tail	0,001904	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	6,25E-06		P(T<=t) two-tail	0,003756		P(T<=t) two-tail	0,003808	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Se*		*Cr*	*Pb*		*Cr*	*Al*
Mean	15937,3	863,1084	Mean	15937,3	105,6316	Mean	15937,3	43,444
Variance	2442275	1385614	Variance	2442275	111037,4	Variance	2442275	769,8025
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	0,702643		Pearson Correlation	-0,07616		Pearson Correlation	0,005586	

Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	9		df	9		df	9	
t Stat	42,76037		t Stat	30,85545		t Stat	32,1593	
P(T<=t) one-tail	5,22E-12		P(T<=t) one-tail	9,66E-11		P(T<=t) one-tail	6,68E-11	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	1,04E-11		P(T<=t) two-tail	1,93E-10		P(T<=t) two-tail	1,34E-10	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Mn	*Pb*		*Mn*	*Al*		*Mn*	*Fe*
Mean	11403	105,6316	Mean	11403	43,444	Mean	11403	122,489
Variance	84777833	111037,4	Variance	84777833	769,8025	Variance	84777833	1906,73
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	0,087019		Pearson Correlation	-0,20572		Pearson Correlation	-0,15126	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	9		df	9		df	9	
t Stat	3,889754		t Stat	3,898964		t Stat	3,87143	
P(T<=t) one-tail	0,001838		P(T<=t) one-tail	0,001813		P(T<=t) one-tail	0,00189	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,003676		P(T<=t) two-tail	0,003625		P(T<=t) two-tail	0,003781	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cu	*Fe*		*Cu*	*Zn*		*As*	*Se*
Mean	795,6243	122,489	Mean	795,6243	22427,9	Mean	0,145	863,1084
Variance	828322,4	1906,73	Variance	828322,4	3,33E+08	Variance	0,005074	1385614
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	0,065068		Pearson Correlation	-0,01499		Pearson Correlation	0,735744	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	

df	9		df	9		df	9	
t Stat	2,343475		t Stat	-3,73882		t Stat	-2,31841	
P(T<=t) one-tail	0,021885		P(T<=t) one-tail	0,002317		P(T<=t) one-tail	0,022802	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,04377		P(T<=t) two-tail	0,004634		P(T<=t) two-tail	0,045604	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Se	*Al*		*Se*	*Fe*		*Se*	*Zn*
Mean	863,1084	43,444	Mean	863,1084	122,489	Mean	863,1084	22427,9
Variance	1385614	769,8025	Variance	1385614	1906,73	Variance	1385614	3,33E+08
Observations	10	10	Observations	10	10	Observations	10	10
Pearson Correlation	-0,39429		Pearson Correlation	-0,07897		Pearson Correlation	0,506351	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	9		df	9		df	9	
t Stat	2,18121		t Stat	1,982479		t Stat	-3,85422	
P(T<=t) one-tail	0,028531		P(T<=t) one-tail	0,039372		P(T<=t) one-tail	0,001941	
t Critical one-tail	1,833113		t Critical one-tail	1,833113		t Critical one-tail	1,833113	
P(T<=t) two-tail	0,057062		P(T<=t) two-tail	0,078745		P(T<=t) two-tail	0,003881	
t Critical two-tail	2,262157		t Critical two-tail	2,262157		t Critical two-tail	2,262157	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Fe*		*Cr*			*As*	*Pb*
Mean	15937,3	122,489	Mean	15937,3		Mean	0,145	105,6316
Variance	2442275	1906,73	Variance	2442275		Variance	0,005074	111037,4
Observations	10	10	Observations	10		Observations	10	10
Pearson Correlation	0,326922		Pearson Correlation	0,704786		Pearson Correlation	-0,04893	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	9		df	9		df	9	
t Stat	32,28479		t Stat	-1,1937		t Stat	-1,00106	

P(T<=t) one-tail	6,45E-11		P(T<=t) one-tail	0,131555	P(T<=t) one-tail	0,171477		
t Critical one-tail	1,833113		t Critical one-tail	1,833113	t Critical one-tail	1,833113		
P(T<=t) two-tail	1,29E-10		P(T<=t) two-tail	0,263111	P(T<=t) two-tail	0,342953		
t Critical two-tail	2,262157		t Critical two-tail	2,262157	t Critical two-tail	2,262157		
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		t-Test: Paired Two Sample for Means			
	Mn	*Zn*		*Cu*		*As*	*Al*
Mean	11403	22427,9	Mean	795,6243	Mean	0,145	43,444
Variance	84777833	3,33E+08	Variance	828322,4	Variance	0,005074	769,8025
Observations	10	10	Observations	10	Observations	10	10
Pearson Correlation	0,08971		Pearson Correlation	-0,55013	Pearson Correlation	-0,20232	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	Hypothesized Mean Difference	0	
df	9		df	9	df	9	
t Stat	-1,76983		t Stat	2,763825	t Stat	-4,93243	
P(T<=t) one-tail	0,055268		P(T<=t) one-tail	0,010987	P(T<=t) one-tail	0,000405	
t Critical one-tail	1,833113		t Critical one-tail	1,833113	t Critical one-tail	1,833113	
P(T<=t) two-tail	0,110535		P(T<=t) two-tail	0,021974	P(T<=t) two-tail	0,000811	
t Critical two-tail	2,262157		t Critical two-tail	2,262157	t Critical two-tail	2,262157	

Table C1*: ANOVA analysis of trace metals in fresh bream

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Cr	6	92,327	15,38783	1,336139
Mn	6	130,754	21,79233	182,0916
Cu	6	7,51	1,251667	1,548337
As	6	1,534	0,255667	0,029805
Se	6	9,3	1,55	1,757613
Pb	6	0,333	0,0555	0,018482
Al	6	324	54	789,504
Fe	6	978	163	9319,088
Zn	6	209,324	34,88733	424,7984

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	131594,2	8	16449,28	13,80981	7,09E-10	2,152133
Within Groups	53600,86	45	1191,13			
Total	185195,1	53				

Table C2*: t-Test analysis of trace metals in fresh bream Bonferroni's correction p = 0,0013158

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Mn*		*Cr*	*Cu*		*Cr*	*As*
Mean	15,38783	21,79233	Mean	15,38783	1,251667	Mean	15,38783	0,255667
Variance	1,336139	182,0916	Variance	1,336139	1,548337	Variance	1,336139	0,029805
Observations	6	6	Observations	6	6	Observations	6	6
Pearson Correlation	0,38128		Pearson Correlation	0,128457		Pearson Correlation	0,666149	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5		df	5	
t Stat	-1,19781		t Stat	21,83447		t Stat	35,33999	
P(T<=t) one-tail	0,142336		P(T<=t) one-tail	1,87E-06		P(T<=t) one-tail	1,71E-07	
t Critical one-tail	2,015048		t Critical one-tail	2,015048		t Critical one-tail	2,015048	
P(T<=t) two-tail	0,284671		P(T<=t) two-tail	3,74E-06		P(T<=t) two-tail	3,41E-07	
t Critical two-tail	2,570582		t Critical two-tail	2,570582		t Critical two-tail	2,570582	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Mn	*As*		*Mn*	*Se*		*Mn*	*Pb*
Mean	21,79233	0,255667	Mean	21,79233	1,55	Mean	21,79233	0,0555
Variance	182,0916	0,029805	Variance	182,0916	1,757613	Variance	182,0916	0,018482
Observations	6	6	Observations	6	6	Observations	6	6
Pearson Correlation	0,85072		Pearson Correlation	0,795302		Pearson Correlation	0,797235	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5		df	5	
t Stat	3,952318		t Stat	3,977587		t Stat	3,977599	
P(T<=t) one-tail	0,005413		P(T<=t) one-tail	0,005278		P(T<=t) one-tail	0,005278	
t Critical one-tail	2,015048		t Critical one-tail	2,015048		t Critical one-tail	2,015048	
P(T<=t) two-tail	0,010826		P(T<=t) two-tail	0,010556		P(T<=t) two-tail	0,010556	
t Critical two-tail	2,570582		t Critical two-tail	2,570582		t Critical two-tail	2,570582	

t-Test: Paired Two Sample for Means

	Cu	*Pb*
Mean	1,251667	0,0555
Variance	1,548337	0,018482
Observations	6	6
Pearson Correlation	0,701323	
Hypothesized Mean Difference	0	
df	5	
t Stat	2,541065	
P(T<=t) one-tail	0,025913	
t Critical one-tail	2,015048	
P(T<=t) two-tail	0,051827	
t Critical two-tail	2,570582	

t-Test: Paired Two Sample for Means

	Mn	*Cu*
Mean	21,79233	1,251667
Variance	182,0916	1,548337
Observations	6	6
Pearson Correlation	0,464016	
Hypothesized Mean Difference	0	
df	5	
t Stat	3,881159	
P(T<=t) one-tail	0,005814	
t Critical one-tail	2,015048	
P(T<=t) two-tail	0,011628	
t Critical two-tail	2,570582	

t-Test: Paired Two Sample for Means

	Cu	*Al*
Mean	1,251667	54
Variance	1,548337	789,504
Observations	6	6
Pearson Correlation	0,285652	
Hypothesized Mean Difference	0	
df	5	
t Stat	-4,65302	
P(T<=t) one-tail	0,002783	
t Critical one-tail	2,015048	
P(T<=t) two-tail	0,005567	
t Critical two-tail	2,570582	

t-Test: Paired Two Sample for Means

	As	*Fe*
Mean	0,255667	163
Variance	0,029805	9319,088
Observations	6	6
Pearson Correlation	-0,16693	
Hypothesized Mean Difference	0	
df	5	
t Stat	-4,12824	
P(T<=t) one-tail	0,00455	
t Critical one-tail	2,015048	
P(T<=t) two-tail	0,009101	
t Critical two-tail	2,570582	

t-Test: Paired Two Sample for Means

	As	*Zn*
Mean	0,255667	34,88733
Variance	0,029805	424,7984
Observations	6	6
Pearson Correlation	0,790462	
Hypothesized Mean Difference	0	
df	5	
t Stat	-4,14321	
P(T<=t) one-tail	0,004485	
t Critical one-tail	2,015048	
P(T<=t) two-tail	0,008969	
t Critical two-tail	2,570582	

t-Test: Paired Two Sample for Means

	Se	*Pb*
Mean	1,55	0,0555
Variance	1,757613	0,018482
Observations	6	6
Pearson Correlation	0,561678	
Hypothesized Mean Difference	0	
df	5	
t Stat	2,918235	
P(T<=t) one-tail	0,016541	
t Critical one-tail	2,015048	
P(T<=t) two-tail	0,033082	
t Critical two-tail	2,570582	

t-Test: Paired Two Sample for Means

t-Test: Paired Two Sample for Means

t-Test: Paired Two Sample for Means

	Cr	*Se*		*Cr*	*Pb*		*Cr*	*Al*
Mean	15,38783	1,55	Mean	15,38783	0,0555	Mean	15,38783	54
Variance	1,336139	1,757613	Variance	1,336139	0,018482	Variance	1,336139	789,504
Observations	6	6	Observations	6	6	Observations	6	6
Pearson Correlation	0,801197		Pearson Correlation	0,047114		Pearson Correlation	0,407677	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5		df	5	
t Stat	42,43064		t Stat	32,44605		t Stat	-3,42099	
P(T<=t) one-tail	6,86E-08		P(T<=t) one-tail	2,61E-07		P(T<=t) one-tail	0,009409	
t Critical one-tail	2,015048		t Critical one-tail	2,015048		t Critical one-tail	2,015048	
P(T<=t) two-tail	1,37E-07		P(T<=t) two-tail	5,22E-07		P(T<=t) two-tail	0,018818	
t Critical two-tail	2,570582		t Critical two-tail	2,570582		t Critical two-tail	2,570582	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Mn	*Al*		*Mn*	*Fe*		*Mn*	*Zn*
Mean	21,79233	54	Mean	21,79233	163	Mean	21,79233	34,88733
Variance	182,0916	789,504	Variance	182,0916	9319,088	Variance	182,0916	424,7984
Observations	6	6	Observations	6	6	Observations	6	6
Pearson Correlation	0,418864		Pearson Correlation	-0,48806		Pearson Correlation	0,93588	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5		df	5	
t Stat	-3,08502		t Stat	-3,33251		t Stat	-3,45261	
P(T<=t) one-tail	0,013657		P(T<=t) one-tail	0,010361		P(T<=t) one-tail	0,009093	
t Critical one-tail	2,015048		t Critical one-tail	2,015048		t Critical one-tail	2,015048	
P(T<=t) two-tail	0,027313		P(T<=t) two-tail	0,020722		P(T<=t) two-tail	0,018186	
t Critical two-tail	2,570582		t Critical two-tail	2,570582		t Critical two-tail	2,570582	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cu	*Fe*		*Cu*	*Zn*		*As*	*Se*

Mean	1,251667	163	Mean	1,251667	34,88733	Mean	0,255667	1,55
Variance	1,548337	9319,088	Variance	1,548337	424,7984	Variance	0,029805	1,757613
Observations	6	6	Observations	6	6	Observations	6	6
Pearson Correlation	-0,14518		Pearson Correlation	0,556054		Pearson Correlation	0,874843	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5		df	5	
t Stat	-4,0962		t Stat	-4,13075		t Stat	-2,6921	
P(T<=t) one-tail	0,004695		P(T<=t) one-tail	0,004539		P(T<=t) one-tail	0,021595	
t Critical one-tail	2,015048		t Critical one-tail	2,015048		t Critical one-tail	2,015048	
P(T<=t) two-tail	0,00939		P(T<=t) two-tail	0,009078		P(T<=t) two-tail	0,04319	
t Critical two-tail	2,570582		t Critical two-tail	2,570582		t Critical two-tail	2,570582	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Se	*Al*		*Se*	*Fe*		*Se*	*Zn*
Mean	1,55	54	Mean	1,55	163	Mean	1,55	34,88733
Variance	1,757613	789,504	Variance	1,757613	9319,088	Variance	1,757613	424,7984
Observations	6	6	Observations	6	6	Observations	6	6
Pearson Correlation	0,566704		Pearson Correlation	-0,33681		Pearson Correlation	0,79081	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5		df	5	
t Stat	-4,69427		t Stat	-4,07743		t Stat	-4,17076	
P(T<=t) one-tail	0,002682		P(T<=t) one-tail	0,004782		P(T<=t) one-tail	0,004366	
t Critical one-tail	2,015048		t Critical one-tail	2,015048		t Critical one-tail	2,015048	
P(T<=t) two-tail	0,005365		P(T<=t) two-tail	0,009564		P(T<=t) two-tail	0,008733	
t Critical two-tail	2,570582		t Critical two-tail	2,570582		t Critical two-tail	2,570582	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cr	*Fe*		*Cr*	*Zn*		*Pb*	*Zn*
Mean	15,38783	163	Mean	15,38783	34,88733	Mean	0,0555	34,88733

Variance	1,336139	9319,088	Variance	1,336139	424,7984	Variance	0,018482	424,7984
Observations	6	6	Observations	6	6	Observations	6	6
Pearson Correlation	-0,18853		Pearson Correlation	0,393275		Pearson Correlation	0,911706	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5		df	5	
t Stat	-3,73682		t Stat	-2,36641		t Stat	-4,16465	
P(T<=t) one-tail	0,006738		P(T<=t) one-tail	0,032119		P(T<=t) one-tail	0,004392	
t Critical one-tail	2,015048		t Critical one-tail	2,015048		t Critical one-tail	2,015048	
P(T<=t) two-tail	0,013476		P(T<=t) two-tail	0,064238		P(T<=t) two-tail	0,008784	
t Critical two-tail	2,570582		t Critical two-tail	2,570582		t Critical two-tail	2,570582	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Cu	*As*		*Cu*	*Se*		*Al*	*Fe*
Mean	1,251667	0,255667	Mean	1,251667	1,55	Mean	54	163
Variance	1,548337	0,029805	Variance	1,548337	1,757613	Variance	789,504	9319,088
Observations	6	6	Observations	6	6	Observations	6	6
Pearson Correlation	0,29628		Pearson Correlation	0,578243		Pearson Correlation	0,376936	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5		df	5	
t Stat	2,025462		t Stat	-0,61802		t Stat	-2,97327	
P(T<=t) one-tail	0,04934		P(T<=t) one-tail	0,281812		P(T<=t) one-tail	0,01552	
t Critical one-tail	2,015048		t Critical one-tail	2,015048		t Critical one-tail	2,015048	
P(T<=t) two-tail	0,09868		P(T<=t) two-tail	0,563623		P(T<=t) two-tail	0,03104	
t Critical two-tail	2,570582		t Critical two-tail	2,570582		t Critical two-tail	2,570582	
t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	As	*Pb*		*As*	*Al*		*Al*	*Zn*
Mean	0,255667	0,0555	Mean	0,255667	54	Mean	54	34,88733
Variance	0,029805	0,018482	Variance	0,029805	789,504	Variance	789,504	424,7984

Observations	6	6	Observations	6	6	Observations	6	6
Pearson Correlation	0,511722		Pearson Correlation	0,755434		Pearson Correlation	0,377554	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5		df	5	
t Stat	3,147482		t Stat	-4,70704		t Stat	1,67952	
P(T<=t) one-tail	0,012725		P(T<=t) one-tail	0,002652		P(T<=t) one-tail	0,076943	
t Critical one-tail	2,015048		t Critical one-tail	2,015048		t Critical one-tail	2,015048	
P(T<=t) two-tail	0,025451		P(T<=t) two-tail	0,005304		P(T<=t) two-tail	0,153887	
t Critical two-tail	2,570582		t Critical two-tail	2,570582		t Critical two-tail	2,570582	

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Pb	*Al*		*Pb*	*Fe*		*Fe*	*Zn*
Mean	0,0555	54	Mean	0,0555	163	Mean	163	34,88733
Variance	0,018482	789,504	Variance	0,018482	9319,088	Variance	9319,088	424,7984
Observations	6	6	Observations	6	6	Observations	6	6
Pearson Correlation	0,198762		Pearson Correlation	-0,22329		Pearson Correlation	-0,29922	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5		df	5	
t Stat	-4,70716		t Stat	-4,13325		t Stat	3,001003	
P(T<=t) one-tail	0,002652		P(T<=t) one-tail	0,004528		P(T<=t) one-tail	0,015032	
t Critical one-tail	2,015048		t Critical one-tail	2,015048		t Critical one-tail	2,015048	
P(T<=t) two-tail	0,005303		P(T<=t) two-tail	0,009056		P(T<=t) two-tail	0,030065	
t Critical two-tail	2,570582		t Critical two-tail	2,570582		t Critical two-tail	2,570582	

Appendix E

Sampling of fish at an open market

Figure 4.11: Prof Luke besides the Sun-drying process of Kapenta similar process for sun-dried bream

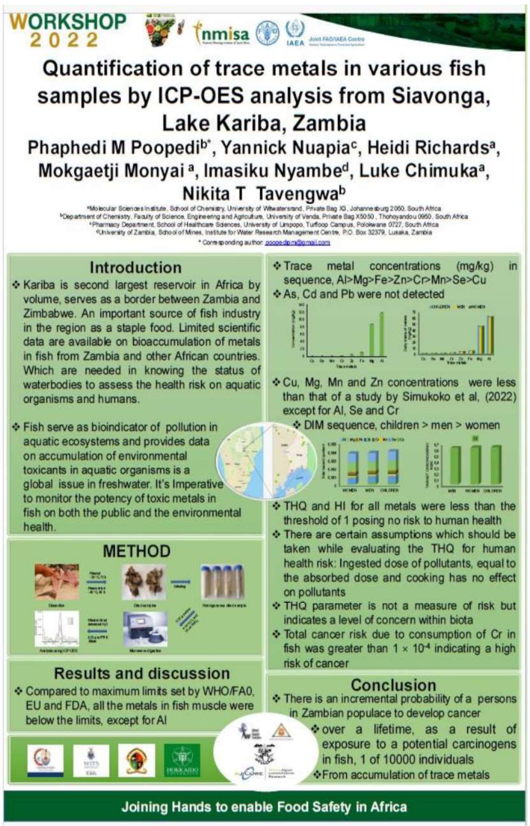
Figure 4.12: Sampling of sun-dried Kapenta at open market

Figure 4.13: Pollution and stagnant near sun-drying of Kapenta open market

Figure 4.14: Kapenta fishing boats (left-side) and mode of transport (right-side) around the lake

Figure 4.15: South African. Japanese and Zambian team on a tour and transport boats

Figure 4.16: Welcome project workshop with different Zambian stakeholders in 2022



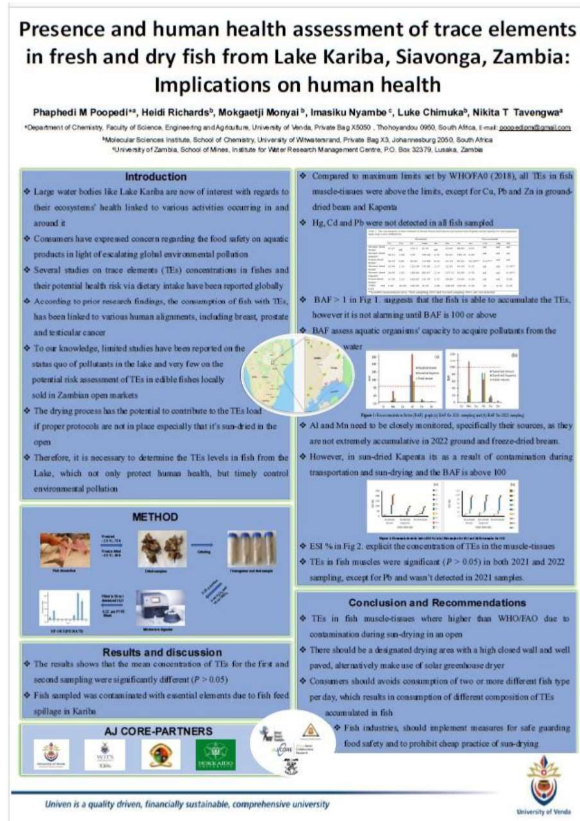


Figure 4.17: Presentation of MSc work at conferences and Postgraduate Workshops at Emperor's Palace, Johannesburg (South Africa) in 2022 and also in University of Limpopo, Auditorium R40, Turfloop Campus, Mankweng, Sovenga (South Africa) 2023, respectively