BINDURA UNIVERSITY OF SCIENCE EDUCATION



FACULTY OF SCIENCE AND ENGINEERING

CHEMISTRY DEPARTMENT

PROJECT TITTLE; COMPARATIVE STUDY OF HEAVY METALS(Lead, Cadmium and arsenic) IN ROADSIDE FRESH FISH, DRIED FISH AND COOKED FISH.

 \mathbf{BY}

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APPROVAL FORM

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In the partial fulfilment of the requirements for the Bachelor of Science Education Honors Degree in Chemistry.

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DECLARATION FORM

I, Nyasha Shirley Makoni hereby certify to Bindura University of Science Education that this dissertation is my original work and that all the materials and academic sources have been properly cited. This work has not been submitted to any academic institute for academic merit.

DEDICATION

I dedicate this academic work to my family and friends who have been there for me and offered me support in every way possible. I give my gratitude to my colleagues for their continuous support.

ACKNOWLEDGEMENTS

Firstly I would like to greatly thank the Almighty God for life. My supervisor Mr Mudavanhu deserves my immense appreciation for assisting me with this project, he took it upon himself and dedicated his time to work together with me to complete this research. I would also like to thank my family for their support throughout my academic career. I want to also thank my lecturers Dr. L. Gwatidzo, Mr Katengeza, Mr N Mudavanhu and the late Professor Mupa.

ABSTRACT

This research focused on determining the concentration of lead, cadmium and arsenic in roadside raw fish, cooked fish and roadside dried fish as well as commercially sold tilapia fish. The fish samples were collected from roadside vendors and analysed using the flame atomic absorption spectroscopy. The level of risks associated with human consumption of fish polluted with heavy metals depend on the type of heavy metal, the amount consumed and the factors such as health status and age. Results obtained showed that the concentrations of lead, cadmium and arsenic in fish significantly varied depending on the cooking method and the source of the fish. The results showed that dried roadside fish had the highest concentration of lead, cadmium and arsenic concentration followed by cooked, raw fish and commercially sold tilapia fish respectively. The levels of cadmium, lead and arsenic did not exceed the maximum permissible limits for human consumption in all the samples of fish. The obtained results of this study suggest that roadside fish has detectable lead, cadmium and arsenic concentrations though in small amounts. The study highlights the importance of regular monitoring of lead, cadmium and arsenic concentrations in fish. The average concentrations of lead, cadmium and arsenic in roadside dried fish were 0.92mg/l, 0.192mg/l and 0.102mg/l respectively. The average concentrations of lead, cadmium and arsenic in roadside fresh fish were 0.87mg/l, 0.180mg/l and 0.080mg/l and respectively. The average concentrations of lead, cadmium and arsenic in cooked fish were 0.50mg/l, 0.150mg/l and 0.050mg/l respectively. The average concentrations of lead, cadmium and arsenic in commercially sold tilapia were -0.005mg/l, -0.008mg/l and -0.007mg/l respectively.

It is recommended to follow recommendations from health authorities, as they give guidance on safe fish, consumption based on age, pregnancy status and other individual factors. Also moderation is key when it comes to fish consumption in order to reduce heavy metal consumption. Consumers should consider other alternative protein sources. Recommendations like cooking fish properly and thoroughly have been suggested in order to help reduce lead, cadmium and arsenic consumption in the human diet as cooking can reduce the amount of heavy metals in some fish. It

is recommended that fish consumers be made aware of the risks that come with the consumption of roadside fish and suitable measures be taken to reduce the exposure to heavy metals.

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Acronyms.

RSF = Roadside Fresh

RSD = Roadside dried

RSC = Roadside cooked

 $Com-Commercially\ sold\ tilapia$

CHAPTER 1

1.0 Introduction

This chapter gives the background of the study, the aim, objectives, justification and problem statement behind the research. It also includes limitations and delimitations of the study.

1.1 Background

Heavy metals are naturally occurring chemical elements with high density and high atomic weight greater than water. Heavy metal contamination in aquatic environments, is a serious environmental and public health concern worldwide (Storelli, 2008). Heavy metals, like mercury, lead, cadmium and arsenic are persistent pollutants that accumulate in fish tissues through bioaccumulation and biomagnification, posing risks to the ecosystem health and human health (Jaishanker et al., 2014). Fish consumption is a major source of heavy metal exposure and the fish can accumulate high concentrations in their tissues (Flegal et al., 2016).

Heavy metals are persistent and toxic pollutants in aquatic environments (Monikh et al., 2019). Fish are susceptible to heavy metal contamination due to absorption of heavy metals from both food and water sources (Omoregi et al., 2018). In addition, roadside environment due to its heavy traffic and emissions is a source of heavy metals in the surrounding ecosystem (Li et al., 2019). Heavy metals originate from natural sources and anthropogenic sources. They originate from natural sources like weathering of rocks and volcanic eruptions as well as anthropogenic sources like industrial activities, mining, agriculture, and urban runoff. Heavy metals contamination in can originate from quite a number of sources such as industrial wastewater, vehicle emissions, and agricultural runoff (Lin et al., 2015).

Aquaculture is widely practise worldwide and is one of the fastest-growing food-producing sectors. High intensity aquaculture involves the addition of large amounts of feed, fertilisers, and chemicals, as well as the use of medicines to reduce disease incidences and increase production. These activities contribute to high fish production but they have created a myriad of environmental concerns. Effluent release from ponds, cages and pen culture systems can degrade the water quality

in aquatic systems. The use of disinfectants, antimicrobials, chemotherapeutants and chemicals can be a serious threat of chemical residue in the environment.

These metals have potential to accumulate in the tissues of aquatic organisms including fish, and they pose significant risks to both environmental and human health through bioaccumulation and bio magnification in the food chain. Heavy metals are of great concern as contaminants in fish and have been subject to much legislation and regulation within the European Union. The main reasons for the analysis of metals in fish have been to protect consumer health, provide informed consumer choice, and maintain protection for the environment.

The regulation EC No. 1881/2006 sets maximum levels for certain contaminants in foodstuffs, including lead, cadmium, and mercury in fish and fish products for human consumption. These maximum levels are set on the basis that the presence of these contaminants should not harm human health and are as low as is reasonably achievable. Many countries including Malaysia have also set standards for metals in fish based on international recommendations. This ensures that fish and fish products meet the requirements of the importing country.

Some countries such as Malaysia have set standards for lead and cadmium in marine fish, which is equivalent of FAO/WHO standards and the European economic communities directive on lead and cadmium in certain foodstuffs. The establishment of these standards has led to studies and research on metals contamination in fish. Fish, a protein is an important component in the diet of many people. Suggestions have been made that moderate fish consumption be encouraged as it makes a significant contribution to a healthy diet.

Fish may contain different contaminants and residues, which come from the aquatic environment and accumulated during the lifespan of the fish, most of which have potentially harmful health effects. Some of the contaminants may be present at low levels that they are of no toxicological significance. Others have a real toxicity on human health. The presence of harmful heavy metals in fish reduces the benefits of consuming fish and may even pose a health risk to the consumer.

A survey done by the food standards Agency of the UK found that many people are not aware of the potential harm that can be caused by eating fish contaminated by environmental pollutants. Suggestions have been made that consumers are provided with information on the levels of contaminants in commercially important fish species, to enable them to make informed choices on

whether or not to consume the fish. The presence of heavy metals in food is an issue of increasing problem, especially in relation to the risks involved to human health.

Over three quarters of heavy metal intake by humans is from fish consumption, due to the chemical form of the metals as well as consumer sensitivity. Toxicity symptoms vary from minor to severe health problems including nervous system, liver and kidney effects and mostly cancer. Regular fish monitoring for heavy metals concentration is necessary as the permissible levels vary. Sampling, preservation and storage procedures in fish involve multiple steps that can affect the concentration of heavy metal concentration in the sample. Fish are used as bio indicators of heavy metals pollution in water bodies. Heavy metals (lead, cadmium and arsenic) are a major environmental concern as they are persistent and tend to be toxic. Fish is a major protein source and can accumulate heavy metals such as lead, cadmium and arsenic from their surroundings through various pathways. Heavy metals such as arsenic, cadmium and lead do not readily break down in the environment and they accumulate in the organisms that are high up in the food chain. Large fish generally have high concentration of heavy metals.

1.2 Aim

The aim of this project is to compare heavy metals (lead, arsenic and cadmium) concentration in roadside fresh fish, cooked and dried fish.

1.3 Objectives

- To determine the concentrations of lead, arsenic and cadmium in roadside fresh fish, cooked fish and dried fish.
- To compare the concentrations of lead, arsenic and cadmium in roadside fresh fish, cooked fish and dried fish.
- To evaluate health risks for human consumption and ecosystem health.

1.4 Problem statement

Fish contamination by heavy metals poses a great threat to human health and ecosystems. roadside fish are commonly consumed in many parts of the world and yet no studies and analysis on their safety has been conducted on them. The aim of this project is to analyse the levels of these heavy metals in fish in order to be able to assess the potential health risks. Increase in urbanization as well as industrialisation has led to an increase in heavy metals being discharged into water bodies from different sources leading to fish contamination, which are an important source of protein in many communities. Lack of extensive studies assessing the levels of heavy metals in fish from different sources disturbs the knowledge of the extent of contamination and its potential health impacts on human consumers (Al-Yousuf et al., 2000). Various anthropogenic activities, including industrial discharges, agricultural run-off and urban waste water discharge, contribute immensely to heavy meatal contamination in aquatic water (Huang et al., 2019)

1.5 Justification

The major reason behind the study of heavy metals present in fish is human health concerns, as fish is an important part of human diet, a protein source, omega-3 fatty acids, vitamins, and minerals. Consumption of fish contaminated with high levels of lead, arsenic and cadmium can cause heavy metal poisoning and various health conditions, particularly for pregnant women,

children and the elderly (Hassan et al.,2017). Regular heavy metal fish analysis and monitoring help ensure compliance with regulations and standards to prevent contaminated fish consumption.

The analysis of heavy metals in fish can help in environmental monitoring as it serves as a bioindicator of environmental pollution. The analysis of heavy metals in fish can help in identifying the sources of heavy metal contamination and this helps to implement effective pollution control. Analysis of heavy metals in fish ensures the quality and safety of fish products on the market. In most cases, roadside fish are sourced from polluted aquatic sources, which could lead to heavy metal accumulation in their tissues (Adeyemi et al., 2018). Therefore, monitoring of heavy metals in fish allows risk assessment to determine the possibility and severity of health effects from exposure to heavy metals. Another crucial reason for the analysis of heavy metals in fish is to raise public awareness about the need food safety and environmental protection. Policymakers can develop guidelines and recommendations for fish consumption basing on the analysis of heavy metals in fish (Hassan et al., 2017).

1.6 Limitations

Despite the importance of analysis of lead, arsenic and cadmium in fish, there are a number of challenges involved with this process. During sample collection and preparation obtaining a representative samples can be difficulty due to factors like variability in heavy metal concentrations between fish species. In addition, sample preparation techniques can be time consuming and labour intensive.

Another challenge that can be faced is that in the analysis of heavy metals in fish, the analytical techniques used typically involves the use of complex techniques such as Atomic absorption spectroscopy, which require skilled personnel, specialised equipment and strict quality control measures in order to get accurate and precise results. Some analytical techniques may have limitations in detection limits and in terms of selectivity and sensitivity.

In addition, the presence of complex matrices such as lipids, organic compounds and proteins in fish tissues can affect detection and quantification of heavy metals. To prevent degradation, contamination and analyte loss there is need for proper storage and preservation methods. There also is the need for a certified standard reference material for heavy metal analysis, which can be limiting to validate analytical methods, instrument calibration to ensure accurate results.

Another limitation is the limitation in the availability of historical data on heavy metal concentration in fish can make it challenging to compare the acquired results and the past trends. The issue of seasonal variations in heavy metal concentrations in fish is not accounted for in the study and this can greatly affect results accuracy. Conducting the study in a certain geographical area may be limiting, as the results may not be generalizable for other geographical areas. Limiting the study to a small sample size may not represent the entire fish population.

Cooking may affect the concentration of heavy metals in fish, as some cooking methods like frying increase levels of some heavy metals (Chin et al., 2015). Drying fish can result in concentrating the heavy metals in the remaining tissue and hence dried fish tend to have higher levels of heavy metal as compared to fresh fish (Wang et al., 2017). This then points out that the cooking method is a very crucial factor to be taken into great consideration when carrying out heavy metal analysis in fish.

1.7 Delimitations

Analysis of heavy metals in fish focuses mainly on a number of contaminants such as mercury, lead, cadmium, and arsenic while other potentially harmful metalloids are not included due to analytical challenges among other factors. Sampling frequency and intensity of sampling for heavy metals analysis may vary due to research objectives and regulatory requirements. Choice of analytical methods and detection limits can affect sensitivity, accuracy and results reliability. In the research, focus is on a specific set of fish species. In the study, boiling is the cooking method to be used and other cooking methods such as grilling and frying were not included. As for heavy metal selection the focus is mainly on lead, arsenic and cadmium and the results may not be a representative of the full range of heavy metals found in fish. Environmental factors, such as water quality, sediment composition will not be considered in the study.

CHAPTER 2 LITERATURE REVIEW

2.0 Introduction

This chapter outlines a brief description on the different heavy metals in fish including the sources of heavy metal contamination as well as the health effects of heavy metal exposure.

2.1 Heavy Metals in Fish

Heavy metals such as lead, arsenic and cadmium accumulate in fish by bioaccumulation in the food chain, waterborne exposure and environmental contamination. The heavy metals enter the food chain through numerous ways, including algae and plankton absorption, which are eaten by fish (Lin et al., 2015).

Studies have shown the widespread heavy metal occurrence and the bioaccumulate nature of these contaminants in aquatic ecosystems (Flegal et al., 2016). Heavy metals from the surrounding fish are accumulated in fish and pose as a potential health risk to human consumption (Wang et al., 2020).

2.1.1 Lead

These mainly come from industrial activities, pollution, and contaminated water sources. Chronic exposure to lead and cadmium can lead to problems on the kidneys, nervous system and bones. Lead can be released into the environment through human activities such as farming, mining, smelting and the use of leaded gasoline. It can be found from pipes and paint. After entering the water sources lead accumulates in the tissues of fish and aquatic organisms.

The consumption of fish contaminated with lead can cause serious health problems such as developmental problems in children. Cooking lead contaminated fish does not remove the lead it actually increases lead concentration as water evaporates and concentrates the remaining lead.

2.1.2 Cadmium

Cadmium gets into the environment through metals, minerals in mining industry and chemical fertilisers in agriculture and cadmium containing products that are discharged into water with wastewater. Cadmium then accumulates in algae and sediment and is absorbed by fish, shellfish, shrimp and crabs in water. Cadmium also affects the structure and function of gills, liver and gonad, and the physiological metabolism and reproductive system of fish. Accumulation of cadmium in fish is influenced by routes and feeding habits.

2.1.3 Arsenic

Arsenic is present in fish due to anthropogenic contamination. There also is the bioaccumulation of arsenic from small prey that are contaminated with arsenic. Inorganic arsenic is carcinogenic and can cause cardiovascular diseases, skin lesions and other health problems. Contamination from arsenic can cause pigmentation changes and hard patches on the hands and feet, developmental effects, diabetes, pulmonary disease as well as cardiovascular disease. Arsenic poisoning can also cause Arsenic-induced myocardial infarction, which can cause excess mortality.

2.2 Sources of Heavy Metals

Release of heavy metals in the aquatic system (Mitra et al., 2022, Taghavi et al., 2023) has detrimental effects on organisms and ecosystems as they are persistent, toxic and accumulate in the environment. Roadside fish vendors sell fish that has higher levels of heavy metals due to exposure to vehicle emissions and industrial emissions. There are quite a number of sources of heavy metals. Some of them are listed below:

2.2.1 Natural Sources:

Other heavy metals occur naturally and are found in rocks, sediments and soils, and are released into the water bodies by processes such as erosion and weathering. Volcanic eruptions also release heavy metals into the environment. In addition to sources of heavy metals, geothermal activities also contribute to heavy metal absorption.

Biomass burning also releases heavy metals such as mercury and arsenic into the environment. Natural geological formations such as mineral deposits contain heavy metals that leach into waterways and accumulate in fish.

2.2.2 Agriculture run-off:

Agriculture runoff has been documented as a known source of heavy metals (Yuliati et al., 2017; Ghorbani et al., 2018). Chemicals used in agriculture such as pesticides and fertilizers are leached into rivers, lakes and oceans and are available to fish. Use of sewage sludge is also a source of heavy metals in fish if the metals are leached into aquatic environments. Once the heavy metals are in water they are absorbed by algae and plankton, which are eaten by fish.

Livestock manure and other organic waste products provide a source of heavy metals. Irrigation drainage and runoff carrying heavy metals from fields get into waterways and subsequently finds its way into waterbodies and is available in fish.

2.2.3 Wastewater Discharge and Sewage:

Wastewater and sewage discharge in water bodies can be a source of heavy metals in fish (Rezzaee et al., 2019; Mohsin et al., 2020). Treatment plants can release heavy metals into water bodies, which is taken up by fish. Aquaculture is widely practised worldwide and is one of the fastest-growing food-producing sectors. Activities such as aquaculture involves addition of large amounts of feed, fertilisers, and chemicals contribute to high fish production but they have created many environmental concerns as they increase heavy metal contamination.

Effluent release from ponds, cages and pen culture systems can degrade the water quality when discharged in aquatic systems. Studies have shown that fish near wastewater discharge sites have higher levels of heavy metals in their tissues (Mohsin et al., 2020; Rashid et al., 2021). Agricultural activities such as irrigation with wastewater effluent causing regular pollutants with heavy metals to groundwater and soil.

2.2.4 Atmospheric Deposition:

Air pollution and vehicle emissions can be a source for heavy metals. Heavy metals deposited in the air from vehicle emissions, industrial activity, biomass burning and volcanic eruptions can travel long distances in the air and are then transported back to the ground in the form of snow, rain and dust. Heavy metals such as mercury and arsenic can be deposited into the water through dust, particulate matter and precipitation.

2.2.5 Industrial Pollution:

Industrial pollution is a major contributory source of heavy metals in fish from roadside). Industrial activities such as mining, smelting, power generation and manufacturing may also contribute to heavy metal deposition in water bodies. These metals then accumulate in the fish tissues through their food chain.

Previous studies have proved that fish caught from places near industrial areas have concentration of heavy metals in their tissues as compared to those caught from places in unpolluted areas (Jia et al., 2016; Rasooli et al., 2018).

2.3 Health Effects of Heavy Metals (Lead, Arsenic and Cadmium) Exposure

Exposure to heavy metals in fish has various health effects and these effects depend on duration of exposure, and the type of heavy metal. Reproductive health can be affected in both male and females causing miscarriages and birth defects. Some heavy metals exposures have been linked to causing cardiovascular diseases such as heart diseases, high blood pressure and stroke.

Heavy metals can weaken the immune system and can cause digestive problems leading to nausea, vomiting, diarrhoea and abdominal pain. Cadmium and lead can cause respiratory problems causing wheezing and shortness of breath. Specific health effects of heavy metals vary depending on the metal exposed to, level and duration of exposure and individual's susceptibility.

Harmful health effects on humans caused by heavy metals (Lead, Arsenic and Cadmium) include:

- ✓ Cancer- long term exposure has been linked to increased cancer risk. Mainly skin cancer has been closely linked to heavy metal exposure.
- ✓ Neurological damage-lead and arsenic can damage brain development, cognitive function and nervous system function.
- ✓ Kidney damage due to prolonged exposure
- ✓ Immune system suppression heavy metals weaken the system making it hard to fight off infections.
- ✓ Respiratory problems- such as bronchitis, wheezing and pulmonary disease.
- ✓ Cardiovascular diseases- exposure to lead and arsenic has been associated with increased risk of cardiovascular diseases.
- ✓ Reproductive issues- heavy metal poisoning can affect fertility and foetal development.
- ✓ Neurodegenerative diseases- mercury and lead have been linked to neurodegenerative diseases like Parkinson's and Alzheimer's.
- ✓ Anaemia- lead and Arsenic interfere with red blood cells production leading to anaemia.

2.3.1 Cadmium

Cadmium damages kidneys and lungs causing respiratory diseases. Prolonged exposure has been linked to causing cancer. Can cause wheezing and shortness of breath. Cadmium also weakens the immune system, reducing its ability to fight off infections.

2.3.2 Lead

Lead poisoning can cause neurological and behavioural problems (CDC, 2020). Lead also causes kidney problems. Lead affects the nervous system causing headaches, behavioural changes and memory loss. In children lead causes cognitive development resulting in learning disabilities. It has been associated with neurodegenerative diseases like Alzheimer's and Parkinson's.

2.3.3 Arsenic

Arsenic causes skin lesions, peripheral neuropathy cardiovascular diseases and cancers of the skin, bladder and lungs (Agency for Toxic Substances and Disease Registry, 2012). Arsenic has been linked to interfering with red blood cells thus causing anaemia. Exposure to arsenic can cause immune system suppression making it hard for the body to fight infections.

CHAPTER 3 METHODOLOGY

3.0 Introduction

This chapter outlines the full procedure carried out in this research. It gives a description of the sample collection, sample preparation, digestion, analysis and the quantitative determination of heavy metals in fish.

3.1 Sample Collection

Samples of raw fish were collected from roadside vendors. We collected the fish in different forms, we had raw, cooked and dried fish.

Raw fish: 10 fresh fish purchased from roadside vendors.

Cooked fish: 10 cooked fish purchased from roadside vendors.

Dried fish: 10 dried fish samples purchased from local markets.

Forceps and clean gloves were used to collect fish samples and placed them in clean plastic bags.

3.2 Sample Preparation

This analytical method used in this study to analyse heavy metals in fish from roadside raw fish, cooked fish and dried fish is the Flame Atomic Absorption Spectroscopy. The different samples were prepared differently.

We carried out the steps below;

3.2.1 Raw fish

We removed the skin, bones, and internal organs. Cut the flesh into small pieces.

3.2.2 Cooked fish

We removed the skin, bones. Cut the flesh into small pieces.

3.2.3 Dried fish

The dried fish was ground to fine powder.

3.3 Weighing, Drying and Grinding

To determine the weight of the fish, samples were weighed.

Fish samples were dried at a low temperature of 60°C, until they reached a constant weight. After drying, the samples were grounded to fine powder by a mortar and pestle.

3.4 Acid Digestion / Sample Digestion

1g of the powdered fish sample was weighed and placed in a digestion vessel. The finely ground powder was digested in a mixture of concentrated nitric acid and hydrochloric acid in the ratio 1:3 using a microwave digestive system. The digestion vessel was left in the microwave oven until the sample was completely dissolved.

A known volume of deionised water was added to the digested samples to dilute them. The process of digestion breaks down the organic matter and releases heavy metals into solution.

A 0,45 µm filter paper was used to filter the digested sample to remove particulate. Deionised water was used to dilute the filtered samples to a known volume. Samples are stored in clean, airtight containers. Samples were kept frozen at -20°C until analysis.

3.5 Analytical Technique

Flame Atomic Absorption Spectroscopy (FAAS) is a widely used and reliable spectroscopy technique as it is relatively affordable and its ease of use and is easy to operate and maintain. FAAS is a versatile technique it can be used to analyse a wide range of heavy metals.it also is a highly accurate technique as its calibration curve are normally linear over a wide range of concentrations. FAAS technique also offers high sensitivity.

3.5.1 Flame Atomic Absorption Spectroscopy (FAAS)

Flame atomic absorption spectrometry works by measuring the absorption of light by atoms in a flame. The technique uses the Beer- Lambert's law. The intensity of the emitted light is directly proportional to the concentration of the heavy metals in the sample. It makes use of a spectrophotometer which measures absorbance of light at specific wavelengths, thereby quantifying the heavy metals.

3.5.2 FAAS Analysis

-The FAAS instrument was initially calibrated using known certified standards of lead, arsenic and cadmium.

-diluted samples were then aspirated into the FAAS flame. The flame excites the heavy metals present causing them to emit different wavelengths of light.

A detector then measured the light intensity emitted proportional to the concentration of the heavy metals present.

3.5.2 Quantitative Analysis

Calculations to determine the heavy metal concentration in the samples were done using the method below;

Concentration (ppm) = (measured intensity/slope of calibration curve)

CHAPTER 4: RESULTS

4.0 Introduction

Chapter four presents As, Cd and Pb concentrations results in mg/l from Roadside fresh, Roadside

dried, Roadside cooked and Commercial tilapia. In order to reduce the risks of errors, experiments

were done triplicate and the average values were used. (Salaam et al.2019). The results are

presented as trendlines tales and bar graphs.

RSF = Roadside Fresh

RSD = Roadside dried

RSC = Roadside cooked

Com – Commercially sold tilapia

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4.1 Calibration Curves

Figure 4.1 Shows the Calibration curve for Cadmium

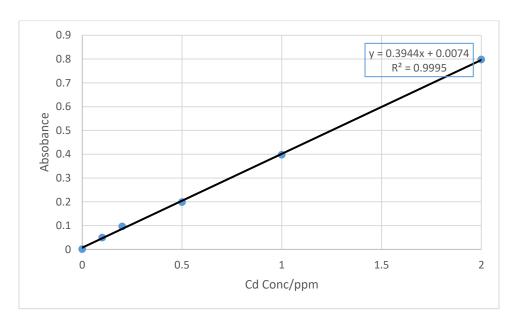


Fig 4.1 Cd Calibration Curve

Figure 4.2 shows the calibration curve for Arsenic

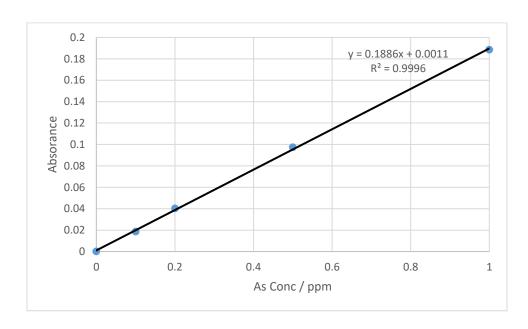


Fig 4.2 Calibration Curve for Arsenic



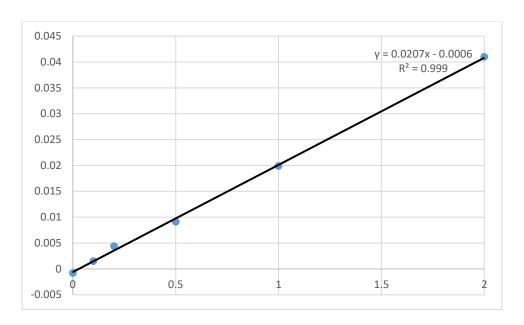


Fig 4.3 Lead Calibration Curve

4.2 Heavy Metal Concentrations Determinations

4.2.1 Pb

Three tests were made for Pb analysis and results are shown in Table 4.1

Table 4.1 Pb determination

	Test 1	Test 2	Test 3	Mean	Sd
	(mg/l)	(mg/l)	(mg/l)	(mg/l)	
RSF	0.87	0.83	0.90	0.87	0.028
RSC	0.53	0.47	0.51	0.50	0.025
RSD	0.91	0.97	0.89	0.92	0.033
Com	0.001	-0.009	-0.007	-0.005	0.004

Table 4. 1 shows that the mean lead concentration of roadside fish was 0.87mg/l, roadside cooked fish was 0.50mg/l, roadside dried fish was 0.92mg/l and commercially sold tilapia had the lowest concentration of -0.005mg/l. Roadside dried fish had the highest concentration of lead, followed by roadside fresh fish, followed by roadside cooked fish and lastly commercially sold tilapia had the lowest concentration of lead.V

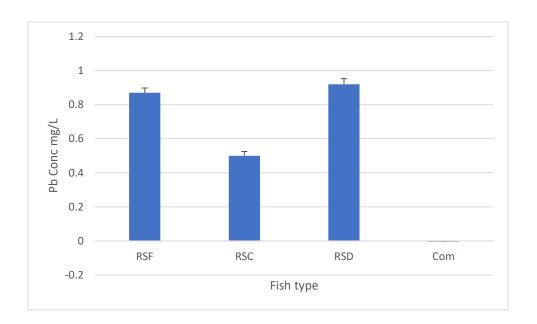


Fig 4.4 The Mean Concentrations of Lead in all the Samples.

4.2.2 Cd

Three tests were made for Cd analysis and results are shown in Table 4.2

Table 4.2 Cd Determination

	Test 1	Test 2	Test 3	Mean	Sd
	(mg/l)	(mg/l)	(mg/l)	(mg/l)	
RSF	0.171	0.183	0.187	0.180	0.007
RSC	0.153	0.147	0.151	0.150	0.002
RSD	0.191	0.197	0.189	0.192	0.003
Com	-0.009	-0.008	-0.007	-0.008	0.001

Table 4.2 shows the results obtained for Cadmium concentration determination. Roadside dried fish had lead concentration of 0.192mg/l, roadside fresh fish had 0.180 mg/l, roadside cooked fish had 0.150mg/l and commercially sold tilapia had -0.008mg/l. Roadside dried fish had the highest cadmium concentration followed by roadside fresh fish, followed by roadside cooked fish and lastly commercially sold tilapia.

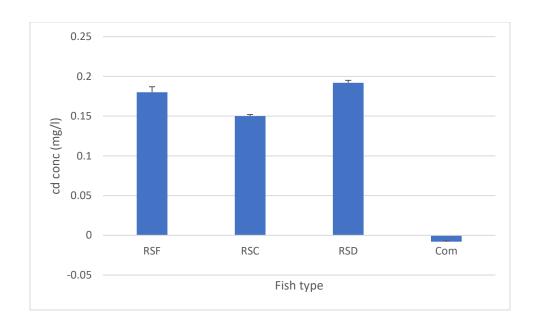


Figure 4.5 The mean concentrations of Cd in all the samples.

4.2.3 As

Three tests were made for As analysis and results are shown in Table 4.3

Table 4.3 As Determination

	Test 1 (mg/l)	Test 2 (mg/l)	Test 3 (mg/l)	Mean (mg/l)	Sd
RSF	0.071	0.083	0.087	0.080	0.007
RSC	0.053	0.047	0.051	0.050	0.002
RSD	0.101	0.107	0.099	0.102	0.003
Com	-0.008	-0.007	-0.007	-0.007	0.0005

Table 4.3 shows the obtained results of Arsenic concentration determination in roadside dried fish, roadside fresh fish, roadside cooked fish and commercially sold tilapia.

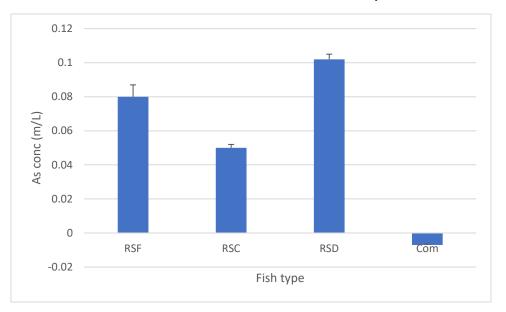


FIG 4.6 Shows Mean Concentration of Arsenic in all the Samples.

Anova test between RSC, RSD and RSF fish showed no significant differences between themselves. Commercially sold tilapia showed lowest detectable metals concentrations for the three metals.

CHAPTER 5 DISCUSSION RECOMMENDATIONS AND CONCLUSION

5.1 Discussion

The results obtained from the study show that roadside fish concentrations of lead, arsenic and cadmium in the fish samples varied. Roadside dried fish had the highest concentrations of all the three metals, followed by roadside fresh fish, followed by roadside cooked fish and the least was commercial tilapia. The average concentrations of lead, cadmium and arsenic in roadside dried fish were 0.92mg/l, 0.192mg/l and 0.102mg/l respectively. The average concentrations of lead, cadmium and arsenic in roadside fresh fish were 0.87mg/l, 0.180mg/l and 0.080mg/l and respectively the average concentrations of lead, cadmium and arsenic in cooked fish were 0.50mg/l, 0.150mg/l and 0.050mg/l respectively. The average concentrations of lead, cadmium and arsenic in commercially sold tilapia were -0.005mg/l, -0.008mg/l and -0.007mg/l respectively.

The results obtained showed that roadside dried, cooked and raw fish are contaminated with small concentrations of lead, cadmium and arsenic, whereas commercially sold tilapia had negative

values indicating that it was not contaminated with heavy metals and is safe for human consumption. The results I obtained in this study are in agreement with the study by (Wang et al.2012), who reported that lead concentrations in roadside raw fish, roadside cooked fish and roadside dried fish were higher than in non-roadside raw fish.

Similarly another analysis conducted by ((Zhang et al. 2018) who reported higher arsenic concentrations in roadside cooked fish than non-roadside raw fish. Both these experimental studies are in sync with this research I conducted as commercially sold tilapia had low concentration of lead, arsenic and cadmium than roadside raw fish, roadside cooked fish and roadside dried fish.

High levels of lead, cadmium and arsenic in dried fish could be due to the fact that drying fish can concentrate the heavy metals in fish. Dried fish is often preserved with salt, which binds to heavy metals and prevents their removal during cooking. Lead, arsenic and cadmium concentrations that were obtained in cooked fish indicates that cooking does not completely remove heavy metals in fish. Lead and other water-soluble metals can be reduced in concentration by cooking, however Arsenic and cadmium may not be reduced but are rather concentrated as water evaporates. (Wei et al. ,2019).

There is a significant difference between commercially sold tilapia and roadside raw, cooked and dried fish lead, cadmium and arsenic concentrations, indicating that commercially sold tilapia was sourced from unpolluted sources whereas roadside fish are contaminated. According to, (Heavy Metals Regulations Legal Notice No 66/2003"), the mean total lead content, as determined by the analysis of the edible parts of the fishery products shall not exceed 0.2ppm (0.2 mg/kg of fresh weight) and the mean total cadmium content as determined by the analysis of the edible parts of the fishery products shall not exceed 0.05 ppm (0.05 mg/kg of fresh weight. This then points out that the fish experimented on were safe for consumption as the levels were below the concentrations considered to be toxic.

5.2 Conclusion

In conclusion roadside fish has high lead, arsenic and cadmium concentration than commercially sold tilapia. Commercially sold tilapia is safe for consumption basing on the results from this research. This clearly shows that roadside fish is not safe for human consumption. Moreover, high

levels of lead, arsenic and cadmium in dried fish indicates that drying fish actually concentrates the heavy metals as water evaporates.

Human consumption of roadside fish especially dried roadside fish, judging by the results obtained in this research poses a great health risk to humans. The risks of lead, arsenic and cadmium in these roadside fish can lead to neurological disorder, reproductive problems and cancer.

The objectives of this study were successfully fulfilled, as we were able to determine and compare the relative concentrations of lead, arsenic and cadmium as well as evaluate the health issues associated with heavy metal contamination

5.3 Recommendations

It is better to limit consumption of roadside dried fish and opt for fish caught in clean water sources such as the commercially sold tilapia. In addition, it is important to thoroughly cook fish to reduce the levels of cadmium, arsenic and lead. Also people should avoid buying fish that is caught near industrial areas or in heavy traffic areas. Regular monitoring and management of atmospheric deposition can assist mitigate lead, arsenic and cadmium pollution in waterways and thus reduce the health effects. There is need to increase public awareness about the risks associated with heavy metals such as lead, cadmium and arsenic and the public be made aware and provided with clear guidance on safe consumption practices. In addition, we should promote sustainable fishing practices in order to maintain health fish populations and reduce environmental contamination.

REFERENCES

Storelli, M. M. (2008). Potential Human Health Risks from Metals (Hg, Cd, and Pb) and Polychlorinated Biphenyls (PCBs) Via Seafood Consumption: Estimation of Target Hazard Quotients (THQs) and Toxic Equivalents (TEQs). *Food and Chemical Toxicology*, 46(8), 2782-2788.

Tejeda-Benitez, L., Flegal, R., Odigie, K., & Olivero-Verbel, J. (2016). Pollution by Metals and Toxicity Assessment Using Caenorhabditis Elegans in Sediments from the Magdalena River, Colombia. *Environmental Pollution*, 212, 238-250.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, Mechanism and Health Effects of Some Heavy Metals. *Interdisciplinary Toxicology*, 7(2), 60-72.

Liu, Q., Liao, Y., Xu, X., Shi, X., Zeng, J., Chen, Q., & Shou, L. (2020). Heavy Metal Concentrations in Tissues of Marine Fish and Crab Collected From the Middle Coast of Zhejiang Province, China. *Environmental monitoring and assessment*, 192, 1-12.

Gabriel, N. N., Omoregi, E., Martin, T., Kukuri, L., & Shilombwelwa, L. (2018). Compensatory growth response in Oreochromis Mossambicus Submitted to Short-term Cycles of Feed Deprivation and Re-feeding. *Turkish Journal of Fisheries and Aquatic Sciences*, 18(1), 161-166.

Li, P., Lin, C., Cheng, H., Duan, X., & Lei, K. (2015). Contamination and Health Risks of Soil Heavy Metals Around a Lead/Zinc Smelter in southwestern China. *Ecotoxicology and Environmental Safety*, *113*, 391-399.

Lin, M., Gui, H., Wang, Y., & Peng, W. (2017). Pollution Characteristics, Source Apportionment, and Health Risk of Heavy Metals in Street Dust of Suzhou, China. *Environmental Science and Pollution Research*, *24*, 1987-1998.

Al-Yousuf, M. H., El-Shahawi, M. S., & Al-Ghais, S. M. (2000). Trace Metals in Liver, Skin and Muscle of Lethrinus Lentjan Fish Species in Relation to Body Length and Sex. *Science of the Total Environment*, 256(2-3), 87-94.

Huang, H., Li, Y., Zheng, X., Wang, Z., Wang, Z., & Cheng, X. (2022). Nutritional Value and Bioaccumulation of Heavy Metals in Nine Commercial Fish Species from Dachen Fishing Ground, East China Sea. *Scientific Reports*, 12(1), 6927.

Da Cunha Martins Jr, A., Carneiro, M. F. H., Grotto, D., Adeyemi, J. A., & Barbosa Jr, F. (2018).

Arsenic, Cadmium, and Mercury-induced Hypertension: Mechanisms and Epidemiological

Findings. Journal of Toxicology and Environmental Health, Part B, 21(2), 61-82.

Chin-Chan, M., Navarro-Yepes, J., & Quintanilla-Vega, B. (2015). Environmental Pollutants as Risk Factors for Neurodegenerative Disorders: Alzheimer and Parkinson Diseases. *Frontiers in Cellular Neuroscience*, *9*, 124.

Jia, Y., Wang, L., Qu, Z., Wang, C., & Yang, Z. (2017). Effects on Heavy Metal Accumulation in Freshwater Fishes: Species, Tissues, and Sizes. *Environmental Science and Pollution Research*, 24, 9379-9386.

Haghdoost, S., Niksokhan, M. H., Zamani, M. G., & Nikoo, M. R. (2023). Optimal Waste Load Allocation in River Systems Based on a New Multi-objective Cuckoo Optimization Algorithm. *Environmental Science and Pollution Research*, *30*(60), 126116-126131.

Mukisa, W., Yatuha, J., Andama, M., & Aventino, K. (2020). Heavy Metal Pollution in the Main Rivers of Rwenzori Region, Kasese District South-western Uganda.

Kumar, V., Parihar, R. D., Sharma, A., Bakshi, P., Sidhu, G. P. S., Bali, A. S., ... & Rodrigo-Comino, J. (2019). Global Evaluation of Heavy Metal Content in Surface Water Bodies: A Meta-Analysis Using Heavy Metal Pollution Indices and Multivariate Statistical Analyses. *Chemosphere*, 236, 124364.

Hu, X., Sun, Y., Ding, Z., Zhang, Y., Wu, J., Lian, H., & Wang, T. (2014). Lead Contamination and Transfer In Urban Environmental Compartments Analysed By Lead Levels And Isotopic Compositions. *Environmental pollution*, 187, 42-48.

Zhang, J., Tan, Q. G., Huang, L., Ye, Z., Wang, X., Xiao, T., ... & Yan, B. (2022). Intestinal Uptake and Low Transformation Increase the Bioaccumulation of Inorganic Arsenic in Freshwater Zebra Fish. *Journal of Hazardous Materials*, 434, 128904.