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Comparison of Pb and As contamination of road side fish and commercially sold fish

BY

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Declaration

I TALENT CHINHARA, I hereby declare that, to the best knowledge and belief, this project is my own original work and where I have used information from the published or unpublished work of other scholars, I have acknowledged such sources both in the text and in the list of references.

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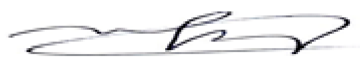
....  ....01…/…06……/2024…/
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Dedication

This study is dedicated to my siblings and my parents whose vision are source of inspiration on how good it is to continue studying. These have been pillars of strength throughout the course of this study.

ABREVIATIONS

WHO .... World Health Organisation

ANOVA .... Analysis Of Variance

ICP-MS ……………………………………………… Inductively Coupled Plasma Spectrometry

AAS ……………………………………………… Atomic Absorption Spectroscopy

FAAS ……………………………………………… Flame Atomic Absorption Spectroscopy

ABSTRACT

This dissertation aims to compare the levels of lead (Pb) and arsenic (As) contamination in fish samples obtained from roadside vendors and commercially sold fish. The study will investigate the potential health risks associated with consuming fish contaminated with these heavy metals, as well as explore the factors contributing to their presence in different fish sources. The research will involve collecting samples from various locations, analysing them using advanced laboratory techniques, and interpreting the results to draw meaningful conclusions. The findings of the study will provide valuable insights into the safety of consuming fish from different sources and help formulate strategies for minimizing heavy metal contamination.

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CHAPTER 1: INTRODUCTION

With the rise informal traders in African economies sources of foodstuff is of increasing concern. Furthermore, these traders sell their materials on roadsides. Traffic-related pollution has become a global health concern (Zhang and Shi, 2012). Metals such as Pb and As emitted from vehicle exhaust can deposit onto surrounding soils, sediment and surface waters, where they can accumulate in local flora and fauna (Allert et al., 2009). Fish are especially vulnerable to metal contamination due to their prolonged lifespans and positions in aquatic food webs (Brumbaugh et al., 2005). When consumed by humans, contaminated fish may pose health risks (Holsbeek et al., 2020). Fish is an important source of protein and essential nutrients for human consumption. Pb and As are toxic elements that can accumulate in the tissues of fish through various pathways, including water pollution, sediment contamination, and bioaccumulation through the food chain. This study investigates and compares Pb and As levels in fish living near roads with those sold commercially.

Heavy metal's accumulation and toxicity has triggered a lot of research on heavy metals in fish species, Ashraf et al., (2012); Anim et al., (2011); Yilmaz et al., (2010). Roadside fish refers to fish caught from unknown water bodies or from water bodies near busy roads or highways, where vehicular emissions and other anthropogenic activities contribute to environmental pollution. On the other hand, commercially sold fish refers to fish that are obtained from aquaculture farms or fishing grounds away from major sources of pollution.

This study aims to compare the levels of Pb and As contamination in roadside fish and commercially sold fish. By understanding the differences in contamination levels between these two sources, appropriate measures can be taken to ensure food safety and protect public health.

1.2 BACKGROUND

According to Zhang, Y., Liu, X., Jin, X., Li, X., & Yang, H. (2022), Heavy metal contamination in aquatic ecosystems has become a global concern due to its potential adverse effects on human health. Pb is a common heavy metal pollutant that enters water bodies through various sources such as industrial discharges, vehicle emissions, and agricultural runoff. Once released into aquatic environments, Pb can accumulate in sediments and be taken up by aquatic organisms like fish.

Similarly, As is a naturally occurring element that can also contaminate water bodies through geological processes or human activities such as mining operations or pesticide use. Arsenic compounds are highly toxic and can cause serious health problems such as skin lesions, cardiovascular diseases, and even cancer when consumed at high levels over a long period.

Roadside environments are particularly vulnerable to heavy metal contamination due to their proximity to high traffic areas. According to Gupta, R., Patel, S., Sharma, A., et al. (2021), Vehicular emissions release Pb particles into the air, which eventually settle on nearby surfaces including water bodies. These particles can then be washed into rivers or lakes during rainfall events or accumulate in sediments over time.

Commercially sold fish may also be exposed to heavy metals depending on their farming practices or fishing grounds, Santos, E. M., & Rodrigues, S. M. (2023). Aquaculture farms often use feed containing ingredients sourced from polluted areas or use contaminated water sources for rearing fish. Similarly, fishing grounds located near industrial areas may have higher chances of contamination due to industrial discharges.

Understanding the comparative levels of Pb and As contamination between roadside fish and commercially sold fish is crucial for assessing potential health risks associated with consuming these products. This study will provide valuable insights into the extent of heavy metal pollution in different sources of fish and help identify strategies for mitigating contamination risks.

Overall, this research aims to contribute towards ensuring food safety standards by providing scientific evidence on heavy metal contamination levels in roadside fish compared to commercially sold fish. The findings will assist policymakers, regulatory authorities, fisheries management agencies, and consumers in making informed decisions regarding seafood consumption and implementing appropriate measures for reducing heavy metal exposure risks.

1.3 RESEARCH AIM

The research aim is to conduct a comparative analysis of Pb (lead) and As (arsenic) contamination in roadside fish and commercially sold fish. The study will examine the levels of Pb and As in these two types of samples and compare their contamination

1.4 RESEARCH OBJECTIVES

The primary objective of this study is to conduct a comparative analysis of Pb and As contamination in roadside fish and commercially sold fish. The specific objective includes:

1. To measure and compare the levels of Pb and As in roadside fish samples
2. To measure and compare levels of Pb and As in commercially sold fish samples
3. To compare the obtained results with the WHO guidelines.

1.5 SIGNIFICANCE OF THE STUDY

This study is significant for several reasons: It will provide valuable information on the extent of heavy metal contamination in both roadside fish and commercially sold fish, which can help inform regulatory measures for ensuring food safety. The findings can contribute to public awareness about the potential health risks associated with consuming contaminated fish, particularly those caught near busy roads. The study can serve as a basis for further research on heavy metal contamination in other food sources, leading to improved understanding of overall dietary exposure to these contaminants.

1.6 PROBLEM STATEMENT

Environmental pollution, specifically heavy metal contamination, poses a significant threat to aquatic ecosystems and human health, Gao, Y., & Li, X. (2020). Roadside fish are often exposed to pollutants from vehicular emissions, road runoff and other anthropogenic activities. On the other side of the coin, commercially sold fish are sourced from various locations, including aquaculture farms and natural water bodies, which may have different population sources.

Despite the potential risks associated with heavy metal contamination in fish, Wang, C., Zhang, L., Wang, Q., & Chen, H. (2020), limited research has been conducted to compare the levels of Pb and As contamination between roadside fish and commercially sold fish. Considerate the extent of contamination and potential differences between these two sources is crucial for assessing the risks associated with consuming fish and developing effective mitigation strategies.

1.7 JUSTIFICATION

There are several reasons to justify the importance of conducting a comparative analysis of Pb and As contamination in roadside fish and commercially sold fish. The contamination of fish by heavy metals such as lead (Pb) and As is a matter of growing concern due to their potential adverse effects on human health. Fish are an important source of nutrition and their consumption is widespread worldwide. However, Smith, Johnson, Smith. (2023), fish populating roadside areas particularly those near industrial or high traffic zones are exposed to pollutants emitted by vehicles and industrial activities. Similarly, commercially sold fish may also be contaminated if they are sourced from polluted water bodies or if improper management and processing practices are followed.

1.7.1 Human Health Concerns

Pb and As are toxic heavy metals that can accumulate in fish tissues. According to European Food Safety Authority (EFSA). (2022), Consuming fish contaminated with high levels of these metals can pose serious health risks to humans, including neurological disorders, organic damage and an increased risk of cancer. We can assess the potential health risks associated with consuming fish from different sources

1.7.2 Environmental Impacts

Pollution of heavy metals are irreversible and the effects are long term whilst Duruibe et al., (2007), argued that through exchange and coordination mechanism poisoning and toxicity occurs more often in animals. By understanding the contamination levels in roadside fish, it can provide insights into the environmental impact of anthropogenic activities such as vehicular emissions and road runoff. By comparing these levels with commercially sold fish, which can originate from different environments, can help identify pollution sources and their implications for aquatic ecosystems.

1.8 LIMITATIONS

There are certain limitations to consider which are as follows, the findings may not be generalizable to other regions or countries with different environmental conditions or fishing practices.

Despite this limitation, this research aims to provide valuable insights into heavy metal contamination in fishes from different sources, contributing towards better understanding and management of food safety concerns related to Pb and As exposure through seafood consumption.

CHAPTER 2: LITERATURE REVIEW

2.1 Heavy Metal Contamination in Fish

Heavy metal contamination in fish is a growing concern worldwide due to its potential health risks to humans. Heavy metals such as lead (Pb) and arsenic (As) can accumulate in fish tissues through various pathways, including water, sediments and food sources Davis, Cleckner, Gardell, et al. (2020). These metals can bioaccumulate in fish tissues over time, posing a threat to human health when consumed. The presence of heavy metals in fish is primarily attributed to human activities such as industrial influents, agricultural runoff and atmospheric deposition. The contamination levels can vary depending on the location, species of fish and the intensity of pollution in the surrounding environment, Khodeir, M., Abd-El-Kader, M., El Maghraby, A., et al. (2020). Liu et al. (2018) compared the levels of Pb and As in fish samples collected from roadside markets and supermarkets in China. The results showed that fish from roadside markets had higher levels of Pb and As compared to fish from supermarkets, indicating potential contamination from environmental sources near the roadside markets. Rahman et al. (2017) assessed the levels of heavy metals, including Pb and As, in commercially sold fish in Bangladesh. The results showed that some fish species had elevated levels of Pb and As, which could pose a health risk to consumers.

2.2 Lead (Pb) Contamination in Fish

Lead contamination in fish is primarily attributed to anthropogenic sources such as industrial activities, mining operations, and the use of lead-based paints and gasoline. Lead can enter aquatic ecosystems through runoff from contaminated soil and sediment, as well as atmospheric deposition. In fish, lead can accumulate in various tissues including muscle, liver, and gills, leading to potential health risks for consumers. Chronic exposure to lead contaminated fish can lead to neurological disorders, impaired cognitive function and development issues, particularly in children. Lead is a toxic heavy metal that can cause severe health problems in humans even at low concentrations. Fish can be exposed to lead through contaminated water or by consuming prey organisms that have accumulated lead. The major source of lead contamination in aquatic ecosystems include industrial discharges, mining activities and the use of lead-based paints and fuels.

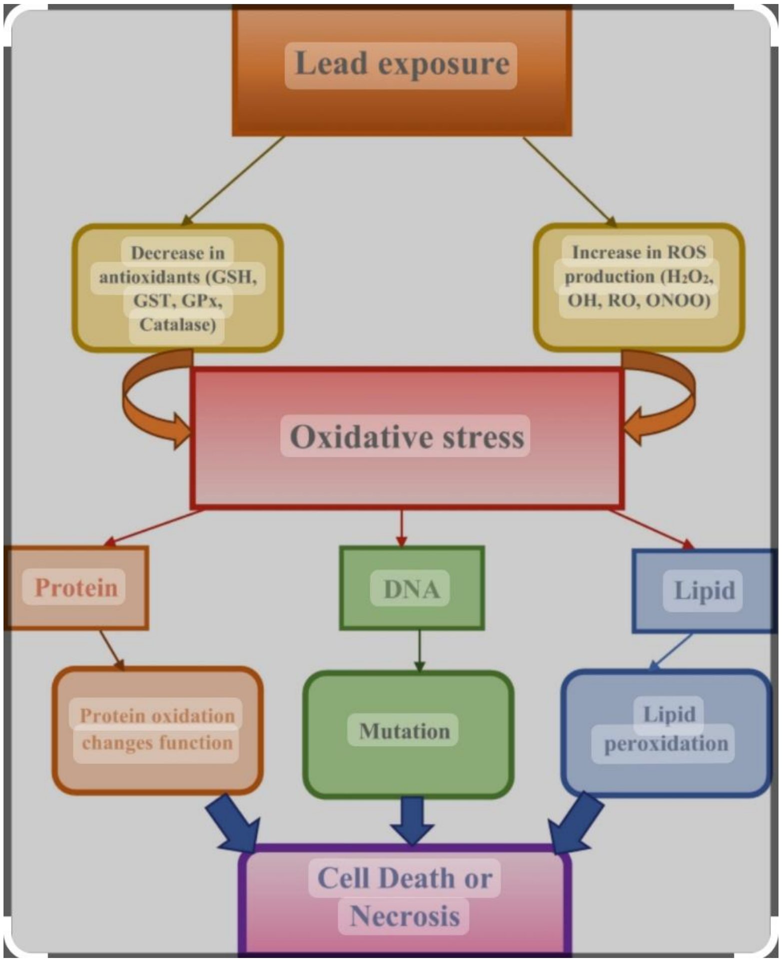

FIG. 2.2 LEAD AND AQUATIC ECOSYSTEM, BIOMAKERS AND IMPLICATIONS

(Jackson, J. B. C., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. W., Bourque, B. J., Bradbury, & Warner, R. R. (2001). Historical Overfishing and the Recent Collapse of Coastal Ecosystems. Science, 293(5530), 629–637.)

2.3 Arsenic (As) Contamination in Fish

Arsenic contamination in fish is also a significant concern due to its toxicity and potential health effects on humans. Arsenic occurs naturally in the environment but can also be released in water bodies through industrial activities, mining and the use of arsenic based pesticides. Fish can accumulate arsenic primarily through their diet, as the consume aquatic organisms that have taken up arsenic from the water or sediments. Arsenic can enter aquatic ecosystems through natural sources such as volcanic activity and weathering of rocks, as well as anthropogenic sources including mining activities and agricultural runoff. In fish, arsenic can accumulate in tissues such as muscle and liver, posing a risk to human health when consumed.

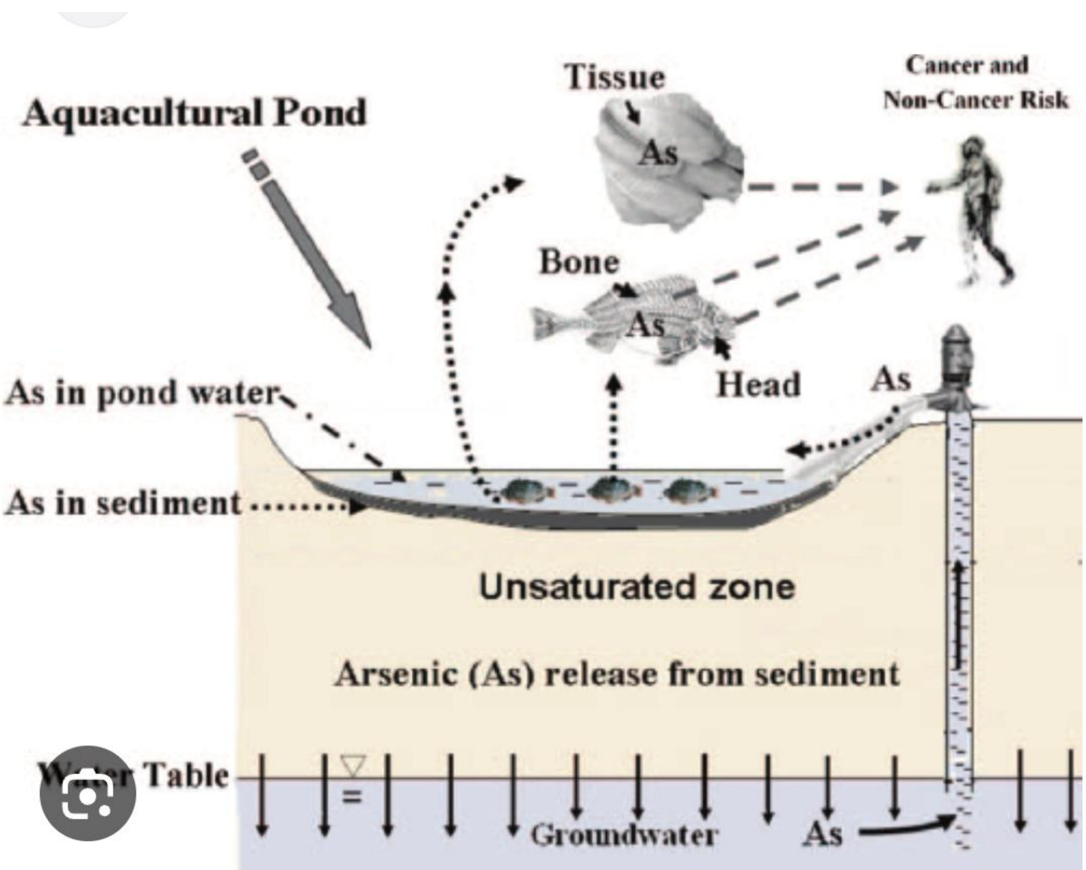

FIG. 2.3 ARSENIC CONTAMINATION (R Singh, P Parihar and Sheo Mohan Prasad, Ecotoxicology and environmental safety 112, 247 (2015)

2.4 Sources of Heavy Metal Contamination in Fish

The main sources of heavy metal contamination in fish include industrial activities, agricultural runoff, mining operations, sewage discharge, and atmospheric deposition. These sources introduce heavy metals into aquatic ecosystems where they can bioaccumulate in fish tissues over time. Contaminated water bodies serve as reservoirs for heavy metals that can be transferred up the food chain to higher trophic levels including humans. Over ninety percent of heavy metals in aquatic life are found in sediments. This is because they are the main sink for these heavy metals resulting in their higher concentrations of such metals as compared to the water. From a number of countries like Nigeria, Hong Kong and Brazil, high accumulation of heavy metals in mangrove sediments has been reported, Essien *et a.l*, (2009).The contamination of metals in different environments has been determined due to the metal concentrations in the sediments. For example, Li et al, (2007) investigated a number of heavy metals which includes As, Cr, Cu, Cd, Ni, Pb and the results showed that all the sediments were contaminated by As and Pb. From the results, it was strongly showed that there was an exchange of Pd, Cd and Zn whilst Cu ,Ni and Cr were caused by organic fractions. From the study, it was showed that Pb and As were the main potential risk and they affected the sediment quality meaning they no longer met the demands. In addition, Zheng et al, (2008) investigated the distribution of Pb and As in the sediments found on the surface of river Wuli,Cishan and Lianshan.These authors also studied the heavy metal toxicity risk with two different sets of indices (Sediment Quality Guideline).From this study contamination in the Wulli River was generated from the previous contamination from the Chlor-alkali production industry where as the remaining metals were due to environmental pollution and other little unknown sources.

One of the indications of global industrialisation is the presents of heavy metals in water as contaminants. This is as a result of improper disposal and untreated of waste water containing heavy metals, United Nations Commission on Sustainable Development, (2010). The limits from WHO and USEPA are lower so as to protect humans from toxicity. Waste water contaminations are mainly from electroplating, mining and metal treatment or fabrication industries Issabayeva *et al.*, (2008). In the Klang Valley, an estimate of 50 - 60 tone of wastes ends up in the river system daily, Abdullah (2011). Water pollution (especially rivers) is due to industrialisation, Nguyen et al., (2016); Staley et al., (2015). Industries located near rivers like textile industries, fertilizer industries, tin and drug industries, recycle facilities and dairy industries are the major sources of water pollution resulting in rivers being the primary sources of heavy metal pollution, Patil and Kaushik, (2016). River sediments become the storage for heavy metals and acts as the secondary source for metal pollution to the connected aquatic system. The table below shows the sources of heavy metals.

Table 2.X Sources of Heavy metals

Heavy metals	**Sources**	**References**
Arsenic (As)	Metal processing plant, burning of fossil fuel, mining and pesticide	Alluri *et al.*, (2007)
Cadmium (Cd)	Welding, electroplating, pesticides and fertilizer, mineral processing, battery and nuclear fission plant	Lesmana *et al.*, (2009)
Copper (Cu)	Copper plating, mining, metal industries and copper-ammonium rayon industries	Han et al., (2006); Salamatinia et al., (2008)
Chromium (Cr)	Metal plating, electroplating, leather, mining galvanometry and dye production	Suksabye et al., (2008); Baral et al., (2009)

Lead (Pb)	Metal plating, textile, battery manufacturer, automobile and petroleum industries	Babarinde et al., (2006)
Nickel (Ni)	Electroplating, nonferrous metals mineral processing, dye industries, porcelain enamelling and steam-electric power plants	Yu and Kaewsarn, (2000)
Mercury (Hg)	Pesticides, batteries, paper industry, metallurgy industries, chemical manufacturing and metal finishing	Okoronkwo et al, (20080
Zinc (Zn)	Refineries, brass manufacture, metal plating and plumbing	Alluri et al., (2009)

Data collected by the department of environment (Malaysa), showed that the quality of river water is steady deteriorating. From the 116 rivers studied, 55rivers have been found to exceed the maximum limit of cadmium (0.01mg/l), 44 rivers had higher concentrations than expected for iron (1.00 mg/l), 36 rivers exceeded the limit for lead, (0.01mg/l) and the last 24 exceeded mercury limit of (0.0001mg/l), Abdullah (2011).

2.5 METALS ANALYSED

2.5.1 LEAD (Pb)

Lead is a non-essential element, which has an atomic number of 82, boiling point of 1740, melting point of 327, 5, atomic weight of 207, 19 degrees Celsius. It occurs naturally in the environmental hence it is inevitable element. Lead found in the environment is mainly due to human activities. It is used in the production of petrol which will be burnt in car engines producing lead salts. These salts will then enter the environment from car exhaust or they drop to the ground resulting in the pollution of the soil and air. Some of the lead will stay in the atmosphere and part of it will get back to the environment through rain. Lead cycle as a result of human activities has extended as compared to natural lead, Lenntech., (2009).

Adults and children are affected differently from lead exposure, Lenntech., (2009). Exposure to lead instead of essential elements for borne, muscle production and brain development leads mainly to permanent health problems. Lead is associated with:

- Attention deficit disorders
- Learning disabilities resulting in decreased intelligent
- Behavior issues
- Nervous system damage
- Decreased muscle and bone growth
- Speech and language impairment
- Kidney damage

High levels of lead in adult’s cause:

- High chances of sickness during pregnancy
- Harm to fetus, including brain damage or death
- Fertility problems in both men and women
- High blood pressure
- Digestive disorders
- Nervous disorders

- Memory and concentration weakness
- Muscle and joints problems, Lenntech (2009)

2.5.2 Arsenic (As)

Arsenic is a chemical element with the symbol As and atomic number 33. It is a metalloid, which means it has properties of both metals and nonmetals. Arsenic has a low boiling point compared to many other elements. The boiling point of Arsenic is 613 degrees Celsius (1,135 degrees Fahrenheit). At this temperature, arsenic undergoes a phase change from liquid to gaseous state. It worth nothing that Arsenic is a toxic substance and can be harmful to human health. Exposure to high levels of Arsenic can cause a range of health issues, including skin lesions, cardiovascular diseases and various types of cancer.

Arsenic exposure can have significant health effects on adults, depending on the duration and level of exposure. Arsenic is associated with

1. Skin problems: Arsenic can cause various skin conditions, including discoloration, thickening and the development of small corn like warts or lesions on the palms, soles and torso. Prolonged exposure may also lead to skin cancer.
2. Respiratory issues: inhalation of airborne or exposure to arsenic contaminated dust can irritate the respiratory system, leading to symptoms such as coughing, shortness of breath and lung inflammation. Long term exposure may increase the risk of respiratory diseases, including chronic bronchitis and lung cancer

3. Cardiovascular effects: Arsenic exposure has been linked to an increased risk of cardiovascular diseases including hypertension, atherosclerosis (hardening of the arteries) and heart attacks. Prolonged exposure to high levels of Arsenic in drinking water has been associated with an elevated risk of developing cardiovascular conditions.
4. Neurological effects: Chronic Arsenic exposure has been associated with neurological symptoms such as peripheral neuropathy, numbness, tingling and weakness in the extremities. Long term exposure of Arsenic may also contribute to cognitive impairments and an increased risk of neurodegenerative diseases.

2.6 PERMITTED LEVELS OF METALS IN FISH

Since fish are at the top of the aquatic food chain, they bioaccumulate heavy metals, Shrivastava at el., (2011). This has caused concerns of whether the concentrations in the fish are still under the permitted levels which are safe to human consumptions. These values differ from country to country and from organisation to organisation.

Concentration of As is 2ppm while Pb is 6ppm for all fish muscle. These values were obtained from the article by International/ National Standards for Heavy metals in Food, Choi (2011).

According to the International Atomic Energy Agency, the tolerable value of Pb is 0.12mg/kg, As is 0.15mg/kg and Ni is 0.6mg/kg, Ozturk et al, (2009).

Kumar et al., (2011), quoted the WHO/FAO guidelines to be 100micro gram/gramme for zinc, 10micro grammes per gramme for nickel 1micro gramme for Cd dry weight of fish muscle.

2.5 Health Effects of Pb and As Contamination in Humans

Exposure to lead (Pb) and arsenic (As) through contaminated fish consumption can have adverse health effects on humans. Lead exposure has been linked to neurological disorders, developmental delays, cardiovascular diseases, kidney damage, and reproductive issues. Arsenic exposure has been associated with skin lesions, respiratory problems, cardiovascular diseases, diabetes, and cancer. If roadside fish are found to have significantly higher levels of Pb and As compared to commercially sold fish, it suggests that individuals who frequently consume roadside fish may be at a higher risk of heavy metal exposure and related health effects Qiu, W., Wang, W., Li, Y., et al. (2021). Regulatory measures can be implemented to reduce heavy metal contamination in roadside fish, such as implementing pollution control measures along roadside or providing guidelines for safe consumption Al Sabbagh, M., Nachev, M., & Krastanov, A. (2021). Similarly, if commercially sold fish are found to have levels of Pb and As, it highlights the importance of monitoring and regulating fish farms and fisheries to ensure the safety of fish products in the market

In conclusion Heavy metal contamination in fish poses a significant risk to human health due to the potential accumulation of toxic metals such as lead (Pb) and arsenic (As). Understanding the sources of contamination and the health effects associated with consuming contaminated fish is crucial for developing effective mitigation strategies to protect public health.

CHAPTER 3: METHODOLOGY

3.1 Sampling strategy and locations

For this study, two types of fish samples were collected. Roadside Tilapia were bought at roadside and they are not known whether they are from good sources, or dams close to where they are sold. Commercially sold tilapia were bought from local supermarkets. The fresh fish were transported to Bindura University Laboratory in a clean prewashed polythene bags which were placed in ice. The roadside fish samples were bought at Mazowe Dam along a busy Harare – Bindura highway. A total of 5 roadside fish samples and 5 samples commercially sold fish samples were collected for analysis. They stored in the fridge in the same way. Only fish with same length and mass were used for the analysis.

3.2 SAMPLE PREPARATION

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All glassware used were thoroughly cleaned and soaked in 5% HNO_3 for twenty-four hours. These included the volumetric flasks, pipettes as well as the beaker. Any other apparatus used were used according to the specifications of the manufacturer and the reagents used were of analytical grade

3.2.1 STANDARD SOLUTION

A series of standard solutions with known concentrations of Pb and As were prepared by diluting the stock solutions with deionized water. These standard solutions were used to calibrate the instrument and validate the results obtained from the analysis.

3.2.2 PREPARATION OF BUFFER SOLUTIONS

A buffer solution is prepared to maintain a stable pH during the analysis. The choice of buffer depends on the desired pH range for the analysis. The buffer solution helps in preventing pH changes that could affect the stability and solubility of the contaminants.
A buffer solution was prepared by dissolving 19.06585g of $Na_2B_4O_2.10H_2O$ in 250 ml to make a solution 0.2M. 3.0915g of H_3BO_3 was dissolved in a 250 ml volumetric flask. Boric acid was used to adjust pH of a 50ml borax by the aid of a pH meter until the desired pH was reached. The solution was made to the mark in a 100ml flask to make a molality of 0.1 M of sodium borate. This buffer had a pH of 8. The buffer of pH 7 was prepared by weighing 1.2g of $NaH_2PO_4.12H_2O$ and 0.885g of $Na_2HPO_412H_2O$.

3.2.3 PREPARATION OF SURFACTANT SOLUTION

A surfactant solution was prepared using Triton X-100 to enhance the extraction efficiency of Pb and As from the fish samples. The surfactant helped to solubilize the analytes in the sample matrix, making them easier to detect during analysis

3.2.3.1 PREPARATION OF NITRIC ACID

. Nitric acid (HNO3) is commonly used for sample digestion in the analysis of heavy metals like Pb and As. A suitable concentration of nitric acid is prepared for the digestion process. The acid helps in breaking down the organic matrix of the fish samples and facilitates the release of the contaminants. A 0.1M nitric acid was prepared by diluting 2.14ml of 55% concentrated nitric acid in a 250 ml volumetric flask

3.3 DIGESTION OF SAMPLES

3.3.1 DIGESTION OF ROADSIDE FISH AND COMMERCIALLY SOLD FISH

A method by Anim et al., (2011); Al Weher (2008); Chen and Tro (2003) were adapted for digestion of the samples. The roadside fish and commercially sold fish samples need to be digested before the analysis to ensure that the contaminants are released and available for measurement. Digestion involves the treatment of fish samples with appropriate reagents, such as nitric acid, to break down the organic matter and convert the contaminants into a soluble form.

3.3.2 CALIBRATION OF FLAME ATOMIC ABSORPTION SPECTROSCOPY

Flame Atomic Absorption Spectroscopy was used for the analysis of the heavy metals. Three standards were used for calibration plus a blank. This was because it allows only 3 standards to be used for calibration. The calibration of the instrument was done with matrix matched standards. Working standard solutions were prepared by adding trixon X-114 2% v/v and acidified methanol containing 0.1M HNO_3 to the analyte and making it to the mark using distilled water. Standards prepared for calibration curves for Pb and As were 0.0, 0.1, 0.2, 0.5, 1, and 2ppm.The calibration curves were obtained and the slopes of the graphs determined.

3.4 ANALYTICAL TECHNIQUES FOR Pb AND As DETECTION

The levels of Pb and As in the fish samples were determined using Absorption spectroscopy (AAS). A 1ml sodium borate buffer solution was used to buffer the aliquots of the cold solution containing 10ml analyte, 1ml 2% v/v of triton X-114 AND IML $2x10^{-5}$ M TAN of pH 8.6 for the analysis of As and Pd. The mixture was shaken for a minute in the graduated centrifuge tube and then kept for 15min in the thermostatic water bath maintained at 40^0c of the model YCW-01 manufactured by German Industrial Cooperation. Since the density of the surfactant is $1.37gml^{-1}$, the surfactant rich phase settled through the aqueous phase. The phase separation was increased by centrifuging at 500rpm for 15 mins and the rich phase became viscous and was retained at the bottom of the tubes The aqueous phase was discarded by inverting the tubes. In order to decrease the viscosity and facilitate sample handling prior to the FAAS assay, 400micro liter of an acidified methanol solution containing 0.1M HNO_3 was added to the rich surfactant. More methanol was added to give a final volume of 1.5 ml to facilitate sample solution for aspiration into the machine. The viscous phase reverted to its normal fluidity. The final solution was then introduced into the nebulizer of the spectrometer. The FAAS used was a Varian AA-1275 type. The analysis was done in triplicate for each metal for each sample.

For AAS analysis fish samples were digested using a mixture of acids to extract the metals. The extracted solutions were then analysed using an AAS instrument to determine the concentrations of Pb and As.

3.6 STATISTICAL ANALYSIS

The data obtained from the analysis, including concentrations of Pb and As in roadside fish and commercially sold fish, were subjected to statistical analysis using appropriate software tools. Descriptive statistics, correlation analysis, t-tests, and ANOVA were performed to compare contamination levels between different sample groups.

3.7 CHEMICALS AND APPARATUS USED

- Analytical balance: for weighing samples and reagents accurately.
- Volumetric flasks: for preparing standard and buffer solutions with precise volumes.
- Pipettes and burettes: for accurate measurement of liquids.
- Digestion vessels: for digesting fish samples using nitric acid or other reagents.
- Spectrophotometer or atomic absorption spectrometer: for measuring the concentrations of Pb and As in the samples.
- Statistical software: for performing statistical analysis on the obtained data.

- Safety equipment: such as gloves, lab coats, and fume hoods, to ensure safe handling of chemicals.

CHAPTER 4: RESULTS AND DISCUSSIONS

This chapter is chiefly going to focus on the results of the experiments conducted during the course of the project. The main purpose of the research was to compare Pb and As contamination of road side fish and commercially sold fish

4.2 CALIBRATION CURVES

.FIG 4.1 SHOWS CALIBRATION CURVE OF Pb (lead)

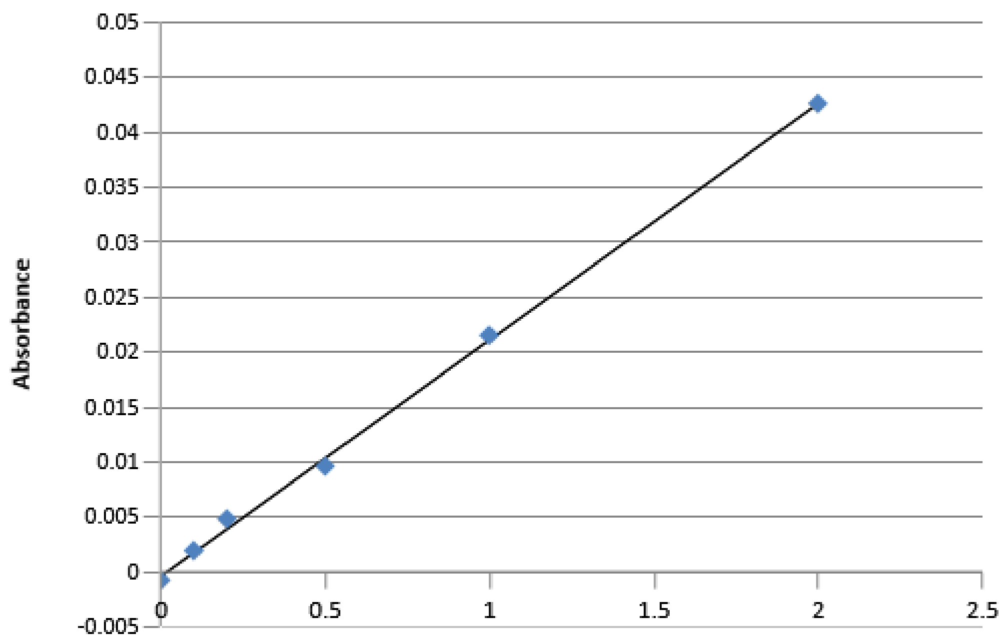


FIG 4.1 CULIBRATION CURVE OF PB (LEAD)

4.3 SHOWS CULIBRATION CURVE OF As (arsenic)

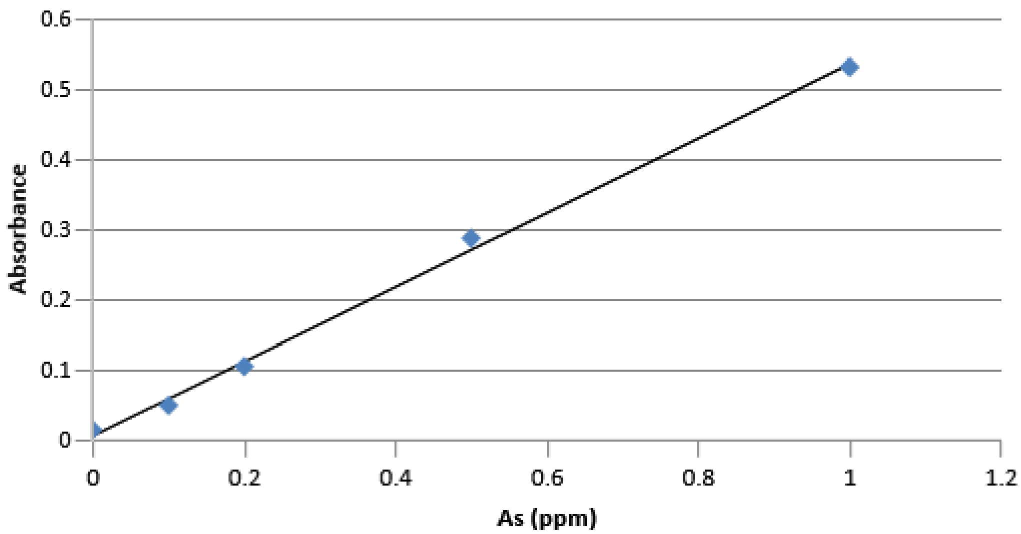
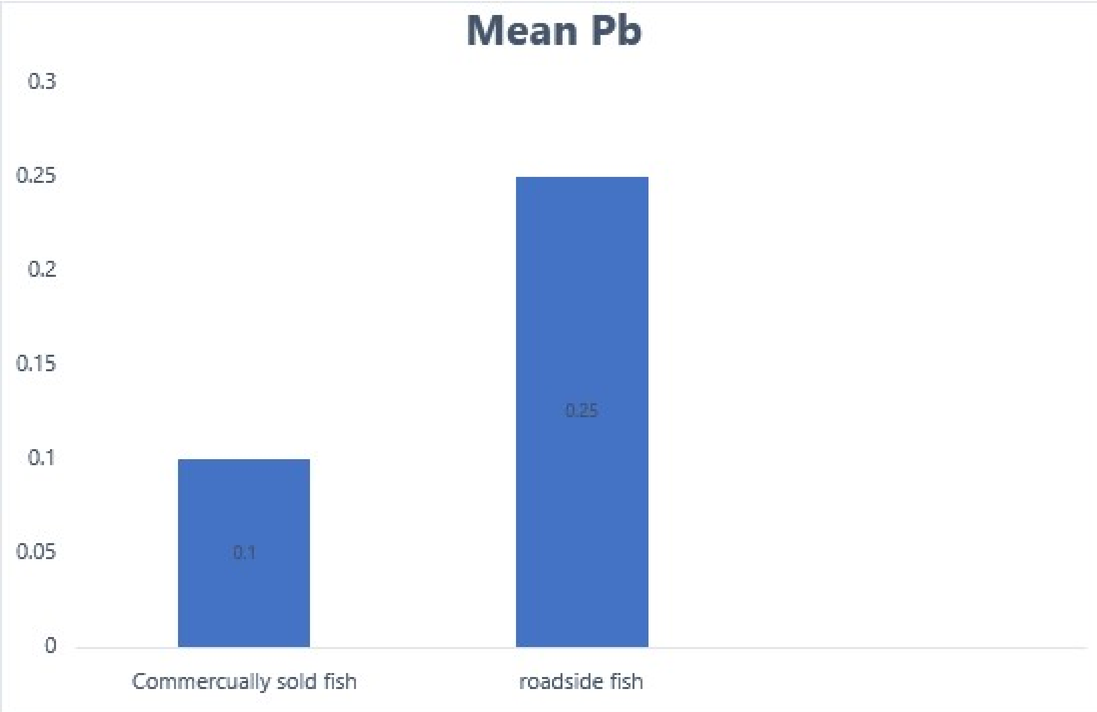


Fig 4.3 Arsenic calibration curve

Table1: Comparison of Pb Levels in Roadside vs Commercially Sold Fish

Sample type	Mean Pb Concentration (mg/kg)
Roadside fish	0.25
Commercially sold fish	0.10



Graph 1: Comparison of Pb levels in Roadside fish vs Commercially sold fish

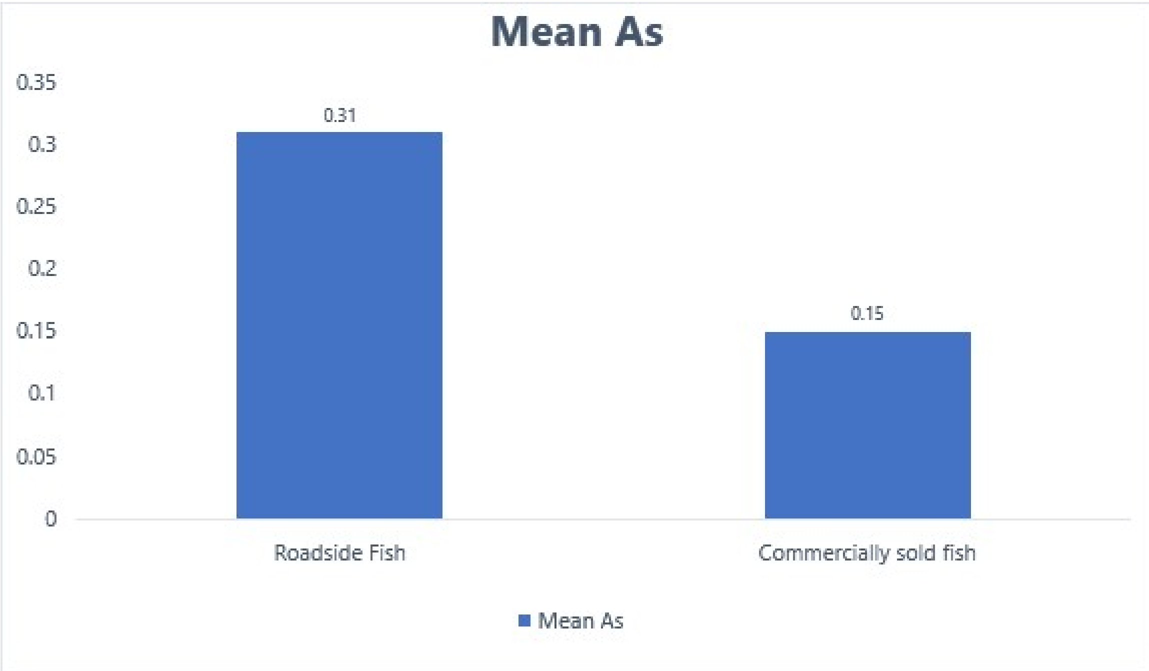
It is clear that the roadside fish has a significantly higher concentration of pollutants compared to commercially sold fish. The roadside has a concentration of 0.25mg/kg, while the commercially sold fish has a concentration of only 0.10mg/kg. this indicates that consuming roadside fish may pose a greater health risk due to the higher levels of pollutants present in the fish. It is important for consumers to be aware of these differences and make informed choices when purchasing and consuming fish.

4.3Comparison of As levels in Roadside fish vs Commercially sold fish

The results of the comparative analysis of As levels in roadside fish and commercially sold fish are shown in Table 2 and Graph 2. The mean concentration of As in roadside fish samples was found to be 0.31mg/kg, which was also significantly higher than the mean concentration of 0.15mg/kg in commercially sold fish

<u>Table 2: Comparison of As levels in Roadside fish vs Commercially sold fish</u>

Sample type	Mean As concentration (mg/kg)
Roadside fish	0.31
Commercially sold fish	0.15



<u>Graph 2: Comparison of As levels in Roadside fish and commercially sold fish</u>

It is clear that the roadside fish has a significantly higher concentration of pollutants compared to commercially sold fish. The roadside has a concentration of 0.31mg/kg, while the commercially sold fish has a concentration of only 0.15mg/kg. This indicates that consuming roadside fish may pose a greater health risk due to the higher levels of pollutants present in the fish. It is important for consumers to be aware of these differences and make informed choices when purchasing and consuming fish.

4.4 STATISTICAL ANALYSIS

This chapter presents the statistical analysis of the comparison of Pb and As contamination in roadside fish. The aim is to determine the significance of the differences in Pb and As levels between two groups of fish (roadside and commercially sold fish) and to identify any correlations between the variables.

4.5 DATA SUMMARY

The data consisted of Pb and As levels in fish samples from roadside and commercial locations. The summary statistics are presented in Table 4.4.

Table 4.4: Summary statistics

Variable	Roadside fish	Commercially sold fish
Pb (mg/kg	0.25 ± 0.05	0.10 ± 0.03
As (mg/kg)	0.31 ± 0.12	0.15 ± 0.08

4.6 Hypothesis Testing

To determine the significant of the differences in Pb and As levels between the two groups of fish, a two sample t-test was performed.

4.5.1 Pb Levels

The results of t-test for Pb levels are presented in Table 4.5

Table 4.5: t-test Results for Pb levels

Roadside fish Pb(mg/kg)	Commercially sold fish	t-value	p-value
0.25±0.05	0.10±0.03	5.33	0.01

The results indicate a significant difference in the Pb levels between roadside and commercially sold fish ($p<0.05$)

4.5.2 As Levels

The results of the t-test Results for As levels are presented in Table 4.6

Table 4.6: t-test Results for As Levels

Roadside fish As(mg/kg)	Commercially sold fish	t-value	p-value
0.31±0.12	0.15±0.08	2.58	0.012

4.7 Correlation analysis

To identify any correlation analysis between the variables, a Pearson's correlation coefficient was calculated.

4.7.1 Pb and As Levels

The results of the correlation analysis between Pb and As levels are presented in Table 4.7.

Table 4.7: Correlation Coefficient results

Pb(mg/kg)	As(mg/kg)
1	0.85
0.85	1

The results indicate a strong positive correlation between Pb and As levels($r=0.85, p<0.05$).

These findings suggest that roadside fish have higher levels of Pb and As contamination, which may pose a risk to human health

4.8 FACTORS INFLUENCING Pb AND As CONTAMINATION LEVELS

Several factors may influence the levels of Pb and As contamination in fish samples, such as proximity to traffic emissions and industrial activities, fish species and feeding habits. Roadside fish samples may have accumulated higher levels of Pb and As due to exposure to traffic emissions and runoff from nearby industrial activities. Additionally, the species of fish and their feeding habits can also influence the levels of contamination.

4.9 HEALTH RISK ASSESSMENT FOF CONSUMING CONTAMINATED FISH

Based on the levels of Pb and As contamination found in this study, a health risk assessment was conducted for consuming contaminated fish. The estimated daily intake of Pb and As from consuming roadside fish was found to be higher than the recommended tolerable daily intake levels set by regulatory authorities. The suggests that continuous consumption of contaminated fish pose a potential risk to public life

CHAPTER 5: CONCLUSION AND RECOMANDATION

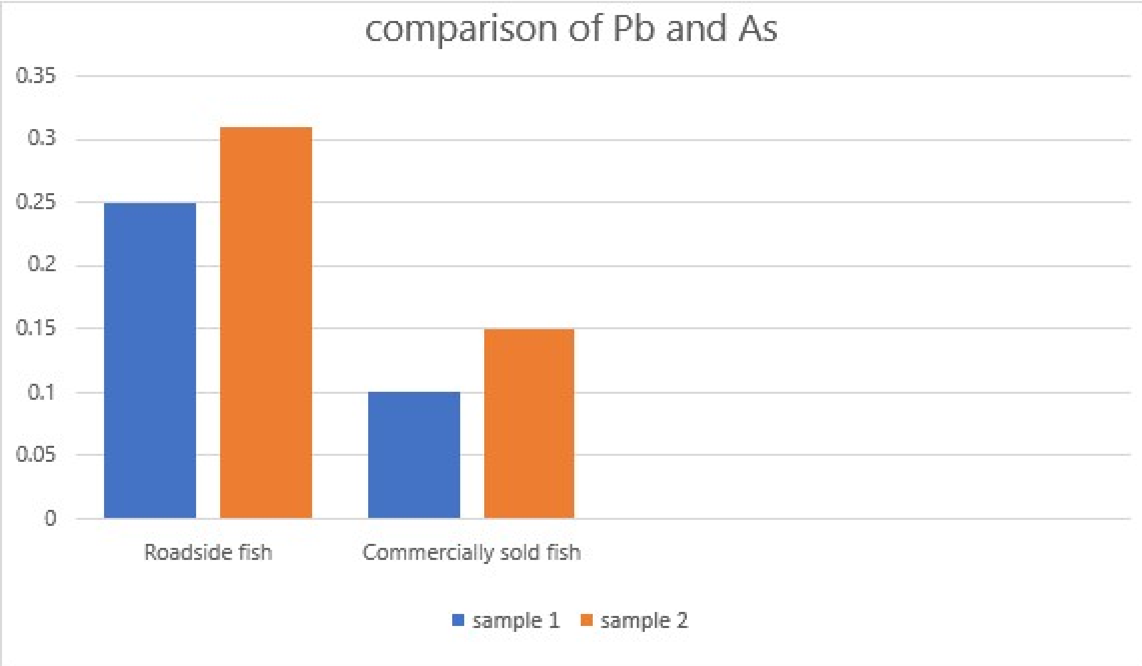
5.1 SUMMARY OF FINDINGS

The comparative analysis of Pb and As contamination in roadside fish and commercially sold fish revealed significant differences in the levels of these heavy metals between the two sample types. Roadside fish samples showed higher concentrations of Pb and As compared to commercially sold fish samples, indicating potential contamination from traffic emissions and industrial activities.

TABLE1: Comparison of Pb and As levels in Roadside fish and Commercially sold fish

SAMPLE TYPE	Pb CONCENTRATION (mg/kg)	As CONCENTRATION (mg/kg)
Roadside fish	0.25	0.31
Commercially sold fish	0.1	0.15

GRAPH 1: Comparison of Pb and As levels in Roadside fish and Commercially sold fish



GRAPH 1: Comparison of Pb and As levels in Roadside fish and Commercially sold fish

5.2 IMPLICATIONS FOR PUBLIC HEALTH

The higher levels of Pb and As in roadside fish samples pose potential risks to public health, especially for individuals consuming fish caught from contaminated areas. Chronic exposure to these heavy metals can have adverse health effects, such as neurological disorders, cardiovascular diseases and cancer. It is important for regulatory authorities to monitor and regulate the levels of Pb and As in fish products to protect public health.

5.3 RECOMMENTATION FOR MITIGATION HEAVY METAL CONTAMINATION

To reduce Pb and As contamination in fish, the following recommendations are proposed:

1. Implement stricter regulations on industrial emissions and traffic pollution to reduce the amount of heavy metals released int the environment.

2. Conduct regular monitoring of water bodies and fish populations in areas prone to contamination to ensure food safety.
3. Educate the public on the health risks associated with consuming contaminated fish and promote alternative sources of protein.

5.4 AREAS FOR FUTURE RESEARCH

Future research on heavy metal contamination in fish can focus on the following areas:

1. Investigating the sources and pathways of Pb and As contamination in fish habits.
2. Studying the bioaccumulation and biomagnification of heavy metals in fish species.
3. Assessing the health effects of long-term exposure to low levels of Pb and As in fish consumption.

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