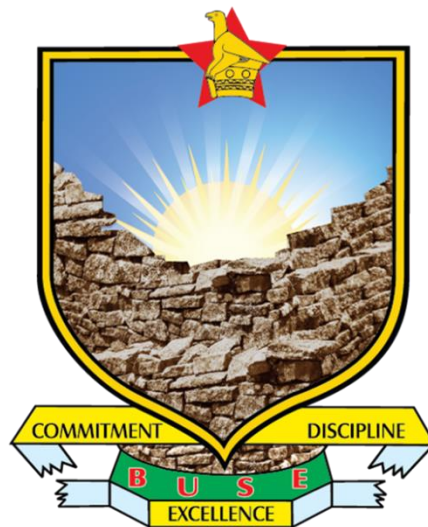


BINDURA UNIVERSITY OF SCIENCE EDUCATION

DEPARTMENT OF NATURAL RESOURCES

**ASSESSING HEAVY METAL CONCENTRATIONS IN SELECTED CROPS GROWN
ON POLLUTED WETLAND SOILS IN BINDURA**



**GOTO NYASHA
(B1850881)**

***A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
OF THE BACHELOR OF ENVIRONMENTAL SCIENCE HONOURS DEGREE IN
NATURAL RESOURCES MANAGEMENT.***

MAY 2022

DEDICATION

This work is dedicated to my parents Mr and Mrs Goto, my deputy parents Mr and Mrs Mugarisanwa and my son Donelle Mufaro.

ACKNOWLEDGEMENTS

I would like to give thanks to the Lord who gave me strength to work on my project. I extend my gratitude to my supervisor Mr. A. Kundhlande for assisting me in supervising my study.

ABSTRACT

This study sought to assess heavy metals concentrations in crops grown on polluted wetland soils and to compare them with World Health Organisation recommended thresholds meant to safeguard human health upon consumption. The selected heavy metals included Zn, Cu, Ni, Cd, Cr, Pb and Fe. The study was carried out at a polluted wetland located near the Bindura University of Science Education Astra campus. Crop produce samples were collected from the wetland garden near Astra Campus and prepared for laboratory analysis following standard procedures. The selected crops included okra (*Abelmoschus esculentus*), vegetable leaves (*Brassica napus*), chillies (*Capsicum frutescenes*), green pepper (*Capsicum annum*), eggplant (*Solanum melongena*), tomatoes (*Solanum lycopersicum*), madhumbe (*Colocasia esculenta*) and sweet potatoes (*Ipomoea batatas*). A quasi experimental design was used in the process. Two factors were designed (plant and growth media). Repeated Measures ANOVA was carried out on the data using Statistical Package for Social Sciences (SPSS) Version 23. World Health Organisation standards were used for benchmarking the concentration of selected trace elements in selected crop produce. Studied results all indicated heavy metal concentrations below the World Health Organization thresholds. In other terms, the results were complying with WHO standards. This study concluded that the studied crops were safe for human consumption and there is also need for other studies to concentrate on heavy metal concentrations in different parts of the plant as they are likely to variably accumulate.

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LIST OF ACRONYMS AND ABBREVIATIONS

Ni	Nickel
Zn	Zinc
Pb	lead
Cd	Cadmium

Cr Chromium

Cu Copper

ANOVA Analysis of Variance

SPSS Statistical Package for the Social Sciences (SPSS)

WHO World Health Organization

CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Vegetables are eatable plant life that stock reserve food materials in their roots, stem, leaves, and/or fruits, where vital dietary rudiments, including iron, vitamins, calcium and protein. Diet rich in vegetables has been informed to reduce the risk of heart illnesses - lung, and esophageal cancers; however, they may pose a danger to human health when contaminated with trace elements.

Heavy metals influence our environment as they are primarily polluting agents in our food source, specifically vegetables. Any element considered toxic or hazardous possibly will be called heavy metal such as lead (Pb), nickel (Ni), Iron (Fe) and only to mention a few. Furthermore, the buildup of high levels of toxic elements by crop plants grown in polluted soils denotes the main passageway for bioaccumulation of these metals into the food web. Other sources of heavy metals in irrigated agriculture include manures, fertilizers, pesticides and airborne contamination from car traffic. The discharge of human and industrial waste has the potential of polluting water. Heavy metals flow in soil and water, which is deeply concerned about public health, farming production and ecological strength. However, there is dearth of information on the intake of some toxic heavy metals by some food crops cultivated in Bindura waters. Accumulation of toxic heavy metals in edible food crops is a potential threat to human and animal health. Hence, a study on food crop in terms of heavy metals accumulation is expedient. In this study, the levels of heavy metals are to be taken from crops grown in polluted waters near Astra campus wet land.

The main sources of pollution are anthropogenic activities which are mostly created by processes in factories, industries, mining, and municipal sewers, among other things (Raju *et.-al.*, 2010). Phosphates, nitrates, sulfates, nitrogen, and total dissolved oxygen are concentrated contaminants in raw sewage that, if not managed, can be detrimental to the environment. Water hyacinth is one of many plants that have been utilized in phytoremediation. Water hyacinth is a nutrient-accumulating plant that aids in the ablation of waste water, which then can be recycled for use in other actions such as crop growing (Dauda, 2010).

Trace elements are categorized as vital and toxic. Fe, Cu, Zn, Co, are necessary for plant growth in adequate amount whereas Hg, Cd, Pb are known as toxic metals. Therefore, the present study seeks to analyze the heavy metal accumulation in crops grown in polluted waters.

1.2 PROBLEM STATEMENT

It is well known that the waters that the crop produce uses for irrigation are heavily polluted as the wetland is the destination site of all wastes from different sources such as mining activities, raw sewage and fertilizer industry located near the garden. These crops, are not only consumed at a household level, but are also sold in the urban markets to unsuspecting consumers who may be subjected to hazards emanating from exposure to heavy metals (Githuku, 2009). The main worry being that these heavy metals if accumulated in the human body in large quantities may lead to serious health problems such as stroke, lung failure and only to mention a few . The problem comes when there is much concentration of heavy metals in the body that will lead to serious health problems. Hence, there is need to assess whether the crop produce have a high concentration of these heavy metals or not so as to determine their safety for consumption by the people.

1.3 JUSTIFICATION

The study will help in identifying the heavy toxic metals found in crops grown in polluted waters and this helps to give heads up to the community on the health risk likely to be faced due to the consumption of such heavy metals. The study is of greater importance as it will also give people the benefit of the doubt in the sense that people will be sure that what they are consuming is safe or not. There is also a need to implement laws governing use of polluted waters for irrigating crops so as to keep the community and the environment safe. Mechanism of waste water discharge constraints are essential in observing Environmental Management Act discarding standards that include “Zimbabwe Effluent Standards for discharge (Effluent and Solid Waste Disposal Regulations Statutory Instrument 6 of 2007) to reduce negative effects to the environment as well as safety of humans”.

1.4 AIM

The aim of the present investigation is to determine the concentration of heavy metals in different crop produce grown on contaminated soils in comparison to WHO thresholds.

1.4.1 OBJECTIVES

- i. To determine selected heavy metal concentrations (Zn,Pb,Fe,Cr,Cd,Ni)in identified crops(*Abelmoschus esculentus*, *Brassica napus*, *Capsicum frutescenes*, *Capsicum annum*, *Solanum melongena*, *Solanum lycopersicum*, *Colocasia esculenta*) and sweet potatoes (*Ipomoea batatas*).
- ii. To compare the heavy metals concentrations with World Health Organisation (WHO) thresholds standards.

1.5 HYPOTHESES

1. There is no significant difference in the effect of heavy metals found in crops grown on polluted waters.
2. There is no significant difference in determining the effectiveness of heavy metal in crop growth.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 WHAT ARE HEAVY METALS

The term “heavy metals” refers to metals that create encounters when released into the environment. Trace metals are environmental pollutants due to their toxicity, perseverance, and bio accumulative nature. Examples of such heavy metals include zinc, copper, iron, manganese, cadmium, chromium, and others (Donjadee *et al.*, 2012). Even though some of them are also crucial to plants for their growth, on the other hand, they mostly become harmful at high concentrations. However, some trace elements, namely cadmium, lead, and chromium are even destructive at low concentration. Heavy metals are not the only problem in wastewater effluent, as it may also contain phosphates, nitrates, and pathogens. Nitrate and phosphate are also necessary nutrients for plant growth. Concentration of these in surface and ground water is dangerous to the environment and could cause serious problems to aquatic life due to their ability to cause eutrophication.

According to Dalton (2012), Nitrates can also harm human beings. Consequently, the concentration of nitrates in potable water is limited to 50 and 10 mg/L for adults and babies, respectively. Phosphate generally arises from the elemental phosphorous and reduces water quality by the disparate development of algae, and its excessive concentration results in the eutrophication process, which decreases the amount of dissolved oxygen in aquatic systems. Due to the dangers posed by partially treated wastewater for irrigation, it is always important to monitor its composition. Therefore, what uttered this research, apart from the fact that partially treated wastewater is released into surface water bodies, is that raw effluent is also used at times to water crops by small-scale farmers. These crops, for example, vegetables apart from being consumed by

farmers, are also sold in local produce markets, which postures a danger to the locals (Onakpa, Njan and Kalu, 2018).

2.2 SOURCES OF HEAVY METALS IN CROP PRODUCE

Heavy metals are naturally occurring elements in the earth's crust and are long-lasting environmental contaminants. They are non-biodegradable and enter the body through food, air, and water, building up in the body over time. Natural and anthropogenic sources can release them unrestrained into the environment. Farming activities, such as pesticide and herbicide application, contaminated irrigation water, municipal garbage used for fertilization, and even mineral fertilizer containing concentrations of heavy metals, are all anthropogenic sources of heavy metal contamination. Direct garbage disposal on farmland, mining activities, the use of lead as an antiknock additive in gasoline, transportation emissions, cigarette smoking, metallurgy and smelting, aerosol cans, sewage discharge, and construction mate are all anthropogenic sources of heavy metals (Ashraf *et.al*, 2021).

2.3 FACTORS THAT AFFECT THE UPTAKE OF HEAVY METALS IN PLANTS

Temperature, moisture, organic matter, pH, and nutrient availability are all factors that influence heavy metal accumulation in plant tissues, according to researchers (Id *et al.*, 2021). Plant species influence heavy metal accumulation, and plant absorption efficiency is controlled by plant absorption or the metals' soil-to-plant transfer factors. Increased lead levels in soils, for example, can reduce soil productivity, whereas extremely low lead levels can obstruct essential plant activities such as photosynthesis, mitosis, and water absorption, leading to toxicity symptoms such as dark green leaves, drooping leaves, stunted growth, and brown short roots. Heavy metals can cause chlorosis, poor plant development, low yield, and nutrient uptake issues.

2.4 TOXICITY OF HEAVY METALS WHEN ACCUMULATED IN HUMAN BODY

When heavy metals are not digested by the body and accumulate in the soft tissues, they become poisonous. Toxicity of heavy metals refers to the negative consequences of excessive exposure or ingestion of levels that exceed the daily recommended limits (Mohamed and Physics, 2001). Even

if individual metals show specific signs of toxicity, gastrointestinal disorders, diarrhea, stomatitis, tremor, hemoglobinuria, ataxia, paralysis, and vomiting are common symptoms of cadmium, lead, arsenic, mercury, zinc, copper, and aluminum poisoning, as are convulsion, depression, and pneumonia when vapors and fumes are inhaled. Children's brains, kidneys, bone marrow, and other systems can be damaged by ingesting or swallowing lead. Blood lead levels as low as 5 g/dL have been linked to developmental problems in toddlers and children. Impaired cognitive function, behavioral difficulties, impaired hearing, and stunted growth are all growing problems. It is thus important to mention that native cuisines typically consumed in Eastern Nigeria may contribute to heavy metal loads, which is important for public health. The accumulation of hazardous materials has both human and plant impacts, according to Khan and Waqas (2015).

2.5 WASTE WATER RECLAMATION

The action or processing of wastewater to make it usable is known as wastewater reclamation. reusability (Richard, 1998). This is a method of supplementing current water supplies. reducing the negative effects of water scarcity Kenya is a resource-rich country. There are only a few developing countries where wastewater reuse is currently used (Jinadasa *et al.*, 2006). While biological sewage treatment reduces the volume of sewage, biodegradation of organic materials and the load of disease-causing organisms it's more difficult to play heavy metal. Having good hygienic conditions with little or no waste. While maintaining the nutrients in the effluent, suspended solids in wastewater When processing wastewater for irrigation, this should be a primary goal (Hartling and Nellor, 1998). Trace elements are non-biodegradable and have long biological half-lives, making them difficult to eliminate from the body (Li *et al.*, 2004). Mercury, cadmium, lead, nickel, chromium, arsenic, and molybdenum are some of the most dangerous heavy metals that come from industrial sources (USDA, 2000). According to Smith (2009), the addition of compost and sewage sludge to a background of heavy metal concentrations increased the amount of these metals in agricultural soil. These metals are also likely to be transported to edible crop portions, he said. A In a separate study in China, it was discovered that irrigation with sewage exacerbated soil problems. Cadmium (Cd), copper (Cu), zinc (Zn), and lead (Pb) contamination (Liu *et al.*, 2000).

CHAPTER 3

3.0 METHODOLOGY

3.1 DESCRIPTION OF THE STUDY AREA

The location of Bindura town is (17° 30' 18"S and 31° 19' 49"E) about 90 km north east of Harare. The area is characterized by hot dry and wet summers (September-March) and cold dry winters (May-August). Mean daily temperature range is 12 -26°C and the average annual rainfall of 810 mm. This research was piloted at Bindura University (Fig 3.1) located (17° 18' 59"S and 31° 19' 22"E).

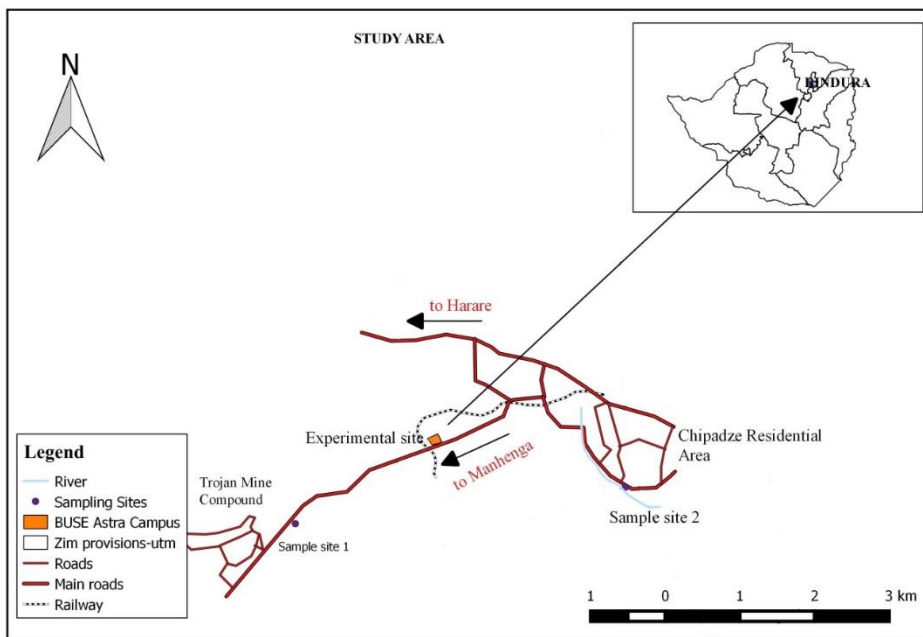


Fig 3.1: Map of the study area

3.1 SAMPLE COLLECTION

Twenty-four fruit samples were randomly collected direct from the garden in polluted waters close to Astra campus. The fruits were chosen from these areas because they are the most consumable fruits grown in polluted waters in Bindura. Another eight fruit samples from each fruit were collected from fruit farms that uses clean water or unpolluted waters to serve as control samples. Crop samples for this research included okra (*Abelmoschus esculentus*), vegetable leaves (*Brassica napus*), chilies (*Capsicum frutescenes*), green pepper (*Capsicum annum*), eggplant (*Solanum melongena*), tomatoes (*Solanum lycopersicum*), madhumbe (*Colocasia esculenta*) and sweet potatoes (*Ipomoea batatas*).

3.2 SAMPLE PREPARATION

The samples were cleaned using tap water to remove soil and other filth, and the eatable parts were split from other portions then they were cut into slices after the covers were taken off. Each of the samples were then placed on bond papers and directed to the oven so as to dry. They were heated at 105 degrees Celsius. The samples were then crashed to powder form and later weighed using an analytical balance machine. The samples were further ashed in the furnace for four hours at 500 degrees Celsius and were left to cool for two hours. After two hours, the samples were removed from the furnace and were placed in a beaker. Purpose of ashing is to remove all organic substances in the samples. Digestion of the samples using a block digester at 500 degrees Celsius was done following the ashing process.

To manage the concentration of heavy metals in the water, calibration curves were created. Examples of solutions Intermediate heavy metal standard solutions (10 mg/L) Stock standard solutions containing 1,000 mg L⁻¹ of Cd, Cr, and Zn were produced. For each metal solution, proper operating standards were created by serialization. Using deionized water, dilute the intermediate solutions. Solution of potassium chloride for determining the concentration of Cr, an ionization suppressor was introduced. The wavelengths, flame color, and slit width were all tuned to fit the apparatus. The operation handbook was then aspirated to improve its sensitivity and operational standards. Atomic absorption spectrometry and their absorbance, one by one, into the flame was captured on film. Each of the calibration curves was plotted.

3.3 RESEARCH DESIGN

Eight sets of experimental designs were set. The experiment was replicated three times and twenty four samples were tested. The duration was 0, 1, 2, 3. The experiment was fixed up in the month of January 2022. The experimental setup was as follows with three replicates each;

1:3 *Capsiucum erutescens* grown in polluted waters + 1(control)

2: 3 *Brassica napus* grown in polluted waters + 1 (control)

3: 3 *Capsium annum* + 1 (control)

4:3 *Solanum melongeria* +1 control

5:3 *Solanum lycopersium* +1 control

6: *Colocasia esculenta* +1 control

7:3 *Ipomoea batatas* +1 control

8:3 *Abelmoschus esculentus*

3.4 EXPERIMENTAL DESIGN: QUASI EXPERIMENTAL DESIGN

The goal of this design is to find a cause-and-effect link between the independent and dependent variables. It's also effective in instances where genuine experiments aren't possible due to ethical or practical considerations. Crop and growth media were the two main contributors. Crops planted in polluted waters comprised component one, while crops grown in unpolluted waters included factor two. A total of twenty-four observations were obtained by replicating each treatment three times. Over the course of three days, samples were taken on a daily basis. As a result, many measurements were taken. The design's structure is depicted in Figure 3.1.

Table 3.1 displays factors, factor levels and treatment combinations

Factor	Factor Level	Treatment Combinations
Crop	Tomatoes	Tomatoes * polluted waters Green paper * polluted waters Vegetables *polluted waters
Growth media	Polluted waters	All crops *polluted waters
	Unpolluted waters	All crops* unpolluted waters

3.5 SAMPLE ANALYSIS

Heavy metals present were analyzed using the Atomic Absorption Spectrophotometer (Varian 2000). Three (3) replicates were made on each sample so as to achieve worth assurance and precision and their mean was used for statistical analysis of results.

3.6 METHODS OF DATA ANALYSIS

Repeated Measures to test for heavy metals in crop produce growing in polluted streams, ANOVA was done on the data using Statistical Package for the Social Sciences (SPSS) Version 23. ANOVA was utilized since the data were interrelated and also because the statistics met the 3 ANOVA rulebooks: Normality, which means the test variables follow a multivariate normal distribution in the population, and sphericity, which means the variances of all difference scores amongst the test variables need to be equal in the population, are all independent and identically distributed variables. Treatment (polluted water-grown crops vs. farm-grown crops (regulator)) was defined as a within-subjects cause, with nutrient content in growth media as a metric. To account for this, a repeated measures ANOVA was performed.

CHAPTER FOUR

4.0 RESULTS

4.1 INTRODUCTION

This chapter gives results on the assessment of selected elements (Cr, Zn, Cd, Ni, Pb, Cd and Cu) in crops grown in polluted waters in Bindura.

The table 4.1.0 below shows the results of the seven elements concentration in selected food crops.

<u>Element</u>	<u>Mean</u>	<u>+ -</u>	<u>SE</u>	<u>pValue</u>
CADMIUM	0.400		0.00881	0.00
CHROMIUM	0.913		0.2157	0.689
ZINC	0.438		0.3101	0.00
NICKEL	0.465		0.00540	0.00
COPPER	0.442		0.0691	0.00
IRON	0.625		0.37327	0.667
LEAD	0.400		0.296	0.00

4.1.1 CADMIUM

The results indicated a significant difference in concentration of cadmium with a p value of 0.00, with a revealed concentration range of 0.01mg/l to 0.09mg/l whilst the highest concentration of cadmium was found in sweet potatoes. The mean cadmium concentration was reported to be 0.400mg/l with a standard value of 0.00881.

4.1.2 CHROMIUM

There was no significant difference in chromium concentration in the food crops as compared to the WHO thresholds. Chromium concentration in food crops had a concentration mean of 0.913mg/l with a range varying from 0.02mg/l to 0.33mg/l. From a universal basis, the Cr mean concentration in food crops is reported to be 66.08mg/kg (Su, Jiang and Zhang,2014).

4.1.3 ZINC

The results showed that there was a significant difference in in concentration as revealed with a p value of 0.00. Zinc concentration varied from 0.13mg/l to 0.22mg/l giving a mean of 0.438mg/l whilst the highest concentration was found in chilies.

4.1.4 NICKEL

There was a significant difference in Nickel concentration with a p value of 0.00. The mean value was 0.0465mg/l as the concentration value ranges from 0.01mg/l to 0.08mg/l as tomatoes marked the lowest concentration whilst madhumbe marked the highest concentration.

4.1.5 COPPER

Results revealed that there was a significant difference in copper concentration as compared to the WHO thresholds. This was shown with a p value of 0.00 and the mean concentration been 0.442mg/l. The concentration of copper in selected food crops ranges from 0.01mg/l to 0.09mg/l whilst okra marked the crop with the highest copper concentration.

4.1.6 LEAD

There was a significant difference in the concentration of lead as compared to WHO thresholds with a p value of 0.00. The mean value was 0.400mg/l .The concentration ranges from -0.10mg/l to 0.14mg/l. To a greater extent, lead was undetectable.

4.1.7 IRON

There was no significant difference in iron concentration as compared to the WHO thresholds as the p value was 0.667 and the mean was 0.625mg/kg.The concentration ranges from -0.36mg/l to 5.13mg/l making chilies the crop with highest iron concentration.

CHAPTER 5

5.0 DISCUSSION

The results showed that Lead concentration which was below the WHO standard of 0.3mg/kg was like that because of less industrial activities around the study area. In this case, the battery industry that mainly released lead for the past years was closed and thus giving one an evidence that that's the reason why lead concentration was even below detected limit. However, other studies observed the concentration of Pb was higher than the maximum limit by WHO (2001), but lower than the values reported by Singh and Kumar (2004). It is well known that exposure to high levels of Pb may cause kidney damage leading to renal failure (Laura et.al 2009; Colgan 2003).

The Cr concentration in the present study were lower than the WHO limits, but in support of the findings given by Inoti, Kawaka *et.al.* (2012) who found Cr concentration in leaf vegetables ranging between 0.89 to 0.99mg/kg. This is because some of the crop produce are not the targeted organs of heavy metal accumulation. Therefore, it is argued that although the crop produce does not contain the heavy metals, it doesn't mean to say that the plant is not accumulated with heavy metals. Hence, the accumulation of heavy metal concentration varies with the plant organ.

Iron concentration was complying with the standards of WHO, 425.5mg/kg because it was observed that the stream that releases water from the mining area downstream does not intersect with the wetland where the study was carried out. From the present study, iron concentration was high in chilies as compared to other crop produce and according to Sign and Kumar (2004), they noted that different vegetables and crop produce have different ability and capacity in accumulating different metals.

Zinc, Nickel and Copper concentrations did not differ and were complying with the WHO limits of 99.4, 67.9 and 73.3 mg/kg respectively. This is because some of the elements are carried downwards as some water continues to infiltrate and flow downwards. Therefore, one can be of view that heavy metal uptake by plants varies with the location where the plants are grown.

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

In this research, the levels of seven trace elements in selected food crops from a polluted wetland garden in Bindura were analyzed. The results showed that Cr, Pb, Cu, Zn, Cd, Fe and Ni were present and they were even below the World Health Organization standards. However, Nickel was below detected limit. Therefore, the results suggested that people are at no well-being risk in consuming food crops from the polluted wetland.

6.2 RECOMMENDATIONS

More trace elements other than the seven elements covered in this research must be examined. This is to offer adequate facts on trace elements found in crops grown in polluted wetlands. Elements such as Arsenic and Mercury are of greater importance since they are mainly prone to mining areas, hence further studies should look at them in particular.

Similar studies should be done to soil, water and the plant part to assess such trace elements concentration up to the crop produce. Human activities that have a habit increasing heavy metals in polluted waters must be restricted.

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APPENDICES

Appendix 1: RESEACHER'S PICTURE IN THE LABORITORY DURING EXPERIMENTS



APPENDIX 2; SPSS DATA BASE OUTPUT

```

NEW FILE.
DATASET NAME DataSet1 WINDOW=FRONT.
T-TEST
  /TESTVAL=0.45
  /MISSING=ANALYSIS
  /VARIABLES=Concentration
  /CRITERIA=CI (.95).
    
```

Notes

Output Created	28-MAR-2022 03:01:06	
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	24
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax	T-TEST /TESTVAL=0.45 /MISSING=ANALYSIS /VARIABLES=Concentration /CRITERIA=CI(.95).	
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.02

[DataSet1]

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
--	---	------	----------------	-----------------

Concentration	24	.6125	1.82863	.37327
---------------	----	-------	---------	--------

One-Sample Test

	Test Value = 0.45					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Concentration	.435	23	.667	.16250	-.6097	.9347

T-Test

```
T-TEST
  /TESTVAL=1.15
  /MISSING=ANALYSIS
  /VARIABLES=Concentration
  /CRITERIA=CI (.95).
```

T-Test

Notes

Output Created	28-MAR-2022 03:09:01	
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	24
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.

Syntax	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
		T-TEST
		/TESTVAL=1.15
		/MISSING=ANALYSIS
		/VARIABLES=Concentration
		/CRITERIA=CI(.95).
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.00

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Concentration	24	.0465	.02648	.00540

One-Sample Test

	Test Value = 1.15					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Concentration	-204.184	23	.000	-1.10354	-1.1147	-1.0924

```
T-TEST
/TESTVAL=0.365
/MISSING=ANALYSIS
/VARIABLES=Concentration
/CRITERIA=CI(.95).
```

T-Test

Notes

Output Created	28-MAR-2022 03:35:06		
Comments			
Input	Active Dataset	DataSet1	
	Filter	<none>	
	Weight	<none>	
	Split File	<none>	
	N of Rows in Working Data File	24	
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.	
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.	
Syntax	T-TEST /TESTVAL=0.365 /MISSING=ANALYSIS /VARIABLES=Concentration /CRITERIA=CI(.95).		
Resources	Processor Time	00:00:00.02	
	Elapsed Time	00:00:00.02	

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Concentration	24	.0438	.15190	.03101

One-Sample Test

	Test Value = 0.365					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Concentration	-10.360	23	.000	-.32121	-.3853	-.2571

```

T-TEST
/TESTVAL=0.365
/MISSING=ANALYSIS
/VARIABLES=concentration
/CRITERIA=CI(.95).

```

T-Test

Notes

Output Created		28-MAR-2022 03:45:27
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	24
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST /TESTVAL=0.365 /MISSING=ANALYSIS /VARIABLES=concentration /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.02

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
concentration	24	.0400	.04317	.00881

One-Sample Test

	Test Value = 0.365
--	--------------------

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
					concentration	-36.874

```
T-TEST
  /TESTVAL=0.2
  /MISSING=ANALYSIS
  /VARIABLES=concentration
  /CRITERIA=CI (.95) .
```

T-Test

Notes

Output Created		28-MAR-2022 03:47:52
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	24
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST /TESTVAL=0.2 /MISSING=ANALYSIS /VARIABLES=concentration /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.00

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
concentration	24	.0400	.04317	.00881

One-Sample Test

	Test Value = 0.2					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
concentration	-18.151	23	.000	-.15996	-.1782	-.1417

T-TEST

```

/TESTVAL=1.5
/MISSING=ANALYSIS
/VARIABLES=concentration
/CRITERIA=CI (.95) .
    
```

T-Test

Notes

Output Created		28-MAR-2022 03:52:21
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	24
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.

Syntax	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
		T-TEST
		/TESTVAL=1.5
		/MISSING=ANALYSIS
		/VARIABLES=concentration
		/CRITERIA=CI(.95).
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.02

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
concentration	24	.0442	.03387	.00691

One-Sample Test

	Test Value = 1.5					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
concentration	-210.580	23	.000	-1.45583	-1.4701	-1.4415

```
T-TEST
/TESTVAL=2.0
/MISSING=ANALYSIS
/VARIABLES=concentration
/CRITERIA=CI(.95).
```

T-Test

Notes

Output Created	28-MAR-2022 03:55:37		
Comments			
Input	Active Dataset	DataSet1	
	Filter	<none>	
	Weight	<none>	
	Split File	<none>	
	N of Rows in Working Data File	24	
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.	
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.	
Syntax	T-TEST /TESTVAL=2.0 /MISSING=ANALYSIS /VARIABLES=concentration /CRITERIA=CI(.95).		
Resources	Processor Time	00:00:00.00	
	Elapsed Time	00:00:00.00	

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
concentration	24	.0400	.14506	.02961

T-TEST

/TESTVAL=0.10

/MISSING=ANALYSIS

/VARIABLES=concentration

/CRITERIA=CI(.95).

One-Sample Test

Test Value = 2.0					
t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
				Lower	Upper

concentration	-66.192	23	.000	-1.96000	-2.0213	-1.8987
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T-Test

Notes

Output Created		28-MAR-2022 03:58:21
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	24
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST /TESTVAL=0.10 /MISSING=ANALYSIS /VARIABLES=concentration /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.02

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
concentration	24	.0913	.10568	.02157

One-Sample Test

	Test Value = 0.10
--	-------------------

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
concentration	-.406	23	.689	-.00875	-.0534	.0359

APPENDIX 2; A PICTURE SHOWING RESEARCHER IN LABORITORY