

**INVESTIGATION OF THE PRESENCE AND HEALTH RISK ASSESSMENT OF
LEAD IN VEGETABLES GROWN IN THE VICINITY OF A LEAD-ZINC MINE
KABWE, ZAMBIA.**

BY

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LUSAKA

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Declaration

I Victor Kwaambwa Kagoli declare that this dissertation is a result of my own original effort and work, and that to the best of my knowledge, the findings have never been previously presented to the University of Zambia or elsewhere for the award of any academic qualification. Where assistance was sought, it has been accordingly acknowledged.

Signature.....

Date 24th December, 2019

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I, the undersigned, certify that this dissertation is a result of the author's own work, and that to the best of my knowledge, it has not been submitted for any other academic qualification within the University of Zambia. The dissertation is acceptable in form and content, and that satisfactory knowledge of the field covered by the dissertation was demonstrated by the candidate through several reviews by the supervisors.

Name of the Supervisor: Dr John Yabe

Signature:

Date:

Abstract

Mining is one of the main sources of heavy metals that present great harm to human health even in low concentrations. Lead (Pb) contamination of food and especially of frequently consumed vegetables is a growing public health concern worldwide. Although levels of exposure in developed countries have declined over the past decades, the same cannot be said of the developing countries. Health risk assessment has increasingly been employed to determine the potential hazards of heavy metal exposure to humans. In this current study, the aim was to investigate the presence and health risk of Pb in vegetables commonly grown in the vicinity of the lead-zinc mine in Kabwe, with the objectives of assessing the Pb levels in garden soils used for growing vegetables and also analyzing Pb levels in the vegetables commonly grown on household backyard garden. Therefore Pb was measured in edible portions of vegetables (giant rape (*brassica napus*) and onion (*allium cepa*)) and soils around the residential areas (Chowa, Kasanda and Mutwewansofu) in the vicinity of the lead-zinc mine plant in Kabwe, Zambia. The results of Pb levels in the backyard soil, where the vegetables samples were randomly picked ranged from 865.7 to 58600mg/kg, all above the maximum permissible limit recommended by WHO/FAO. The potential health risk of Pb contamination to the local population via vegetable consumption was evaluated. The concentrations in edible portions of vegetables were 2.14 (0 to 9.657 mg/kg) onion (*allium cepa*) and 2.35 (0 to 9.8 mg/kg) giant rape (*brassica napus*) respectively. Approximately 71% of the Pb in the vegetables samples exceeded the maximum allowable concentrations by WHO/FAO of 2015. The calculated estimated daily intake or the estimated daily dose of Pb from both onion and giant rape were also higher than the permissible tolerable daily intake PTDI- 0.0035 mg/kg/day of Pb in both adult and children. Target hazard quotient THQ value >1 were also estimated in both children and adults, therefore indicating that, there is a health risk from consumption of these vegetables in these sampled residential areas in the vicinity of the Pb-Zn mine in Kabwe.

Dedication

I dedicate this work to my wife Faith, my children, my parents, my siblings, relatives and friends.

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Acronyms/Abbreviations

AAS – Atomic Absorption Spectrophotometer

BLL – Blood Lead Levels

CDC – Centre for Disease Control

CEP – Copper belt Environmental Project

CSO – Central Statics Office

ECZ – Environmental Council of Zambia

F.A.O – Food and Agriculture Organization

JECTA – Joint Expert Committee on Food Additives

MAC – Maximum Allowable Concentration

Pb – (Plumbum) Lead

PTDI – Permissible tolerable daily intake

US – United States

USEPA – United State Environmental Protection Agency

WHO – World Health Organization

XRF – X-Ray Fluorescent Machine

ZCCM – Zambia Consolidated Copper Mine.

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CHAPTER ONE

Introduction

1.1 Lead

Heavy metal pollution is public health concern, with health implications for human and animals. Exposure to toxic metals such as lead (Pb) can cause serious health problems (World Bank, 2003). Lead is a ubiquitous toxic metal and is understood to be one of the earliest metals discovered by the human race. Distinctive properties of Pb, such as high malleability, softness, low melting point, ductility and resistance to corrosion, have resulted in its extensive usage in different industries like automobiles, paint, ceramics, plastics, etc. This widespread use has led to a diverse rise in the occurrence of free Pb in biological systems and the inert environment. Lead has very well-known toxicological manifestation as it is regarded as a potent occupational toxin. The prime reason for lead's prolonged persistence in the environment is its non-biodegradable nature (Kalia and Flora, 2005).

As suggested by various authors, Pb may contaminate humans from various pathways including: inhalation of airborne Pb particulates, consumption of water or food contaminated by Pb, and ingestion (due to contamination of hands or other objects) of soil or dust contaminated with Pb (U.S.Department of Health,1998). Several studies have indicated that young children have an increased risk due to their greater propensity for placing non-food objects into their mouths and the vulnerability of their developing nervous system. Lead may be present on plant surfaces on account of atmosphere deposition; but its presence in internal plant tissues indicates biological uptake from the soil and leaf surface (Holmgren *et al*, 1993). Lead may be taken up in edible vegetables and fruits from the soil via the root system, by direct foliar uptake and translocation within the plant. The amount of Pb in soil that is bio-available to vegetable plants depends on factors such as cation exchange capacity, pH of the soil, amount of organic matter present and type of fertilizer added to the soil (Holmgren *et al*, 1993). However, it has been documented that mining is one of the major sources of Pb pollution (Yabe *et al.*, 2010). In Kabwe metal concentrations are highest near the mining area and decrease outward. This suggests that pollution is as a result of mining and smelting operations (Tembo, Sichilongo and Cernak, 2006). Sources of Pb in humans include soils, water, food stuff including vegetables grown in polluted

areas (Basu, Mazumdar and Goswami, 2013). Acute toxicity is related to occupational intense exposure of short duration reaching blood lead levels (BLLs) of 100–120 µg/dl. On the other hand, chronic toxicity is much more common and occurs after repeated exposure over a prolonged period, BLLs being of 40–60 µg/dl. If not treated in time, persistent vomiting, encephalopathy, lethargy, convulsions, delirium, and coma can occur (Flora et al. 2006; Pearce 2007).

1.2 Statement of the Problem

Kabwe town was the country's major producer of lead and zinc minerals, where mining and smelting facilities ran almost continuously from 1902 to 1994 without addressing the potential dangers of lead contamination.

In addition to the loss of employment and income generation opportunities following the closure of the mine in Kabwe, it left an unattended legacy of environmental pollution in certain parts of the town, especially communities immediately surrounding the mine. In trying to supplement the insufficient income and household food security, the affected communities embarked on increased gardening activities in their backyards. Consumption of vegetables grown within the household back-yards contributes to their daily food ration; the most common ones being onion (*Allium cepa*) and rape (*Brassica napus*) which is locally known as five years due to its long lifespan.

It has also been observed that due to the impact of the smelting/reprocessing of Pb tailings, the Pb content in soils in polluted areas is extremely high. Studies done in catchment areas of Kabwe recorded median Pb concentrations of 3008 mg/kg in Kasanda Township, 1233 mg/kg in Chowa Township and 1148 mg/kg in Mutwe Wansofu (Nakayama *et al*, 2010) exceeding recommended levels for residential areas (< 400 ppm). The pathways of human Pb exposure are mainly through ingestion of Pb-contaminated soil or food including vegetables, likely to cause acute and chronic intoxication. Depending on the type of plant species and different root systems, the quantity of Pb uptake, may vary considerably; The FAO/WHO maximum allowable concentration of Pb in vegetables is 0.3 to 1.0 mg/kg. Through plant uptake, Pb enters the food chain which then ends up being ingested by humans through contaminated food crops. It is argued that once ingested,

Pb accumulates in the bones of its host's skeleton and is subsequently remobilized along with the phosphates from the bones which exert a toxic effect (Nachiyunde, 2013). Exposure to Pb results in non-specific clinical conditions including abdominal pains, neurological symptoms, seizures, anemia and headache (Pearce, 2007). While there is lack of systematic data on health impacts, local health officials in the four critically contaminated catchment areas in Kabwe, reported high numbers of such clinical conditions, especially in children below 15 years (World Bank, 2016).

1.3 Justification

The route of Pb exposure in humans in Kabwe has not been fully established. The current study was undertaken to determine the risk exposure to human during their day to day consumption of leaf and tuber vegetables grown in backyard gardens. According to the (WHO/FAO), the acceptable Pb levels in residential soil and vegetables are less than 400 mg/kg and 0.3 mg/kg, respectively. Despite extensive Pb contamination in residential soils, no research has been done in Kabwe to investigate Pb exposure through consumption of locally grown vegetables and associated health risks in communities in the vicinity of the Pb-Zn mine.

The current study therefore, analyzed the Pb levels in both tubers and leaf vegetables as to determine the type of vegetables with the highest Pb levels and estimated the daily in-take of Pb by household members through consumption of these vegetables. Findings of the current study are expected to help policy makers to come up with effective remedial measures to reduce the exposure to humans.

1.6 Hypothesis

H_0 : It is hypothesized that, there is no relationship between Pb in soils and vegetables commonly grown in the vicinity of the Lead-Zinc Mine in Kabwe.

H_0 : It is hypothesized that, there is no relationship between health risk and Pb in vegetables commonly grown in the vicinity of the Lead-Zinc Mine in Kabwe

1.7 General Objectives

To investigate the presence and health risk of Pb in vegetables commonly grown in the vicinity of the Lead-Zinc Mine in Kabwe.

1.8 Specific Objectives

1. To assess the Pb levels in the garden soils used for growing vegetables.
2. To analyze Pb levels in the vegetables commonly grown in backyard gardens.

1.9 Scope of the Study

The main focus of the current study was on the categories namely; soils in backyard gardens and commonly grown vegetables i.e. (giant rape (*Brassica napus*) and Onion (*Allium cepa*)). Giant rape represented leaf vegetables and onion represented tuber vegetables.

1.10 Operational Definitions

Public Health: The art and science of preventing disease, prolonging life and promoting health through the organized efforts of society.

Risk Assessment: A method for estimating the probability or likelihood of a specified type of harm occurring to an individual or a population.

Uptake: Is the process of physically absorbing something into a living organism

Vicinity: A neighbourhood, or the area surrounding a particular place

CHAPTER TWO

LITERATURE REVIEW

2.1 Empirical Literature Review

The accumulation of toxic metals in the human food chain is recognized as a public health hazard worldwide. Metal pollution has been associated with anthropogenic activities, such as effluents and emissions from mines and smelters, that often contain elevated concentrations of toxic metals including Pb, Cd, Hg and As. As such, widespread metal contamination has frequently been reported in regions with long histories of mining, where high concentrations of metals contaminate water, soils, and vegetation. In a review by Yabe *et al.* (2010), contamination of food animals, fish, soil, water, and vegetables with Pb and Cd has reached unprecedented levels over the past decade in some parts of Africa. As a result, human exposure to toxic metals has become a major health risk (Yabe *et al.*, 2015).

Lead (Pb) is one of the most common contaminants in urban soils. Several studies have proposed health based soil Pb guidelines. For example, Madhavan *et al.* (1989) proposed a maximum permissible soil Pb concentration of 600 mg/kg assuming that the exposure would increase blood lead by 5 µg/dl, which has been set as the level of concern by CDC. Also notable is a review that proposed a guideline of <100 mg/kg based on data from several studies, with an added margin of safety, and the assumption that 10 µg/dl is safe (now known to be at odds with clinical studies). Gardening in contaminated soils can result in Pb transfer from soil to humans through vegetable consumption and unintentional direct soil ingestion (Attanayake *et al.*, 2014).

Analytical data of Pb contents in vegetables, the number of vegetables studied per group and the statistical significance between study and control group was done in Kolkata (India). The study revealed that values of Pb contents in the edible parts of ten types of vegetables showed that the maximum allowable limits for Pb were exceeded in the majority of samples. In root vegetables, especially radish and carrots, maximum allowable limits of Pb were exceeded by up to 3 times. The authors assumed that those remarkably high Pb contents of radish and carrots collected from the study area were probably related to their own physiological milieu (Basu, Mazumdar and Goswami, 2013).

The accumulation of Pb in fruits and vegetables is normally high, Vegetables such as cabbage and cauliflower Pb is high because of large portion of Pb absorbed by trees which is stored from air deposition. The Pb content in these vegetables was seen to be almost 4 times the maximum allowable limit. The higher concentrations of Pb in vegetables were attributed to their location and proximity to the highway. The vegetable samples obtained from the highway side had significantly higher ($p < 0.0001$) Pb concentration than those obtained from a local market, where the source of vegetable supply was from a region free from industrial pollution (Basu, Mazumdar and Goswami, 2013). The average content of Pb in leaves of spinach was reported to be slightly higher than the maximum allowable limit, but 2-times higher when compared with the control group. Chilly, ladies finger and tomato showed low Pb levels, suggesting that there was a resistance towards Pb accumulation. The results of the study revealed that continuous consumption of those vegetables would inevitably result in hazardous consequences (Basu, Mazumdar and Goswami, 2013).

A case study by (Orisakwe *et al.*, 2017) in Nigeria among three communities showed the diverse concentration of Pb in agricultural soils. The most Pb contaminated vegetables in Dilimi were tomatoes, cabbage and lettuce with mean concentrations of 0.83, 2.1 and 2.42 mg/kg, respectively. In Bukuru mean concentrations of 1.78 and 1.89 mg/kg for carrot and cabbage, respectively showed the highest contamination. Also in Barkin Ladi mean levels of 1.36 mg/kg (pepper) and 1.72 mg/kg (green beans) showed the highest concentration when compared with others. Leafy vegetables in Dilimi had higher concentrations than other vegetables, probably suggesting a predominance of aerosol deposition. Vegetable Pb levels in that study were similar to the findings reported in Bangladesh (Jolly, Islam and Akbar, 2013; Latif *et al.*, 2018), Pakistan (Mahmood and Malik, 2014) and Romania (Harmanescu *et al.*, 2011). The mean levels of Pb observed in vegetables were all above 0.3 - 1.0 mg/kg, the maximum allowable concentration (MAC) of Pb in vegetables (FAO-WHO, 2001).

In the case of Kabwe, the maize cob, which is a popular staple food crop consumed by Zambians was found to be easily contaminated, but not from the soluble Pb going through root system, but from atmospheric deposition (Nachiyunde *et al.*, 2013). Once it was discovered that the maize cobs were being contaminated due to atmospheric Pb pollution, it gave a better understanding as

to why ingested locally grown maize in certain areas had higher levels of Pb exposure than in other areas. It is a common practice among most households in Kabwe to grow food crops in their backyards so as to supplement their household food security, and because of this, any backyard garden exposed to atmospheric Pb pollution was likely to get contaminated (Nachiyunde *et al.*, 2013).

The toxic effects of Pb in food have been reviewed several times by the FAO/WHO Joint Expert Committee on Food Additives (JECFA). Chronic exposure to Pb at relatively low levels can result in damage to the kidneys and liver, and to the reproductive, cardiovascular, immune, hematopoietic, nervous, and gastrointestinal systems. Short-term exposure to high amounts of Pb can cause gastrointestinal distress, anemia, encephalopathy, and death. The most critical effect of low-level Pb exposure is reduced cognitive and intellectual development in children.

According to Yabe *et al.*, (2015), childhood lead poisoning is a serious public health concern worldwide. Blood lead levels exceeding 5µg/dl are considered elevated. In Kabwe, the capital of Zambia's Central Province, extensive Pb contamination of township soils in the vicinity of a Pb-Zn mine and posing a serious health risk to children has been reported. Although Pb exposure was significantly reduced through Copperbelt Environmental Project (CEP) interventions, recent sampling by Pure Earth Organization (formerly Blacksmith Institute) revealed dangerously high Pb levels. Of the 196 blood samples that were analyzed in 2014, ninety-eight percent had BLL which were above 20µ/dl. Twenty-six percent had BLL above 65µ/dl (Blacksmith Institute, 2014). This by far exceeded, the CDC set levels of 5µ/dl for children aged 1- 5years (Bennett, *et al*, 2015). Similarly, Yabe et al. (2015) recorded high BLLs in children under the age of 7 years in townships around the mine, where blood samples were collected and analyzed using an ICP-MS analyzer. All of the sampled children had BLLs exceeding 5 µg/dl. Therefore, children in those areas could be at serious risk of Pb toxicity as 18% of the sampled children in Chowa, 57% (Kasanda) and 25% (Makululu) had BLLs exceeding 65 µg dl. Eight children had BLLs exceeding 150 µg/dl with the maximum being 427.8 µg/dl. Despite such alarming revelations, Pb exposure still remains a public health problem in Kabwe and intervention should take account of environmental management focusing on the continuous process of environmental monitoring of Pb levels (Mbewe *et al.*, 2016)

CHAPTER THREE

Methods and Materials

3.1 Research Design

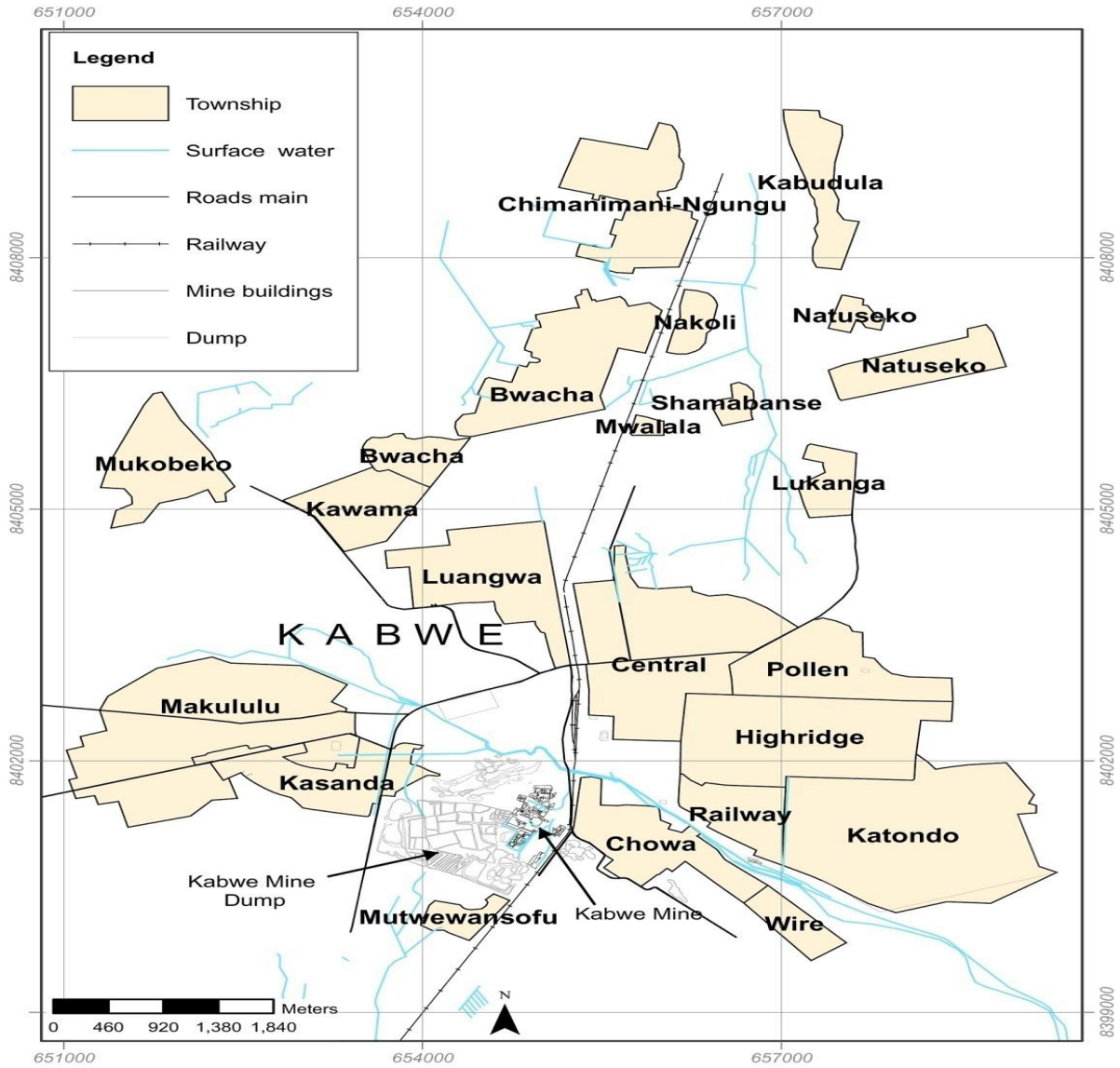
The current study was an ecological study design predominantly quantitative approach, as it brought out the current status of the Pb contamination in Kabwe Town. The use of quantitative approaches enabled the current study to investigate the presence and health risk of Pb in vegetables grown in the vicinity of the lead-zinc mine in Kabwe-Zambia, through the use of statistical inferences.

3.2 Study Area and Research population

The study was carried out in Kabwe town (Figure 4.1) particularly in Kasanda, Mutwewansofu and Chowa Townships (Figure 4.2). The townships are in the vicinity of the Pb-Zn mine with soils extensively contaminated with Pb (Nakayama *et al*, 2010). Kabwe Town is located about 130 km north of the capital city Lusaka, is the provincial headquarter of the central province which is one of the 10 other provinces in Zambia. Kabwe is the 4th largest town in Zambia with population over 200, 000. The major economic activity of Kabwe town was mining and manufacturing before closure the aligned industries.

The town was the country's major producer of Pb and Zn minerals. Mining and smelting facilities ran almost continuously from 1902 to 1994 without addressing the potential dangers of Pb contamination. This was because Kabwe's mining and mineral processing operations were undertaken during a period when statutory environmental controls within the mining sector were non-existent (MENR, 1994).

The closure of Kabwe mine brought about challenges such as scavenging of scrap metals on old dump sites, trespassing within the caving areas; children playing in the abandoned contaminated drainages without restriction, vandalism of the remaining structures of historical significance and illegal allocation of contaminated land near the mine area and along the Kabwe central canal waterway (ECZ, 2004). Currently a few companies are engaged in recycling of metals and Pb on small scale basis providing some commercial activities.



Source: ZCCM-IH. Figure 3.1, showing the major settlements in Kabwe both planned and unplanned, and the location of the former Lead- Zinc mine.

**SATELITE IMAGE SHOWING STUDY AREA OF LEAD CONTAMINATION IN THE
3 SAMPLED RESIDENTIAL AREAS**



Figure 3.2, Google Earth Map taken by the Principle Investigator, showing exactly the 3 sampled residential areas

3.4 Sample Size

A total of 102 soil and vegetables samples were collected from 14 area sections of the selected townships. These sections were divided according to the township sizes. The larger the township the bigger the sample size. Soil and vegetable samples were randomly selected in each section of the township from household's backyard gardens.

Table 3.1, Sample selection and sample size

Area Sampled	Sampled Section	Name of Sample & Number Collected			Sample Size
		Soil	Rape	Onion	
Kasanda-Mine	Section B	2	2	2	6
	Section D	2	2	2	6
	Section E	2	2	2	6
	Section F	3	3	3	9
	Section G	2	2	2	6
	Section H	2	2	2	6
	Section K	2	2	2	6
	Section M	3	3	3	9
Chowa	Section 1	3	3	3	9
	Section 2	3	3	3	9
	Section 3	3	3	3	9
	Section 4	3	3	3	9
Mutwewansofu	Section A	2	2	2	6
	Section B	2	2	2	6
Total		34	34	34	102

3.5 Sampling Procedure

In the current study the sampling procedure was based on a purposive sampling. The rationale for choosing this approach was that the study had to select households with vegetable gardens in Pb contaminated soils.

3.6 Data Collection Techniques (Instruments)

In the current study, paired vegetables, (leaf and tuber vegetables) and soil samples were collected from the backyard gardens in Chowa, Kasanda and Mutwewansofu mine townships. These were analyzed at the Food and Drugs laboratory and/ or School of Mining engineering Laboratories. The procedure involved a thorough washing of the vegetables with tap water and distilled water, then crushed using a Pb free blender into the desired size. About 5g of the crushed sample was weighed into a clean and dry crucible. It was thereafter heated at 600 C° for 6 hours (Amin *et al*, 2013). Hydrochloric acid and nitric acid were mixed in 5ml proportion each which became a yellowish solution. To clear the yellowish coloration of the solution, 20ml of hot water added, and then filtered and transferred volumetrically into 100ml flask, distilled water was added to make it up to a 100ml mark, and was agitated to mix.

The absorbance was read in the whole 100ml solution by the Atomic Absorption Spectrophotometer (AAS- Perkin elmer model- 700). In addition, soil samples from the gardens were analyzed using an X-ray Fluorescence Machine (XRF- XLT 793W) to determine the concentration of Pb in the soil.

Below was the formula used to obtain the concentration after analysis by AAS.

$$\text{Concentration mg/kg} = \frac{A - B}{W} \times 100 \%$$

A= Absorbance of sample

B= Absorbance of the blank

100= Solution volume.

W= Weight

3.7 Data Processing

The quantitative data was analyzed using the Statistical Package for Social Science (SPSS Version 21) software. Data presented in the form of frequencies, percentages, cross tabulation, and regression analysis, to establish relationships among variables. Regression analysis was done to test the significance of relationships between variables

3.8 Data Analysis

The data was analysed using quantitative approaches, which provided numerical evaluation of this current research report

3.9 Ethical Issues

The study protocol underwent ethical review.

3.10 Validity and Reliability

Validity has been defined as the degree to which a test measures what it purports to be measuring. In other words, it is the degree to which results obtained from the analysis of the data

actually represents the phenomenon under investigation (Orodho, 2004). Validation of the current study results was done using standards and also certified reference materials.

3.11 Reliability

The current study ensured reliability by standardizing the instrument. The research tools were tested using SPSS version 21 to reduce biases during analysis. This was done to determine the stability of the data collection tool.

3.12 Model Specification

3.12.1 The Level of Lead in Leaf Vegetables

In order to produce an empirical study, the current study established functional relationship for the level of Pb in leaf vegetables and Pb levels in the soil. Therefore, the functional model specification was represented mathematically as follows:

$$LLV = f(LCS \text{ and } e) \dots\dots\dots (1)$$

Where;

LLV - Level of Lead in Leaf Vegetables,

LCS - Lead-Contaminated Soil,

T – Tuber, and e - the error term.

To help establish the relationship and significance of the variables in the objectives of the current study and to also superimpose the testing of the hypothesis, the following econometric model was expressed stemming from (1) above:

$$LLV = \beta_0 + \beta_1 LCS_1 + e \dots\dots\dots (2)$$

Where;

LLV - Level of Lead in Leaf Vegetables,

LCS - Lead-Contaminated Soil and e - the error term.

β_0 = Constant term

β_1 = the coefficient of the independent variable (LCS) in the model and $\beta_1 > 0$

3.12.2 The Level of Lead in Tubers

In order to produce an empirical study, a functional relationship for the level of Pb in tubers and Pb levels in the soil was established. Therefore, the functional model specification is represented mathematically as follows:

$$LLT = f(LCS \text{ and } e) \dots\dots\dots (1) \text{ Where;}$$

LLT - Level of Lead in Tuber,

LCS - Lead-Contaminated Soil,

T – Tuber, and e - the error term.

To help establish the relationship and significance of the variables in the objectives of the current study and to also superimpose the testing of the hypothesis, the following econometric model was expressed stemming from (1) above:

$$LLT = \beta_0 + \beta_1 LCS_1 + e \dots\dots\dots (2) \text{ Where;}$$

LLT - Level of Lead in Tubers

LCS - Lead-Contaminated Soil and e - the error term.

β_0 = Constant term

β_1 = the coefficient of the independent variable (LCS) in the model and $\beta_1 > 0$

3.12.3 Health Risk of Lead

In order to produce an empirical study, a functional relationship for the Health Risk of Pb was established. Therefore, functional model specification is represented mathematically as follows:

$$\text{HRL} = f(\text{GVLPV}, e) \dots\dots\dots (3) \text{ Where;}$$

HRL - Health Risks of Lead,

GVLPV - Growing of Vegetables in Lead Polluted Vicinities and e - the error term.

To help establish the relationship and significance of the variables in the objectives of the current study and to also superimpose the testing of the hypothesis, the following econometric model was expressed stemming from (1) above:

$$\text{HRL} = \beta_0 + \beta_1 \text{GVLPV} + e \dots\dots\dots (4) \text{ Where;}$$

HRL - Health Risks of Lead,

GVLPV - Growing of Vegetables in Lead Polluted Vicinities and e - the error term.

β_0 = Constant term

β_1 = the coefficient of the independent variable (GVLPV) in the model and $\beta_1 > 0$

3.13 Health risk assessment calculation

In order to evaluate the daily or long-term potential health risk of hazardous exposure to (Pb) via consumption of vegetables grown in Chowa, Kasanda-Mine and Mutwewansofu mining communities, the Estimated Daily Dose (EDD) and Target Hazard Quotient (THQ), which are models developed by the US EPA to assess risk of exposure to contaminants were calculated. If THQ value is > 1 , there could be a potential health risk associated with the pollutant. On the other hand, if THQ value is < 1 , then, there is no obvious risk.

3.13.1 Estimated Daily Dose (EDD)

Daily intake of Pb was calculated using the equation below;

$$EDD = \frac{C_{metal} \times IR \times EF \times ED}{BW_{average\ weight} \times AT_n}$$

Where;

C is the mean concentration of Pb in vegetable

IR is the daily vegetable intake by the exposed population: 350g in adults and 220g in children (Song *et al*, 2015).

EF is the exposure frequency- 365 days (USEPA, 2011).

ED is the exposure duration of 61.8 years as the average life expectancy rate for a Zambian Adult according to World Health Organisation (WHO) statistics 2015 (www.worldlifeexpectancy.com/zambia-life-expectancy, 27 November 2017).

BW is the average weight of local residents 60kg for adults and 16kg for children,

AT is the average exposure time for non-carcinogens (exposure days within whole lifetime)
22,557 days = 365 x 61.8

3.13.2 Target Hazard Quotient (THQ)

The Target hazard quotient was calculated using the following equation;

$$THQ = \frac{EDD}{RFD}$$

Where;

RFD is the chronic oral reference dose for Pb 0.0035 mg/ kg/day (USEPA, 2003).

3.13.3 Bio-Concentration Factor (BCF) calculation

Soil to plant metal transfer factor was calculated as the ratio of Pb concentration in plants to Pb concentration in soils. The BCF was calculated by using the following equation as follows;

$$\text{BCF} = \frac{C_{\text{vegetables}}}{C_{\text{soil}}}$$

Where C vegetable is the total concentration of Pb in the vegetable (mg/ kg dw), and C soil is the corresponding heavy metal concentration in the soil habitat of the vegetable (mg/ kg).

3.14 Identification of Variables

Equation 2

- Independent Variable – Lead-Contaminated Soil, and e - the error term.
- Dependent Variable – Sources of Pb in Vegetables.

Equation 4

- Independent Variable – Growing of Vegetables in Pb polluted vicinities.
- Dependent Variable – Health Risks of Pb

CHAPTER FOUR

Results

The content of Pb varied ranging from 865.7 – 586000 mg/kg soil, 0 - 9.657 mg/kg onion and 0 – 9.84 mg/kg giant rape.

Table 4.2: Pb concentration <0.1 & 0.3 mg/kg and >0.1 & 0.3 mg/kg

	Pb < 0.1 & 0.3 mg/kg	Pb >0.1 & 0.3 mg/kg
Onion	11	23
Giant Rape	9	25
Total	20	48
Percentage	29%	71%

FAO/WHO- 2015 Standards (MAC).....0.3 mg/kg leaf vegetables and 0.1 mg/kg root/tuber

EU (MAC)0.3 mg/kg

Findings from the current study revealed mean concentration of Pb in soil of 6155.43 mg/kg indicating that concentrations of Pb in soil were higher than the maximum allowable limit of 600 mg/kg of Pb by WHO/FAO. The mean concentration of Pb in onion and rape were found to be 2.14 mg/kg and 2.35 mg/kg, respectively. These concentrations in onion and rape were higher than the WHO/FAO (2015) maximum allowable limit of 0.3 mg/kg for leaf vegetables and 0.1mg/kg for root/tuber vegetables. This implies that, the Pb levels in the vegetables commonly grown in backyard gardens posed a health.

4.2 Regression Analysis

4.2.1 Regression Analysis for Onion

Table 4.3 ANOVA^a

Model	Sum of Squares	df	Mean Square	F	Sig.
1 Regression	25.926	1	25.926	4.296	.046 ^b
Residual	193.134	32	6.035		
Total	219.060	33			

a. Dependent Variable: Conc (Pb) for onion (mg/kg)

b. Predictors: (Constant), Results from XRF soil (mg/kg)

Table 4.4: Model Summary of the effect of Soil Pb on Concentration of Pb in Onions

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.344 ^a	.118	.091	2.457

a. Predictors: (Constant), Results from XRF soil (mg/kg)

b. Dependent Variable: Conc (Pb) for onion (mg/kg).

Table 4.5 Coefficients^a

Model		Unstandardized Coefficients		Standardized	t	Sig.
		B	Std. Error	Coefficients		
1	(Constant)	1.739	.464		3.745	.001
	Results from XRF soil (mg/kg)	6.563E-05	.000	.344	2.073	.046

a. Dependent Variable: Conc (Pb) for onion (mg/kg)

4.2.1.1 Constant Interpretation

The intercept value of 1.739 was found and it meant that if the influence of soil was held constant, the concentration of Pb in onion will be 1.739.

4.2.1.2 Coefficients Interpretation

4.2.1.2.1 The Influence of Pb Concentration in Soil

The coefficient of 6.563E-05 was found as a partial regression coefficient of Pb concentration in soil and indicates, if the Pb in soil increases by 1%, then Pb in onion would increase by 656%. This meant that, soil played an essential role in the concentration of Pb in onion.

4.2.1.3 Coefficient of Determination R-Squared (r^2)

The coefficient of variation for this econometric model was found to be 0.118. This implied that 11.8 percent of the disparity of Pb concentration in onion was attributed to the variation of Pb concentration in soil and the remaining 88.2 % of the variables in the model were not explained.

4.2.1.4 F-test and its significance

The F-test showed that the relation of the disparity in the regression to the ratio of the variation of the residual or errors was found as 4.296. This value found was greater than the tabulated F-

value of 0.046 at 0.05 level of significance. This implied that the econometric model was of good fit.

Table 4.6: Mean Concentration of Pb in Onion (Tuber Vegetables)

Residuals Statistics					
	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	0	10	2.14	.581	34
Residual	-3.047	7.252	.000	2.510	34
Std. Predicted Value	-1.657	1.657	.000	1.000	34
Std. Residual	-1.195	2.845	.000	.985	34

a. Dependent Variable: Conc (Pb) in onion (mg/kg)

The mean concentration of Pb in onion was found to be 2.14 mg/kg and this meant that, the concentration of Pb in onion was very higher than the maximum allowable limit of 0.1 mg/kg by WHO/FAO- 2015.

4.2.2 Regression Analysis for Rape

Table 4.7: The Effect of Pb in soil on the Concentration of Pb in Rape (Leaf Vegetable)

Model Summary^b				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.498 ^a	.248	.224	2.481

a. Predictors: (Constant), Results from XRF soil (mg/kg)

b. Dependent Variable: Conc (Pb) for rape (mg/kg)

Table 4.8: ANOVA^a

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	64.860	1	64.860	10.541	.003 ^b
	Residual	196.901	32	6.153		
	Total	261.760	33			

a. Dependent Variable: Conc (Pb) for rape (mg/kg)

b. Predictors: (Constant), Results from XRF soil (mg/kg).

Table 4.9: Coefficients

Model		Unstandardized Coefficients		Standardized	t	Sig.
		B	Std. Error	Coefficients		
1	(Constant)	1.707	.469	Beta	3.642	.001
	Results from XRF soil (mg/kg)	.000	.000	.498	3.247	.003

a. Dependent Variable: Conc (Pb) for rape (mg/kg)

2.2.1 Constant Interpretation

The intercept value of 1.707 was found indicating that if the influence of soil was held constant, the concentration of Pb in rape will be 1.707.

4.2.2.2 Coefficients Interpretation

4.2.2.2.1 The Influence of Pb Concentration in Soil

The coefficient of 0.000 was found as a partial regression coefficient of Pb concentration in soil indicating that: if the Pb in soil increased by 1 %, there would be no Pb increase in rape. This meant that, soil did not have an essential role in concentration of Pb in rape.

4.2.2.3 Coefficient of Determination R-Squared (r^2)

The coefficient of variation for this econometric model was found to be 0.248. This implied that 24.8 % of the disparity of Pb concentration in rape was attributed to the variation of Pb concentration in soil and the remaining 75.2 % of the variables in the model were not explained.

4.2.2.4 F-test and its significance

The F-test shows that the relation of the disparity in the regression to the ratio of the variation of the residual or errors was found as 10.54. This value found was greater than the tabulated F-value of 0.003 at 0.05 level of significance. This implied that the econometric model was of good fit.

Table 4.10: Mean Concentration of Pb in Rape (Leaf vegetables)

Residuals Statistics^a					
	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	0	10	2.35	.640	34
Residual	-2.507	7.719	.000	2.743	34
Std. Predicted Value	-1.657	1.657	.000	1.000	34
Std. Residual	-.900	2.771	.000	.985	34

a. Dependent Variable: Conc (Pb) for rape (mg/kg)

The mean concentration of Pb in rape was found to be 2.35 mg/kg indicating that, the concentration of Pb in rape was higher than the maximum allowable limit of 0.3 mg/kg by WHO/FAO-2015 and EU.

4.3 Correlation Analysis

4.3.1 Correlation Analysis for Vegetables

Table 4.11: Correlation between Soil and the Vegetables (Leaf and Tuber Vegetables)

Correlations				
		Results from XRF soil (mg/kg)	Conc (Pb) for onion (mg/kg)	Conc (Pb) for rape (mg/kg)
Results from XRF soil (mg/kg)	Pearson Correlation	1	.344 [*]	.498 ^{**}
	Sig. (2-tailed)		.046	.003
	N	34	34	34
Conc (Pb) for onion (mg/kg)	Pearson Correlation	.344 [*]	1	.261
	Sig. (2-tailed)	.046		.136
	N	34	34	34
Conc (Pb) for rape (mg/kg)	Pearson Correlation	.498 ^{**}	.261	1
	Sig. (2-tailed)	.003	.136	
	N	34	34	34

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.01 level (2-tailed)

Findings in table 4.1.1 shows that, there was a strong positive correlation between soil and the concentration of Pb in rape with Pearson correlation of 49.8 % as compared to the concentration of Pb in onion that had a Pearson correlation of 34.4%.

Table 4.12: Correlation between Sampling Areas and the Vegetables (Leaf and Tuber Vegetables)

		Conc (Pb) for onion (mg/kg)	Conc (Pb) for rape (mg/kg)	Sampling Area
Conc (Pb) for onion (mg/kg)	Pearson Correlation	1	.261	.249
	Sig. (2-tailed)		.136	.155
	N	34	34	34
Conc (Pb) for rape (mg/kg)	Pearson Correlation	.261	1	.241
	Sig. (2-tailed)	.136		.170
	N	34	34	34
Sampling Area	Pearson Correlation	.249	.241	1
	Sig. (2-tailed)	.155	.170	
	N	34	34	34

Findings in table 4.1.2 shows that, there is a strong positive correlation between Sampling area and the concentration of Pb in onion with Pearson correlation of 24.9 % as compared to the concentration of Pb in rape which had a Pearson correlation of 24.1%.

4.4 Health Risk Assessment for Rape

$$EDD = \frac{C_{metal} \times IR \times EF \times ED}{BW_{average\ weight} \times AT_n}$$

$$\text{Rape EDD (Adults)} = \frac{2.35\ mg/kg \times 3500\ mg \times 1 \times \left(\frac{1}{365\ day}\right)}{60kg \times 1\ days}$$

$$= \underline{\underline{0.375571}}$$

$$\text{Rape EDD (Children)} = \frac{2.35\ mg/kg \times 2200\ mg \times 1 \times \left(\frac{1}{365\ day}\right)}{16kg \times 1\ days}$$

$$= \underline{\underline{0.885274}}$$

Estimated Daily Dosage (EDD), is the model developed by the USEPA to assess risk of exposure to contaminants. Findings in the current study revealed that EDD values were > 0.0035 mg/kg/day, indicating that, there could be a potential health risk of Pb exposure from rape consumption in both adults and children. This was in relation to the chronic oral reference dose for Pb of 0.0035 mg/kg/day (USEPA, 2003).

4.5 Health Risk Assessment for Onion

$$EDD = \frac{C_{metal} \times IR \times EF \times ED}{BW_{average\ weight} \times AT_n}$$

$$\begin{aligned} \text{Rape EDD (Adults)} &= \frac{2.14\ mg/kg \times 3500\ mg \times 1 \times \left(\frac{1}{365\ day}\right)}{60kg \times 1\ days} \\ &= \underline{\underline{0.342009}} \end{aligned}$$

$$\begin{aligned} \text{Rape EDD (Children)} &= \frac{2.14\ mg/kg \times 2200\ mg \times 1 \times \left(\frac{1}{365\ day}\right)}{16kg \times 1\ days} \\ &= \underline{\underline{0.806164}} \end{aligned}$$

Estimated Daily Dosage (EDD) is the model developed by the USEPA to assess risk of exposure to contaminants. From the results obtained in the current study, EDD values > 0.0035 mg/kg/day were recorded, indicating that, there could be a potential health risk of Pb exposure from onion consumption in both adults and children. This was in relation to the chronic oral reference dose for Pb of 0.0035 mg/kg/day (USEPA, 2003).

4.6 Target Hazard Quotient (THQ)

$$THQ = \frac{EDD}{RFD}$$

$$\text{Rape THQ (Adult)} = \frac{0.375571}{0.0035\ mg/kg/day} = \underline{\underline{107.306}}$$

$$\text{Rape THQ (Children)} = \frac{0.885274}{0.0035 \text{ mg/kg/day}} = \underline{\underline{252.9354}}$$

$$\text{Onion THQ (Adult)} = \frac{0.342009}{0.0035 \text{ mg/kg/day}} = \underline{\underline{97.71686}}$$

$$\text{Onion THQ (Children)} = \frac{0.806164}{0.0035 \text{ mg/kg/day}} = \underline{\underline{230.3326}}$$

Target Hazard Quotient (THQ), is the model developed by the US EPA to assess risk of exposure to contaminants. Results obtained from the current study, revealed THQ values > 1, indicating that, there could be a potential health risk of Pb contamination in both adults and children.

4.7 Bio-Concentration Factor (BCF) calculation

$$\text{BCF} = \frac{C_{\text{vegetables}}}{C_{\text{soil}}}$$

$$\text{Rape BCF} = \frac{2.35}{17.50} = \underline{\underline{0.1342857143}}$$

$$\text{Onion BCF} = \frac{2.14}{17.50} = \underline{\underline{0.1222857143}}$$

Findings from the calculation above shows that, rape and onion were slightly contaminated with Pb, given that, the Rape BCF and Onion BCF values ranged between 0.10 - 0.25. The conclusion was based on data from the Department of Petroleum Resource (DPR, 2002) as the permissible level of bioavailable Pb in Nigeria soil, since Zambia has no permissible levels of bioavailable Pb in soil.

Table 4.13: Mean Concentration of Pb in Soil

Descriptive Statistics						
	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Results from XRF soil (mg/kg)	34	866	58600	6155.4	13505.466	182397609.8
Valid N (listwise)	34					

The mean concentration (6155.43 mg/kg) of Pb in soil in the current study was higher than the maximum allowable limit of 600 mg/kg of Pb by WHO/FAO.

4.8 Hypothesis

H_0 : It was hypothesized that, there was no relationship between health risks and Pb in vegetables commonly grown in the vicinity of the Lead-Zinc Mine in Kabwe.

4.9 Hypothesis testing

The mean concentration of Pb in onion was found to be 2.14 mg/kg, which is higher than the maximum allowable limit of 0.1 mg/kg in root/tuber by WHO/FAO-2015. Therefore, the study rejected the null-hypothesis and accepted the alternative one, which stated that, there was a relationship between Pb in onion which is commonly grown in the vicinity of the Pb-Zn Mine in Kabwe.

The mean concentration of Pb in giant rape was found to be 2.35 mg/kg, which is also higher than the maximum allowable limit of 0.3 mg/kg in leaf vegetables by WHO/FAO-2015. Therefore, the study rejects the null-hypothesis and accepts the alternative one, which stated that, there was Pb contamination in giant rape which was commonly grown in the vicinity of the Pb-Zn Mine in Kabwe. The study further rejected the null- hypothesis and accepted the alternative one, which stated that there was a relationship between health risk and Pb in vegetables commonly grown in the vicinity of the Pb- Zn mine in Kabwe, because the THQ and the EDD showed that there was a potential health risk of consuming vegetables grown in those three areas. The results of the study were in line with result found by Komex International Ltd; ZCCM Investment Holdings PLC (2014), which found that the soil Pb levels remained relatively high because the residential areas were built on what used to be the mine's waste rock and were nearer to the mine area with mean concentration of 6155.43 mg/kg which was higher than the maximum allowable limit of 600 mg/kg of lead by WHO/FAO.

CHAPTER FIVE

Discussion

This current study demonstrates that Pb pollution in soil in the vicinity of the Pb-Zn mine in Kabwe is significantly high, ranging from (865.7 – 58600 mg/kg) with a mean concentration of 6155.43 mg/kg. This indicates that concentrations of Pb in soil were higher than the maximum allowable limit of 600 mg/kg of Pb by WHO/FAO guidelines, and the same limit value proposed by Madhavan *et al.* (1989) as a maximum permissible soil Pb concentration. The permissible level of 600 mg/kg is said to provide for a "safe" level that would contribute to not more than 5 µg /dl to total blood Pb of children less than 12 years of age and therefore providing a reasonable certainty that the adverse health effects will not occur (USEPA, 2003). However, the study showed that all the soil Pb levels were above the permissible level as stated above, thereby reasonably ascertaining the adverse health effect. Similar reports of significant high levels of Pb in the soil in these areas in the vicinity of the Pb-Zn mine was documented by Nakayama *et al* (2010) and Tembo *et al* (2006).

Results from the current study further agrees with the assertion made by Reilly *et al* (2018) that, “in certain housing areas of Kabwe, the recommended tolerable soil Pb levels of 400 mg/kg is clearly exceeded”. According to the data in the current study spread in appendix 2 and table 3.1, Kasanda area has the highest soil pollution levels followed by Chowa and then Mutwewansofu, which results are in line with findings by Komex international ltd; ZCCM Investment Holding PLC (ZCCM-IH), (2014) and therefore this study agrees with its assertion that, “much of the Pb in the soil found in the three residential areas being focused on, mainly originated from the smelter stack, the mine used to emit it`s pollutants into the atmosphere and through the canal flooding in a case of Chowa”. ZCCM-IH, further reported that “Kasanda area was not only experiencing contamination from the emissions settling on the ground, but more worrying, also from foundation on which the township was built, which used to be the mine waste rock. It therefore resonates well with the findings of this current study that found the highest Pb sample in Kasanda of 58600 mg/kg.

The current study has revealed that, 71 % of the vegetables samples were above the MAC and the mean concentration of Pb in onion and rape were found to be 2.14 mg/kg and 2.35 mg/kg, respectively. These concentrations in onion and rape were higher than the WHO/FAO (2015) maximum allowable limit of 0.3 mg/kg for leaf vegetables and 0.1 mg/kg for root/tuber vegetables. This implied that, the Pb levels in the vegetables commonly grown in backyard gardens posed a health hazard to the consumers. Using the results of this current study, Pb in vegetables and Pb in soil, a simple regression model was developed to find out the effect of Pb in soil on the concentration of Pb in vegetables grown in the vicinity of Pb- Zn mine areas in Kabwe.

The findings revealed that Pb in soil from the sampled areas had influence on the concentration of Pb in vegetables. Similar studies conducted by Chen *et al* (2013) in China (in vegetables grown around battery production area) and Orisakwe *et al* (2017) in Nigeria (in vegetables from Artisanal mining site of Dilimi, Bukuru and Barkin Ladi, north central Nigeria), showed that heavy metals in soil including Pb had influence in the concentration of these heavy metals including Pb in the vegetables grown in the same contaminated soils.

However, in the current study more influence was observed in tuber vegetables (onion) than in leaf vegetables (giant rape) having coefficient values of 6.563 and 0.000 respectively. This indicated that the edible part of onion absorbs or takes up Pb more easily than the edible part of giant rape. The reasons of this difference in uptake of Pb by these edible parts of the vegetables may have many scientific explanations that need to be evaluated in future studies. Some assumed explanations could be that, the giant rape may have developed a failure of Pb up-take in soil due to high levels of phosphorus in the stem that inhibit the uptake of Pb (Brown *et al*, 2003). The other possibility could be that giant rape stores much Pb in the stem, which was not included in the analysis as it is not an edible part of the vegetable. Findings in the current study were interesting as the mean value of Pb in leaf (giant rape- 2.35 mg/kg) was higher compared to tubers (onion- 2.14 mg/kg), in contrast to the higher influence of soil Pb in tuber than leafy vegetables. Furthermore the current study revealed that there was a strong positive correlation between Pb concentration in soil and in giant rape with a Pearson correlation of 49.8 % as compared to the concentration of Pb in onion and soil which had a Pearson correlation of 34.4 %.

This paradox justifies the possibility of other factors or mediums that influence levels of Pb in leaf than in tubers and these could be atmospheric Pb or dust particles (Wander, 2016).

The health risk of Pb in vegetables was evaluated using the Estimated Daily Dosage (EDD) or estimated daily intake, Target Hazard Quotient (THQ) and Bio-Concentration Factor (BCF) calculations.

Findings from the current study revealed that EDD values were > 0.0035 mg/kg/day (US EPA, 2003), the permissible tolerable daily intake, and it can therefore be concluded that, there could be a potential health risk of Pb in rape and onion in human consumers. THQ values were > 1 , thereby indicating that there could be a potential health risk of Pb in both adults and children. The calculation on BCF shows that, rape and onion were slightly contaminated with Pb, because, the rape BCF and onion BCF values ranged between 0.10 - 0.25. The conclusion was reached through the usage of data from the Department of Petroleum Resource (DPR, 2002) as the permissible level of Pb in Nigeria soil, since Zambia did not have permissible levels of Pb in soil.

CHAPTER SIX

Conclusion and Recommendation

6.1 Conclusion

1. Pb levels in the samples of soil from the three residential areas exceeded the maximum allowable concentration (MAC) for both residential and agriculture criteria, set by WHO/FAO and USEPA, therefore are considered potential health risk to the public.

2. Most of the edible portions of the vegetables sampled had Pb concentration above the maximum allowable concentration, thereby posing a potential health risk to the local population via vegetables consumption.

However, the uptake of Pb through the root system was significantly low going by the results of the bio-concentration factor calculation, which indicated that both giant rape and onion were slightly contaminated and also the simple linear regression scatter plot that showed that the soil Pb contributed to the Pb content in vegetables, however, there could be other factors that contribute to the levels of Pb in vegetables than does the soil Pb.

6.2 Recommendations

For the purpose of phytoremediation, more studies must be done to determine the soil chemistry i.e. cation exchange capacity, pH of the soil, amount of organic matter present and type of fertilizer applied to the soil in backyard gardens without which the plants that will be identified for phytoremediation would fail to absorb the Pb, because it could not be bioavailable.

Following the results in giant rape and onion that showed more Pb content in giant rape and yet more absorption through root system in onion, there is need to investigate factors that facilitated more uptake of Pb in onion than in giant rape, and further investigation of the presence and levels of phosphorus in giant rape, because phosphorous inhibits the bioavailability or absorption of Pb. However, it should be noted that giant rape has more content of Pb, which implies that there other source of contaminations other than soil which sources must be investigated and established.

The current study has revealed that there is potential health risk of Pb exposure via vegetable (giant rape and onion) consumption to the local population in the vicinity of the Pb-Zn Mine in Kabwe. It is therefore recommend to the policy makers and other key stakeholders to ensure that no vegetables are grown for consumption from these areas till proper remedial measure are provided.

7.0 References

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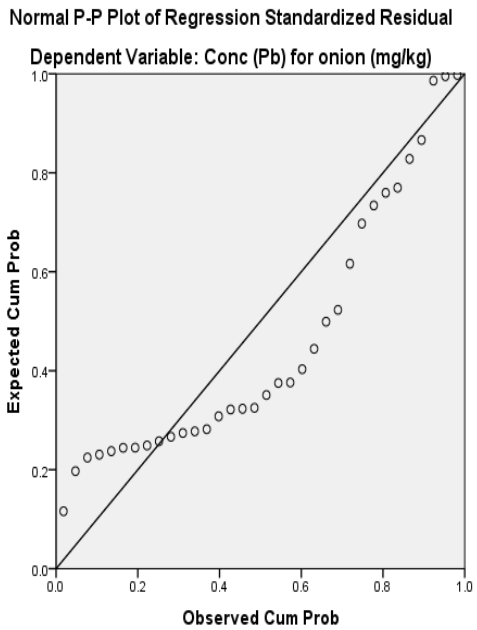
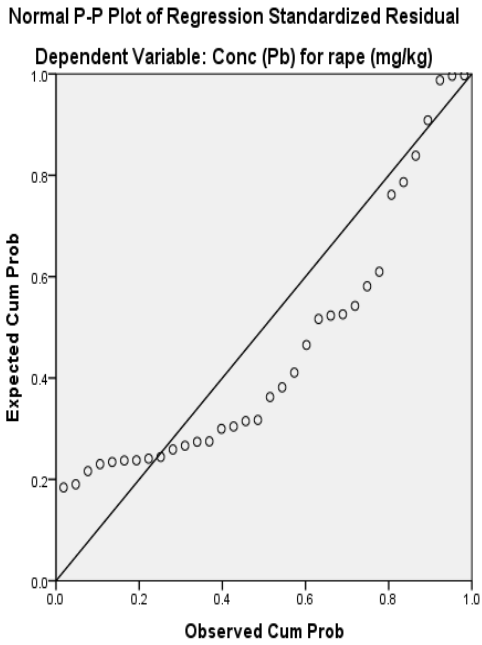
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Appendix 1: Simple linear regression scatter plots for giant rape and onion Pb concentration through root uptake.



Appendix 2: XRF and AAS analysis results

S/N	Sample : No	Result from XRF	Results from AAS						
			Soil (mg/kg)	Onion			Rape		
				Weight (g)	Absorbance	Conc (mg/kg)	Weight (g)	Absorbance	Conc (mg/kg)
1	1	1432	0.935	0.0119	0	6.4065	0.0461	0.447	
2	2	1879	4.9553	0.0389	0.434	6,0269	0.0397	0.37	
3	3	1793	1.6937	0.0522	2.050	2.4800	0.0716	2.19	
4	4	5406	3.0979	0.0592	1.350	5.4723	0.0639	0.85	
5	5	2883	1.6900	0.0717	3.210	1.4733	0.0366	1.30	
6	6	1516	4.8634	0.2376	0	3.1015	0.8795	9.08	
7	7	1551	7.5323	0.149	0	3.7877	0.3029	0	
8	8	1427	4.1647	0.3392	0	6.039	0.45	0	
9	9	1337	5.2725	0.6333	0.67	4.0241	0.5861	0	
10	10	2343	4.0336	0.7199	3.022	12.1823	0.8396	1.98	
11	11	8961	2.4632	0.4019	0	9.2804	0.3556	0	
12	12	1511	7.0010	0.0358	0	9.9829	0.5763	0	
13	13	1156	5.1895	0.5291	0	7.097	0.1947	0	
14	14	58600	4.5725	0.9433	7.55	1,4435	0.74	9.84	
15	15	1171	4.7761	0.6764	1.64	8.0169	0.5291	0	
16	16	1553	18.7155	0.7515	1.43	3.2763	0.5628	2.43	
17	17	3825	6.1804	0.6227	2.26	15.881	0.6405	0.99	
18	18	3646	11.033	0.1074	0	18.626	1.339	4.59	
19	19	3025	7.22	0.56	1.06	4.50	0.43	0	
20	20	4569	16.552	1.264	4.702	5.2672	0.4203	0	
21	21		3.3894	0.4780	0	4.501	0.578	6.28	
22	22	4521	9.1586	1.18	9.657	9.987	0.833	5.39	
23	23	1681	2.70	0.5414	9.1	17.407	0.4233	0.74	
24	24	1742	11.2398	0.434	1.242	13.4645	0.443	1.099	
25	25	5369	16.346	0.4458	0.92	12.500	0.3998	0.84	
26	26	2725	8.983	0.593	4.523	13.184	0.376	1.437	
27	27	2072	11.6465	0.314	1.558	3.9618	0.4999	9.21	
28	28	2799	2.6872	0.1616	0.99	4.5136	0.2972	3.59	
29	29	1714	9.0158	0.696	5.645	7.2748	0.2693	1.067	
30	30	2035	5.8317	0.3073	2.06	7.108	0.5515	5.13	
31	31	2304				15.8647	0.7444	3.51	
32	32	865.7	20.188	0.4416	1.26	13.0639	0.6367	3.44	
33	33	12000	6.2439	0.0917	0	6.44	0.2642	2.00	
34	34	1273	4.99	0.0823	1.98	9.902	1.98	1.98	

Appendix 3: Blank numbers used to calculate concentration (mg/kg), after AAS results

S/N	Samples	Blank used for calculation
1	1 to 5	0.0174
2	6 to 15	0.5980
3	16 to 20	0.4831
4	21 to 25	0.295
5	26,29,30,,32 (31- 0.00)	0.1869
6	33,27,28	0.135