BIOAVAILABILITY OF HEAVY METALS IN SOILS THAT ARE IN CLOSE PROXIMITY TO MINE TAILINGS IN COPPERBELT, ZAMBIA

By
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DECLARATION

I, Belinda K. Kaninga, hereby declare that all the work presented in this thesis is my own original work and has not been submitted for a degree award in any other university.

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CERTIFICATE OF APPROVAL

This thesis of **Belinda K. Kaninga** was approved as fulfilling the requirements for the award of the degree of **Doctor of Philosophy in Soil Science** by the University of Zambia.

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ABSTRACT

Mine tailings are a significant source of heavy metal contamination for soils in their proximity. In Zambia's Copperbelt Province, historical and current mining operations have resulted in large outputs of tailings. Food crops grown on contaminated soils may be a conduit for transfer of heavy metals to the food chain. Current soil assessments of risk are based on total soil concentrations and yet only the bioavailable fractions are potentially taken up by plants. This study aimed to evaluate the effect of lime and manure amendments on the bioavailability of heavy metals in contaminated soils near mine tailings, and their uptake by maize and pumpkin. A field experiment was conducted in an agricultural settlement area around a large mine tailings dam in Kitwe Zambia and a complementary pot experiment with soil from the study area was conducted in the glass house at Zambia Agriculture Research Institute. Plots located within 200 m and others between 300-400 m away from the tailings dam were arranged in a Randomised Complete Block Design. The treatments of i) lime, ii) manure, iii) lime and manure, iv) control with no amendment and replicated four times were assigned to the plots. Lime was applied at 2 ton ha^{-1} and manure at 5 ton ha^{-1} at the onset of the study, and data on bioavailable fractions of Cadmium, Copper, Nickel, Lead and Zinc were collected over two cropping seasons (2016-17 and 2017-18). Bioavailable Cd, Cu, Ni, Pb and Zn in the soil were determined by extraction with DTPA and 0.01 M $Ca(NO_3)_2$ before, and after soil was amended, and all elemental determinations were done by ICP-MS. Total elemental concentrations in crops were determined by ICP-MS following microwave-assisted acid digestion.. Data were analysed with a linear mixed model to account for non-homogeneity as a result of differences in plots within a field, differences between fields and the hierarchical nature of the study where repeated measurements were taken in the same plots over 2 seasons. For the pot experiment, chinese cabbage was planted as a test crop and data were used for validation of the Windermere Humic Aqueous Model seven (WHAM VII). The soils were highly contaminated with Cu but not Cd, Ni, Pb and Zn. When compared to unamended plots, lime application with/ or without manure reduced bioavailable Cd, Cu, Ni, Pb and Zn. The response to lime was greater in soils with an initially acidic pH than in those with approximately neutral pH values. Manure reduced DTPA extracted Cd but increased Pb. In alkaline conditions manure increased soluble Cu and Pb. On amended soils only the concentration of Cu and Pb in pumpkin

leaves were above the FAO /WHO safe limit. In maize grain all the five heavy metals were below this limit. The accumulation of heavy metals in crops corresponded with the bioavailable fraction of the heavy metals in soil. The soils and subsequently the crops were more highly contaminated with Cu than other elements, therefore only the results of cu were used to validate the model. The best prediction of Cu concentration in the soil solution was with DTPA extracted Cu concentration as an input to WHAM VII. Free ion Cu was the most predominant species at pH below 5. Above pH 5.5, Cu speciation in solution was dominated by colloidal fulvic acid. Fulvic and Humic acids were the dominant solid sorption phases, with humic acids dominating more at pH<6. Iron oxides were dominant at around neutral pH. The model's prediction of soil solution Cu was overestimated at higher pH and particulate Fe and Al oxides concentrations, and lower particulate humic acid content. The concentration of Cu in the Chinese Cabbage did not corelate with either the measured or the predicted soil solution concentrations. The study adds new understanding of the soil factors driving bioavailability of Cu in tropical soils. These findings can be applied to devising modelling approaches for predicting crop uptake from soils.

DEDICATION

To my girls Bwalya, Mwaba, and boys Mwiza, Siulapwa,
you guys are the best!

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ABBREVIATIONS AND ACRONYMS

AMD	Acid Mine Drainage
ANOVA	Analysis of Variance
ASV	Anodic Stripping Voltammetry
ATSDR	Agency for Toxic Substances and Disease Registry
BGS	British Geological Survey
CCME	Canadian Council of Ministers on the Environment
CEC	Cation Exchange Capacity
CRM	Certified Reference Material
DCB	Dithionite-Citrate-Bicarbonate
DGT	Diffuse Gradient in Thin film
DMT	Donnan Membrane Technique
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DTPA	Diethylenetriamine pentaacetate
EDTA	Ethylenetriamine pentaacetate
FA	Fulvic Acid
FAAS	Flame Atomic Absorption Spectrophotometer
FAO	Food and agriculture Organisation
HA	Humic Acid
ICP-AES	Inductively Coupled Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission spectrometry
ICP-QQQ-MS	Triple quadruple Inductively Coupled Plasma Mass spectrometry
IMO	International Maritime Organization
LOI	Loss on Ignition
MMCR	Mount Makulu Central Research
MP-AES	Microwave Plasma Atomic Emission Spectroscopy
NMR	Nuclear Magnetic Resonance
PHE	Potentially Harmful Element
RCBD	Randomised Complete Block Design
REML	Residual Maximum Likelihood
SEM	Scanning Electron Microscopy
SGV	Soil Guideline Value
SOM	Soil Organic Matter
TEA	Triethanolamine
TENORM	Technologically Enhanced Naturally-Occurring Radioactive Material
UHMI	Ultra High Matrix Introduction
UNEP	United Nations Environment Programme
US EPA	United States Environmental Protection Agency
USDA	United States Department of Agriculture
WHAM	Windermere Humic Aqueous Model
WHO	World Health Organisation
XAFS	x-ray absorption fine structure
XRD	X-ray diffraction
ZARI	Zambia Agriculture Research Institute

CHAPTER ONE

1. INTRODUCTION

1.1. Background

The term 'heavy metals' has been defined by many authors based on high density (Järup 2003; Tchounwou *et al.*, 2012; Helmenstine 2014), atomic weight (Duffus and Templeton 2002; Thornton 1995; Tchounwou *et al.*, 2012), and atomic number (Bennet 1986). Appenroth (2010) opposed the definitions based on physical properties as heavy metals occur in nature as compounds and not pure elements, therefore, the characteristics of their pure elements would have no bearing on the physiological effects of their compounds. Nevertheless, the composition of this group of elements is fairly well-understood, and the term is still widely used. Therefore, for this study, heavy metals are defined as elements of the periodic table with high specific gravity (approximately 5 g cm^{-3} and above) and toxic to living organisms at relatively low concentrations.

Heavy metals are of environmental significance due to their detrimental effect on human health and the ecosystem. Some of these metals are necessary for biological functions but are detrimental at excessive concentrations. The study focussed on cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). According to UNEP (1973), all of these metals are among the most hazardous elements. At low concentrations, Cu, Ni, and Zn are essential nutrients for humans and/or plants, while Cd and Pb have no recognized biological function though they can still be absorbed by biological systems.

Cadmium is a highly toxic element with no recognized function in both plants and humans. It is harmful to plants at low concentrations in the growth media and has a maximum permissible limit of 3 mg kg^{-1} in agricultural soils (WHO, 1993). Its toxicity symptoms in plants include; chlorosis of leaves, wilting, and generally reduced growth (Yadav, 2010). In humans, Cd is absorbed into the bloodstream and transported to the liver where it forms complex metallo-protein molecules which accumulate in the kidney (Godt *et al.*, 2006). Accumulation of excess metallo-protein complexes in the kidney may induce calcium (Ca) excretion, therefore causing irreversible kidney damage (Barbier *et al.*, 2005). Another health problem associated with Cd in humans is fragile bones, leading to osteoporosis and other bone maladies (Kasuya *et al.*, 1992). A classic demonstration of this is in the Japanese, Jinzu basin, where people on a long-term diet of Cd-rich rice suffered bone disorders (Tsuchiya, 1969). Cadmium has also been associated with cancer (ATSDR, 2012).

Copper is an essential nutrient for both plants and humans where it serves as a co-factor in several enzymatic reactions necessary for their growth and development (Pilon *et al.*, 2006; Uauy *et al.*, 1998). In plants, it is involved in photosynthesis, respiration, protein and carbohydrate synthesis, pollen formation, and cell wall lignification. When present in toxic concentrations, it restricts root growth while promoting excessive lateral root growth (Yruela, 2005). Excess accumulation by plants is known to induce other micronutrient deficiencies (Martins and Mourato, 2006). According to WHO (1993), the maximum total permissible limit for Cu in agricultural soils is 100 mg kg^{-1}. In humans, Cu is involved in maintaining the skin, bones, blood and blood vessels, liver, and central nervous system. High accumulation of Cu in the body has been associated with kidney disease (Mahurpawar, 2015), liver cirrhosis, and brain disorders (Wilson, 1912).

Nickel was the last element proved essential among the 17 known essential plant nutrients (Bhalerao, 2015). However, its functions in plants are quite extensive. It is a catalyst in the hydrolysis of urea to ammonia in protein synthesis. It is also involved in nitrogen fixation, and for this reason, it is particularly vital to legumes. Moreover, in a recent study by Fabiano *et al.* (2015), it was indicated that Ni may also be useful in antioxidant metabolism by plants under stressful conditions. Ni is required in low concentrations despite all these functions, and toxicity has been reported at tissue concentrations above 10 mg kg^{-1} dry weight (Alam *et al.*, 2007; Bhalerao, 2015). At these levels, the plant may exhibit leaf chlorosis, wilting, reduced growth, and yield (Hassan *et al.*, 2019). The maximum permissible limit of Ni in agricultural soils is 50 mg kg^{-1} (WHO, 1993). The essentiality of Ni in human nutrition has not yet been established (Genchi *et al.*, 2020), however oral exposure to toxic levels has been associated with allergies (Duda-Chodak and Blaszczyk, 2008), digestive problems (Das *et al.*, 2008). It is also a known carcinogen (Chervona *et al.*, 2012).

Lead is not essential for plants, animals, and humans and is highly toxic even at low concentrations. It enters the plant system mainly through the roots, where it is stored and restricted from translocating to aerial parts of the plant by the barrier action of endodermal cells (Sharma and Dubey, 2005). However, at higher Pb concentration, the barrier is weakened, and Pb is transported to various tissues where it induces its toxicity by displacing essential metal ions from functional metallo-protein complexes. This results in distortion of the structure and function of these molecules, leading to inhibition of vital metabolisms such as photosynthesis and respiration and a reduction in uptake of essential elements (Silva *et al.*, 2015). All these ultimately result in reduced plant growth. Symptoms of toxicity in plants are leaf chlorosis, stunting, and malformed roots (Sharma and Dubey, 2005). Levels above 100 mg kg^{-1} are considered excess for agricultural soils (WHO, 1993). Pb has deleterious effects on various, if not all, major organs of the human body (Pearce, 2007). When Pb enters the body, it is absorbed in the blood stream, where it binds to red blood cells and transports to major organs such as the brain, liver, and kidneys (Pearce, 2007; WHO, 2016). About 90 and 70 percent of absorbed Pb in adults and children, respectively, diffuses into bones where it is stored for a very long time (WHO and Regional Office for Africa, 2015). It causes toxicity by inducing oxidative stress due to its interference with the mechanisms that regulate oxidizing species in cells (Gillis *et al.*, 2012). It has been associated with degeneration of the central nervous system, especially in children (Barry, 1981).

The function of Zn in plants is similar to that of Cu in many ways. It is a constituent of enzymatic systems involved in synthesizing proteins, carbohydrates, and chlorophyll (Roohani *et al.*, 2013). Therefore, its deficiency reduces the growth and development of the plant. Particularly, the plant may exhibit symptoms such as chlorosis on leaves, necrotic spots, and cupping of tips (Hafeez *et al.*, 2013). Similarly, excess Zn concentration causes reduced plant growth and may show similar symptoms to its deficiency. Although toxicity concentrations vary among plant species and physiological period at the time of exposure, typical phytotoxicity occurs around 500 mg kg^{1} (Hafeez *et al.*, 2013). However, according to WHO (1993), the maximum total permissible limit for agricultural soils is 300 mg kg^{-1}. Zinc toxicity may also induce deficiency of other micronutrients with similar absorption mechanisms such as Cu and Fe. In humans, Zn is found in all body cells where it is involved in protein synthesis, DNA metabolism, maintaining good immunity, cell division, and growth (Cousins, 1996). When undersupplied, humans may suffer from Zn deficiency, which may manifest in susceptibility to diseases due to impaired immune function, skin ailments, reduced cognitive function, brain disorders, and overall growth suppression, especially in children (Roohani *et al.*, 2013). When in excess, toxicity, may occur although it is rare. Chronic effects have been observed over long-term exposure (Fosmire, 1990), while acute toxicity occurs after short-term, high-dose exposure (Plum *et al.*, 2010). Perhaps the most severe effect of Zn toxicity is that it induces Cu and Fe deficiency (Plum *et al.,* 2010), hence may present symptoms of their deficiencies.

Although heavy metals are relatively scarce in the earth's crust, high concentrations of both essential and toxic heavy metals do occur especially in areas where anthropogenic activities, such as mining, have disturbed the natural balance of these metals in soils (Kříbek *et al.,* 2010; Lindahl, 2014). During the mining process, until disposal of wastes such as tailings, heavy metal pollution of soil and various other environmental media occurs (Tepanosyan *et al.*, 2018). Tailings are fine grainy material formed after the beneficiation of ores. Tailings usually contain significant amounts of heavy metals that may be transferred to surrounding land as they disperse, contaminating the soils (García-Giménez and Jiménez-Ballesta, 2017; Gevorgyan *et al.*, 2015; Li and Yang, 2008). Mine tailings are undoubtedly a significant contributor to soil contamination with heavy metals as there are at least 3500 tailings storage facilities globally (IMO and UNEP, 2012).

The accumulation of heavy metals in soils around tailing piles is of public health concern because unplanned settlements of poor agrarian communities emerge on such land. In the Copperbelt of Zambia, agricultural activities can be seen around active and old tailing dumps (Kříbek *et al.*, 2014). Farming near mine waste dumps is not unique to Zambia and is common in most other mining districts in sub-Saharan Africa. For example, crop production has been reported around the Kilembe Cu mine in Uganda (Mwesigye *et al.*, 2019) and in Namibia at Kombat (Mileusnić *et al.,* 2014) and Berg Aukas (Mapani *et al.,* 2009). However, there is an imminent risk of excessive uptake of heavy metals by crops grown on contaminated soils. Studies have reported a high concentration of toxic heavy metals in food crops grown under polluted environments (Qu *et al.,* 2012; Woldetsadik *et al.,* 2017; Zia *et al.,* 2017). For example, Qu *et al* (2012) reported that locally grown vegetables were among the major exposure pathways for Pb, Cd, and mercury in villages near a Pb-Zn mine in China. Dinis and Fiúza (2011) reported that food crops that were grown on soils that were contaminated by atmospheric deposition was a major exposure route for Cd. Thus, soil to crop transfer has been identified among the major pathways by which humans are exposed to heavy metals (Attanayake *et al.*, 2014; Gallacher *et al.,* 1984; Makokha *et al.,* 2008).

1.2. Statement of the problem

In Zambia, several studies have reported heavy metal contamination of soils around the mine wastes in Kabwe and the Copperbelt, but they have mainly focused on the total element concentrations (Ikenaka *et al.*, 2010; Nachiyunde, 2013). However, the bioavailable fraction, which is the portion of an element that is in a form that living organisms can take up, is the fraction that poses a risk to humans and other living organisms. To determine the bioavailable heavy metal fractions in soils, extraction with various reagents such as dilute acids, chelating agents, and salt solutions is carried out. However, these different protocols give varying results. Therefore, the available index related to crop uptake remains a contentious issue. The link between concentrations in the soil and crop uptake has not been clearly defined.

It is well acknowledged in the literature that plants take up elements from the soil solution (Cancès *et al.,* 2003; Degryse *et al.,* 2003; Smolders *et al.,* 1998), and so measuring its concentration would give a good indication of the potential risk of heavy metals. However, direct measurement of the soil solution requires sophisticated and expensive equipment. Even where sampling of the soil solution is managed, the concentrations of the heavy metals are at very low detection levels (parts per billion/ million) and so would require spectrometric equipment with low detection limits, such as ICP-MS. Most analytical laboratories however in developing countries do not have such equipment due to budget cost constraints. Therefore, there is a need for alternative accurate indirect methods for determining the concentration of the soil solution.

Geochemical speciation models have successfully been used to determine the soluble concentration in soils (Buekers *et al.,* 2008; Mao *et al.,* 2017; Marzouk *et al.,* 2013). One such model is the Windermere Humic Aqueous Model (WHAM), which has undergone several refinements from when it was first developed (Lofts and Tipping, 2011; Tipping, 1998, 2002). The latest version, WHAM VII, has been successfully used to predict the soil solution concentration (solubility), solution speciation, and solid-phase fractionation (Li *et al.,* 2017; Mao *et al.,* 2017; Zhang *et al.,* 2015) in soils. But like most models which are available so far, WHAM VII was developed based on data from temperate regions. Due to extreme differences in the environmental conditions which influence the formation of soils between the tropics and the temperate regions, their soils vary in terms of the major sorption phases including major clay types, amount of organic matter and dominant (hydr)oxides. Therefore, there would be need to validate models developed based on temperate soils if they are to be used under tropical settings.

1.3. Justification of the study

Although there is an imminent risk of exposure to hazards, including heavy metals in mine waste materials, human settlements near tailing dumps and other mine wastes have been reported in most urban areas in Africa (Kneen *et al.,* 2015). By 2011, the area around three abandoned tailings storage facilities in Witwatersrand, South Africa was home to about 589,889 people and the number was projected to rise in subsequent years (Kneen *et al.,* 2015). An estimated 1.6 million people collectively live in informal and formal settlements on or directly next to mine dumps in South Africa (Kneen *et al.,* 2015). In Zambia's Kabwe town, an estimated 300,000 people are exposed to the Pb contamination from mining tailings (Burga and Saunders, 2019). The Copperbelt Province of Zambia has a population density close to 80 persons per square kilometer against a national average of about 20 persons per square kilometer (CSO, 2013). Although the contamination of soils due to mining activities has been reported worldwide, it is a matter of urgency in developing countries in Africa where the continuously rising population has put pressure on food and nutrition security to the extent that even unsuitable land near tailings and other mine waste dump is cultivated (Mapani *et al.*, 2009). Therefore, studying the potential risk of uptake of heavy metals by crops grown in these localities becomes pertinent.

1.4. Aim and Objectives

The main objective of the study was to evaluate the effect of selected soil management practices on the bioavailability of heavy metals in soils near mine tailings. The following were the specific objectives;

1. To determine the concentration of heavy metals at varying distances from the mine tailings dam
2. To determine the effect of lime and manure on the bioavailable concentration of heavy metals
3. To determine the effect of lime and manure amendments on the uptake of Cd, Cu, Ni, Pb and Zn by maize and pumpkin
4. Evaluate the performance of the WHAM VII model for prediction of Cu fractions in tropical soils

1.5. Structure of the presentation of the thesis

Chapter one presents an introduction of the study by giving; some background information on functions and detrimental effects of Cd, Cu, Ni, Pb and Zn on humans and plants, the problem which the study addressed, the justification and the objectives of the study.

Chapter two is a review of literature highlighting the gaps in the study of heavy metals in soils of tropical nature. It includes a discussion of the impact of disposed mine tailings on their immediate surrounding, the processes leading to heavy metal contamination of soil by mine tailings, soil-to-crop transfer mechanisms, and methods for determination of bioavailability including predictive modelling.

Chapter 3 describes all the materials and methods used to achieve the aim of the study, including; soil characterisation, field and pot experimentation, sampling and sample processing, determination of total and labile heavy metal concentrations, data analysis and evaluation of WHAM VII model.

Chapter 4-6 is a presentation of the results and discussion of the specific experiments conducted in this study. Chapter four and five are based on the field experiment conducted in Kitwe, Mugala farm area, with lime and manure treatments. In contrast, chapter 6 is based on the pot experiment conducted in the glasshouse at ZARI Chilanga, using soils collected from the study site. In Chapter four, the bioavailability of Cd, Cu, Ni, Pb, and Zn was investigated using DTPA and dilute calcium nitrate extractions followed by analysis by ICP-MS. The effect of locally recommended soil management practices, of liming acidic soils to increase pH to agronomic range and addition of manure to evaluate bioavailability of soil-borne heavy metals were investigated. Chapter five presents an assessment of soil-plant relations and uptake of Cd, Cu, Ni, Pb, and Zn by maize and pumpkin leaves. Concentrations of heavy metals in the plants were correlated to available soil fraction (determined by DTPA and 0.01M $Ca(NO_3)_2$ extractions) in the soil. The effect of liming and manure on uptake of heavy metals by the crops was equally investigated. In Chapter six the bioavailability of Cu is presented for the wider farm area of the study site. The WHAM VII geochemical speciation model was used to study the partitioning of Cu into solid and liquid phases in order to understand the drivers of its bioavailability in the soil.

Chapter seven presents a synthesis of the most important results from the study into a conclusion and lessons learned.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Introduction

In many parts of Africa there are large deposits of metalliferous mine tailings which have caused serious contamination of soil and other environmental media. In Zambia's Copperbelt alone, over 9,125 ha of land are occupied by tailings (Lindahl, 2014) and about 75 ha in Kabwe are contaminated by a combination of several mine wastes (Leteinturier *et al*., 2001). The problem is on a greater scale in South Africa, where gold mine tailings alone occupy about 18,000 ha of land (Rösner *et al*., 1998). In a review on the "Nature and Articulation of Ethical Codes on Tailings Management in South Africa" Poswa and Davies (2017) concluded that the current legislation on tailings management is ineffective and both compliance and compliance monitoring by the mining community and authorities, respectively, are weak. Although such studies have not been conducted in other African countries, the same can be said for them owing to the level of environmental degradation which can be attributed to mine tailings.

Mine tailings are a source of heavy metals which may end up in soils and other ecosystems. However, only the bioavailable fraction of the heavy metals presents a threat to humans and other living organisms, through the food chain. Therefore, knowledge of the bioavailability of heavy metals, and the factors that influence bioavailability in areas where risk is probable is important for risk assessment.

This review provides a discussion of the bioavailability of heavy metals in soils that are contaminated by mine tailings, with emphasis on the mechanisms in tropical soils, covering: (1) the processes leading to soil contamination by heavy metals, (2) bioavailability of heavy metals and (3) methods for determination and assessment of bioavailability and subsequent evaluation of predictive modelling of soil dynamics for heavy metal transfer to crops.

2.2. Impact of mine tailings on soils

2.2.1. Chemical characteristics of mine tailings

Environmental problems associated with tailings lie in their chemical constituents, because ore extraction leaves a multi-elemental waste containing potentially harmful elements (PHE). For example, Sracek *et al*. (2010), Mileusnić *et al*. (2014) and Gevorgyan *et al*. (2015) reported high concentrations of heavy metals in mine tailings in Zambia, Namibia and Armenia respectively. Table 2-1 summarises reported concentrations of heavy metals in tailings from selected mines in sub-Saharan Africa, Asia and Eastern Europe and mean values for the Earth's crust. A measurable impact on adjacent agricultural soils has been observed globally (Table 2-2). For example, Li and Yang (2008) reported concentrations of soil Cd, Cr, Cu, Zn, Pb and Mn significantly greater than background values, by factors of 5000, 200, 50, 100, 700 and 4000 percent respectively, due to enrichment by tailings from a Mn mine in China. Soils around a Cu mine in Armenia were contaminated with Cu, Pb, As, Co, Ni and Zn from tailings (Gevorgyan *et al*., 2015). Kříbek *et al*. (2010) reported widespread Cu contamination of Oxisols in the Zambian Copperbelt by mining by-products, including tailings, while historical Pb mining in Zambia's Kabwe town has left a legacy of mine waste piles; soils in Kabwe are reportedly among the most Pb polluted soils worldwide (Nriagu 1992; Leteinturier *et al*., 2001; Water Management Consultants *et al*., 2006; Ikenaka *et al*., 2010; Yabe *et al*., 2010). In Ghana, elevated concentrations of As, Cu, Pb and Zn were found in soils around Obuasu gold tailings dam (Antwi-Agyei *et al*., 2009). In South Africa's Gauteng, soil contamination by As and other heavy metals around gold tailings piles has been widely reported (Rösner and Van Schalkwyk 2000; Olobatoke and Mathuthu 2016; Ngole-Jeme and Fantke 2017). In Namibia's Otavi Mountainland, soils around Kombat Mine are reportedly contaminated with Cu and Pb, attributed to tailings which have been disposed of improperly (Mileusnić *et al*., 2014). Mapani *et al*., (2009) equally found high concentrations of Pb, Zn, V, Cu, Cd and As in agricultural soils near a tailings storage facility at the abandoned Berg Aukas mine.

Table 2-1: Heavy metal concentration of some mine tailings

Mine/ Location	Mined ore	Heavy metals mg kg^{-1}					Reference
		Cd	Cu	Ni	Pb	Zn	
Mindolo/ Zambia	Cu	NA	1,600-2,781	NA	NA	NA	Sracek *et al*., 2010
Berg Aukas/ Namibia	Pb, V, Zn	352	184	NA	<10	52,100	Mapani *et al*., 2009
Chambishi/ Zambia	Cu	NA	795-10,000	NA	NA	NA	Sracek *et al*., 2010
Kabwe/ Zambia	Pb, Zn		129-2,320		4,900-42,200	45576-64740	Leteinturier *et al*., 2001
Kombat/ Namibia	Cu	BD–7.6	64-9,086	0.2-7.9	710-5007	49-157	Mileusnic *et al*., 2014
Anka/ Zamfara Nigeria	Ag	1.78-10	NA	NA	1694-4152	358-1600	Uriah *et al*., 2014
New Zurak/ Nigeria		4.6	36	21	825	9.39	Uriah *et al*., 2014
Pingu/ China	Mn	7.6 ±0.8	86 ± 15	NA	239 ± 26	198 ± 11	Li and Yang 2008
Chockan/ Armenia	Cu	NA	320	35	12	125	Gevorgyan *et al*., 2015
Earth crust mean (ECM)		0.2	45-70	84	13-16	70-132	Yobouet *et al*., 2010

**NA: not reported*

Mine tailings in some geological settings including the Katanga basin in DR Congo and Zambia can have technologically enhanced naturally-occurring radioactive material (TENORM) which includes uranium(U) and radon (Ra) (Katebe *et al*., 2008). In the Zambian Copperbelt, Lark *et al*. (2017) observed a decreasing trend in soil U with increasing spatial distance from Cu mine tailings, indicating that the tailings were the source. Tailings may also be a source of acid mine drainage (AMD). This is particularly a characteristic of tailings from sulphidic mineral ores when metal sulphides, e.g., Pyrite (FeS_2), are exposed to air and water and become oxidised in a reaction in which sulphuric acid is also a product as shown in Eq. 1.

$$FeS_2 + \frac{15}{4}O_2 + \frac{7}{2}H_2O \leftrightarrow Fe(OH)_3 + 2H_2SO_4 \qquad \text{Eq. 1}$$

Table 2-2: Heavy metal concentrations of soils near mine tailings

Mine/Location	Heavy metals mg kg^{-1}					Reference
	Cd	**Cu**	**Ni**	**Pb**	**Zn**	
Kitwe, Zambia	0.28	1,250	15	10	10	Kříbek *et al*., 2010
Chambishi, Zambia	<0.8	7,600	38	65	52	Kříbek *et al*., 2010
Mufulira, Zambia	<0.8	1,650	<5	<10	5	Kříbek *et al*., 2010
Chingola, Zambia	<0.8	40,200	10	18	144	Kříbek *et al*., 2010
Kabwe, Zambia	7.1 ± 9.9	572 ± 597	47 ± 9	7,076 ± 8,644	16,991 ± 20,614	Ikenaka *et al*., 2010
Obuasu, Ghana	NA	39.6-71.4	NA	24.2-38.7	72.6-168.1	Antwi-Agyei *et al*., 2009
Johannesburg, South Africa	NA	4-39	0-72	3-49	5-168	Olobatoke and Mathuthu 2016
Krugersdorp, South Africa	0.1-12	4-1350	1-4,400	0.4-72	47-5340	Ngole-Jeme and Fantke 2017
Kombat/ Namibia	BD	74-150	7.6-11	71-164	31-62	Mileusnic *et al*., 2014
Berg Aukas/Namibia	5.4-387	6-327	NA	1,157-34,400	2,047-216,000	Mapani *et al*., 2009
Pingu/China	4.2 ± 0.8	40.7 ± 2.9	NA	170.7 ± 7.1	136.7 ± 7.1	Li and Yang 2008
Chockan/ Armenia	NA	20-320	18-35	4-12	90-125	Gevorgyan *et al*., 2015
WHO/FAO Maximum Permissible Limits	3	100	50	100	300	FAO/WHO 1985, 1992/93
Canadian Soil Quality Guidelines for agricultural soils	1.4	63	50	70	200	CCME 2007

**NA: not reported, BD: below detection, #largely contaminated with arsenic*

In acidic conditions, heavy metals can be mobilised and transferred into other environmental media including soils and water systems. Otherwise, tailings with a large capacity to neutralise acidity have a neutral to basic pH as is the case with Mindolo tailings in Kitwe-Zambia where the main underlying geology is a calcitic and dolomitic shale (Kříbek *et al.*, 2010). Sracek *et al.* (2010) observed that at neutral pH, heavy metals were immobilised due to a precipitation reaction with hydrous oxides of iron, changes which will also influence the stability of these precipitates. Moreover, lime ($CaCO_3$) is often added to tailings prior to disposal as a way of reducing the formation of acid mine drainage (AMD). However, when the tailing materials are transported to surrounding land by erosion forces (wind or run-off), the heavy metals may become bioavailable, especially under acidic conditions (Rieuwerts *et al.*, 1998).

2.2.2. Transfer of heavy metals from tailings to soils

The transfer of heavy metals from tailings to soils on adjacent land may be attributed to the mechanical deposition of the tailings material onto the soil surface largely by water and wind erosion (Blight 1989; Mileusnić *et al.*, 2012). Chemical transfer through dissolution in AMD is another possible process but mechanical transfer has been reported as the major pathway (Mileusnić *et al.*, 2012). Amponsah-Dacosta (2015) reported that annual erosive losses from South Africa's gold tailings range from 257 – 316 ton ha^{-1} and cited other works that reported even higher rates of erosive losses. In dry environments, tailings dams or impoundments tend to dry out hence leaving the material prone to wind erosion. Increasing levels of heavy metals towards tailing dams, and in the direction of the wind, have been reported in Zambia and Nigeria (Tembo *et al.*, 2006; Uriah *et al.*, 2014; Lark *et al.*, 2017). The particle size of the tailings material is crucial as the finer material is carried further by wind. Moreover, finer tailings material may have a higher metal load due to higher specific surface area. Water erosion through run-off causes significant transfer of tailings material onto surrounding land (Blight 1989). With run-off, larger particles that cannot be carried by wind are moved and deposited down slope. In addition to the effects of erosive water, mine waste water has been used for irrigation by communities living near tailings dams. This water carries dissolved metal ions (Michelutti *et al.*, 1995), which are subsequently adsorbed and accumulated in soils. Heavy metals are non-degradable, tending to accumulate and persist in soils. Thus, heavy metal loading of soils near tailings must inevitably increase with time. In view of this, measures to prevent erosion by both water and wind, such as slope terracing (Blight 1989; Blight and Caldwell 1984), vegetation techniques (Pepper *et al*., 2012) and surface covers (Amponsah-Dacosta 2015) have been studied. Combined vegetative and cover techniques were observed to reduce erosion by about 90 percent (Amponsah-Dacosta 2015). However, the implementation of these interventions is hampered by their substantial cost.

2.2.3. Retention of heavy metals in tropical soil

The ability of soils to accumulate not only plant nutrient elements but also heavy metals has implications for agriculture and environmental sustainability. Soil is a major reservoir for heavy metals through its various constituents, such as soil organic matter (SOM), (hydr)oxides of Al, Fe and Mn, clays and other amorphous soil minerals, as shown in Figure 2-1. It is noteworthy that heavy metals are natural constituents of soils by virtue of their release from weathering of rocks in addition to the dispersion of tailings as discussed in this review. Heavy metals are retained in soils by: (i) non-specific exchange reactions between the soil solution and charged soil geocolloidal surfaces, (ii) specific adsorption (chemisorption) on soil geocolloidal surfaces, (iii) co-precipitation with anions (Sherene 2010) and fixation into the structure of the soil minerals (Bradl 2004). The retention and accumulation of metals in all soil components is reversible but ease of desorption differs according to the type of bonding involved.

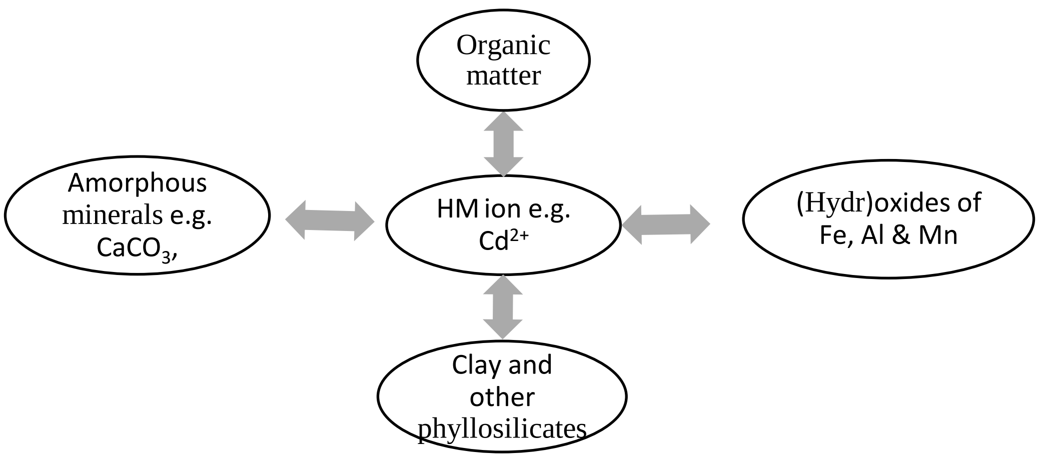


Figure 2-1: Soil components that retain heavy metals

Geocolloidal components which give soils high cation retention capacities include: SOM, 2:1 clays and (hydr)oxides of Al, Fe and Mn. Thus, soils with larger contents of SOM and 2:1 clays have greater potential to retain heavy metals, thereby immobilising them. Typical tropical soils (Ultisols, Oxisols, Alfisols) are low in SOM and are dominated by 1:1 clays, like kaolinite, and hydr(oxides) of Al, Fe and Mn. This gives tropical soils a low CEC and they are, therefore, relatively ineffective at reducing lability of heavy metals. Moreover, the negative charge on kaolinitic clays and oxides are pH-dependent, tending to reduce even further in acidic conditions, which is the predominant condition in these soils. However, soil management practices that can increase the SOM content and increase pH have the potential to offset this problem. In a recent study by Ramos *et al*. (2018), the CEC of Oxisols in Brazil increased by 25 percent with every 1.8 kg m^{-2} of stored organic carbon due to a 'no-tillage' system. According to a study by Soares and Alleoni (2008), the mean contribution of soil organic carbon to the CEC of Brazilian Oxisols and Ultisols was 44 times higher than the contribution from the clay fraction. Other studies have shown that lability of heavy metals is reduced in soils which have been treated with organic inputs (Oliver *et al*., 2005; Angelova *et al*., 2013), such that uptake by plants grown in such soils is equally reduced (Angelova *et al*., 2010). Similarly, amendments that increase the pH of soils have been reported to increase retention of heavy metals.

Often, pH is cited as the most important factor affecting metal bioavailability in soils owing to its key role in determining the solubility and transport of heavy metals (Rieuwerts *et al*., 1998). A direct relationship exists between retention of cationic metals and pH in soils (Laing *et al*., 2008). Tlustoš *et al*. (2006) noted significant reductions in the mobile fractions of Zn, Cd and Pb in Cambisols treated with liming material to increase pH from 5.7 up to 7.3. Additionally, higher solubility of Pb, Cd and Zn were observed under acidic conditions (pH = 3.3–5) than in alkaline conditions at pH = 8 (Chuan *et al*., 1996). Lombi *et al*. (2003) tested lime, beringite, and red mud for their effect on lability of Cd, Cu, and Zn in polluted soils from France. Further, Lombi *et al*. (2003) reported significant reduction in lability of the heavy metals largely due to increased metal adsorption and, precipitation of metal hydroxides and carbonates at high pH. Similar results have been widely reported and collated in several reviews (Fijałkowski *et al*., 2012; Rieuwerts *et al*., 1998; Sherene 2010; Smith and Huyck 1999; Violante *et al*., 2010). Mobilisation of metals at lower pH is attributed to reduced surface negative charge and the resultant increased competition for sorption with hydrogen ions (Rieuwerts *et al*., 1998; Smith 1999). Conversely, adsorption, complexation and precipitation processes of heavy metals are increased at higher pH due to increased surface negative charge which increases cation adsorption, formation of metal hydroxy cations (e.g. MOH^{+}) which have greater affinity for adsorption than ligand-free cations (e.g. M^{2+}), and precipitation of metal cations with hydroxyl ions to form insoluble hydroxides (Tlustoš *et al*., 2006). Although most of the studies which have been cited here were from non-tropical regions, altering the soil pH with liming amendments is likely to be very effective in tropical soils where most of the charge on the soil colloids is pH-dependent.

2.3. Bioavailability of heavy metals in soil

The bioavailable portion of a heavy metal in soil is the fraction of that element which is potentially available for uptake by an organism resulting in either improved growth or induced toxic effects. This section, however, only focuses on bioavailability in relation to plants, specifically known as phyto-availability. An element in soil, whether essential or not, has to be in a chemical form that is accessible by the plant for it to be taken-up through the roots. This concept has been well understood and applied in the field of soil fertility especially in relation to major plant nutrients, but surprisingly overlooked for potentially harmful elements (PHEs) such as heavy metals whose soil guidelines values (e.g. CCME, 1996; 2006, FAO/WHO Codex Alimentarius) still exist as total soil metal concentrations. Some regional bodies such as the EU and individual countries, in the developed world are working on specifying bioavailable limits for various land uses. Moreover, most soil guidelines values (SGVs) which are available in the literature (total concentrations) are based on data from temperate regions, but bioavailabilities of heavy metals in temperate and tropical soils are very likely to be different due to varying soil properties. Therefore, SGVs specified in bioavailable terms should be much more universally applicable compared to SGVs expressed as total soil metal concentrations.

2.3.1. Soil components and their bioavailability

Chemical elements in the soil, including heavy metals, can be classified as occurring in three operational fractions, namely: ‘inert’, ‘labile’ and ‘soluble’ (Figure 2-2). Inert elements are strongly held on, or within soil particles and are only released upon weathering of soils. The labile fraction constitutes the elements which are surface-adsorbed and are in equilibrium with the soil solution which contains the soluble elements. The soluble fraction is thus immediately bioavailable, although the degree of bioavailability of the dissolved constituents may vary. These three fractions are not fixed but are constantly changing with respect to soil properties and time. Transition from the inert to the soluble fraction is long-term as it depends on the rate of weathering of soil minerals. According to Kabata-Pendias (2004), it may take several years or even decades. Conversely, labile elements move into the solution fraction in response to a concentration gradient and this process may occur within days to hours (Shi *et al*., 2008). Therefore, the labile and soluble fractions make up the potentially bioavailable fraction of elements in soil, this fraction is the target for chemical extractions of soil with weak acids, neutral salts and chelating agents to estimate the plant-available concentrations. However, the effectiveness of these extractants is highly dependent on their interaction with soil constituents and they may either under-estimate or over-estimate the bioavailable concentrations (Ayoub *et al*., 2003) depending on their extraction power.

Numerous studies have investigated heavy metal fractions in soil and uptake by plants to answer the question ‘which fraction is the driver for bioavailability?’ with variable results. Some have suggested that the labile fraction is the determining factor (Domergue and Vedy 1992; Labanowski *et al*., 2008), while others have reported better correlations between the soil solution concentration and the bioavailable concentration (Bonten *et al*., 2008; Houba *et al*., 1986; Zhang *et al*., 2016). Moreover, other studies have shown the free ion concentration to be the best predictor of bioavailability in the soil (Zhang *et al*., 2001). On the other hand, a number of studies have shown no clear correlation with any of the extractable fractions (Angelova *et al*., 2010; Chenery *et al*., 2012), but none of the cited studies included extraction with a weak salt solution.

Obviously, the solution concentration being the medium from which plants absorb these elements should give a good estimate of the bioavailable concentration. It is also possible that 'solution speciation' to resolve the free metal ion concentration (M^{2+}) provides an even better measure as it represents the actual absorbed form of heavy metals. Unfortunately, direct measurements of both solution concentration and speciation are not straightforward as they require sophisticated equipment, which are not always available in the laboratories of all African countries. Thus, relatively simple chemical extractions are still widely used to estimate available nutrients and heavy metals. The best extraction to predict bioavailability would be one that mimics the soil solution as much as possible (Kabata-Pendias 2004). However, a thorough assessment of the risk posed by heavy metals in soil should include characterisation of its labile concentration, solution concentration and speciation. This is because the intensity factors (total solution concentration and M^{2+} ion concentration) give an accurate estimate of the immediate bioavailability and the labile quantity present the longer-term (potential) availability and reflects the ability of the soil to replenish metal ions in the soil solution. Heavy metal bioavailability must depend on both the immediate soil solution concentration and the capacity of the solid phase to replenish the soil solution (Violante *et al.*, 2010). Therefore, characterisation of the solid fraction is important in heavy metal risk assessment studies in soils.

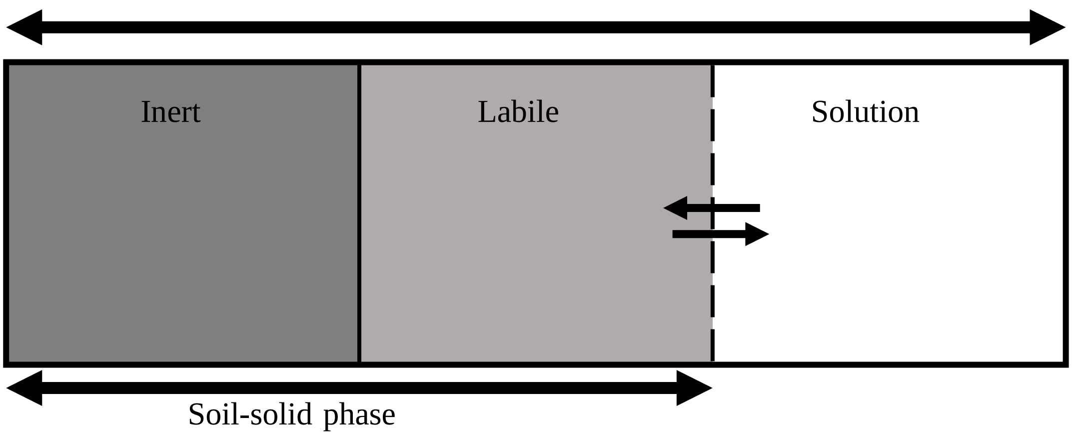


Figure 2-2: Schematic diagram of the three soil metal fractions

2.3.2. Solid-phase heavy metal fractionation

Heavy metals in soil are retained on various soil solid components that vary in degree of reactivity and solubility, and hence bioavailability. Therefore, identifying and quantifying the particular heavy metals which are associated with the different soil solid phases would give an indication of their relative bioavailability. Typically, five heavy metal fractions have been operationally defined according to an extraction regime, namely: exchangeable, specifically adsorbed (acid soluble), Fe and Mn (hydr)oxides bound (reducible), organic matter and sulphide bound (oxidisable) and residual (Tessier *et al*., 1979; Ma and Rao 1997; Li and Thornton 2001). Table 2-3 shows the soil-solid fractions and their relative bioavailabilities. The bioavailability of these fractions is said to reduce in the order they are listed (Ma and Rao 1997). However, this depends largely on the extractants used, which should provide an estimate of the fraction involved. Some metals may be bound to less soluble components despite being classified under the more bioavailable fractions and vice versa. For instance, in calcareous soils, Pb may occur as sparingly soluble cerussite ($PbCO_3$) which may be extracted in the second phase (acid soluble) of the extraction procedure, thereby overestimating the bioavailable fraction. Moreover, these fractions are only operationally defined as the amount extracted depends on the strength and specificity of the extractant used, and how it interacts with the soil characteristics. For instance, Marzouk *et al*. (2013a) observed that the labile fraction of Pb in calcareous soils corresponded to the exchangeable fraction only while that of acidic soils corresponded to the combination of the exchangeable, acid soluble and reducible fractions.

Table 2-3: Soil heavy metal fractions and their relative bioavailability (Tessier *et al.*, 1979)

Step No.	Soil fraction	Relative bioavailability
1	Exchangeable	Readily bioavailable
2	Acid soluble	Very bioavailable
3	Organic bound	Moderately bioavailable
4	Fe/Al/Mn oxide bound	low bioavailability
5	Residual	Non-bioavailable

Organically bound metals may be more or less bioavailable depending on the fraction of SOM they are bound to, such as fulvic acid (FA), which is highly soluble, and humic acids which are less soluble (Temminghoff *et al.*, 1997) al. 1994). In their assessment of the effect of molecular weight of dissolved organic matter (DOM) on bioavailability of Cu, Wang *et al*. (2010), concluded that high molecular weight DOM forms stable complexes with copper ions, which are not readily bioavailable to plants. Moreover, low molecular weight DOM had less capacity to complex Cu, therefore leaving most of the Cu in solution as free ions (Cu^{2+}). In response to such anomalies, expanded fractionation schemes (Table 2-4) have been defined in the literature (Krishnamurti *et al*., 1995; Krishnamurti and Naidu 2000; Fedotov and Miró 2007). The organic and metal oxide bound fractions are further subdivided into readily soluble and more strongly bound ones. These multi-phase fractionation schemes are theoretically more informative but whether the results obtained are more accurate than procedures with fewer phases is doubtful owing to the reported drawbacks of fractionation schemes, such as non-specificity of reagents and re-adsorption/redistribution of elements during extraction. All these fractions can be conveniently classified into 2 broad categories namely, detrital and non-detrital, according to whether solid phase dissolution is required or not, respectively, to mobilise the associated heavy metals (Elliott *et al.*, 1990). Non-detrital fractions include the exchangeable and acid extractable, while detrital fractions are those that are; complexed by organic or inorganic ligands, precipitated and part of the solid structure of soils. Non-detrital are, therefore, the readily mobile fractions while detrital are mobilised over time due to changes in key soil properties and environmental conditions such as pH and redox potential (Violante *et al*., 2010). Labanowski *et al*. (2008) studied heavy metal mobility by kinetic extractions and equally reported the occurrence of more labile and less labile fractions of heavy metals representing mobile and less mobile fractions.

Table 2-4: Expanded soil heavy metal fractions and their relative bioavailabilities (Krishnamurti and Naidu 2000)

Step No.	Soil fraction	Relative bioavailability
1	Exchangeable	Easily bioavailable
2	Specifically adsorbed/Acid soluble	Easily bioavailable
3	Organic complexes	Readily bioavailable
4	Easily reducible metal oxides	Readily bioavailable
5	Organic compounds and sulphides	Poorly bioavailable
6	Amorphous mineral colloids	Poorly bioavailable
7	Crystalline Fe oxides	Poorly bioavailable
8	Residual	Non-bioavailable

The lability of soil heavy metals depends primarily on the stability of their associations with the soil solid phase but can also be influenced by their source i.e. whether geogenic (from parent material) or anthropogenic. According to Kelepertzis and Stathopoulou (2013) and Kelepertzis and Argyraki (2015), geogenic heavy metals are less soluble than those of anthropogenic origin because geogenic heavy metals may be incorporated into the structure of the soil, unless otherwise released due to weathering. Anthropogenic heavy metals, on the other hand, are likely to be bound to less stable fractions, thereby being more bioavailable. Adriano (2001) reviewed a number of studies that showed more bioavailable Cd in soils impacted by mining activities than in relatively unimpacted soils. Thus, the risk posed by heavy metals contamination of soils by mine tailings is again emphasised as these heavy metals are extrinsic and so may be comparatively loosely bound. Additionally, heavy metals added to soils will occupy the strongest adsorption sites first and as their concentration increases progressively, weaker sites are occupied. Therefore, lability would be expected to increase as the concentration of added heavy metals increases (Marzouk *et al*., 2013a). However, ageing (fixation) reactions in soils which are affected by anthropogenic heavy metals have been shown to reduce the soil solution concentration of metals (Buekers 2007). Thus, Mao *et al*. (2015), reported that lability (of Cu) was dependent on contact time between the soil and its contaminant. Ageing is a slow process whereby adsorbed ions on the soil solids move into more immobile fractions and are no longer in equilibrium with the soil solution. Iron (hydr)oxides were identified as the major surfaces involved in irreversible fixation of heavy metal in soil (Buekers *et al*., 2008), hence fixation could be an important reaction in highly weathered tropical soils where (hydr)oxides of Fe are dominant.

Apart from soil reaction, heavy metal concentration and nature of sorption surfaces, lability of heavy metals may also differ depending on the element involved. For instance, the lability of elements with small ionic radii such as Ni, Cu and Zn may be reduced due to fixation on Fe (hydr)oxide in soils with high concentrations of these oxides (Mao *et al*., 2017). Copper has a high affinity for SOM and so its lability could be reduced in soil with high SOM content (Smolders *et al*., 2012). Lability of Pb is variable in soils, with the highest values reported at about 40 to 60 percent of the total concentration in peat and non-organic soils (Shaheen 2009; Atkinson *et al*., 2011; Chenery *et al*., 2012) and lower in soils with high phosphate concentrations where it may precipitate as pyromorphite (Sauvé *et al*., 1998). Contrastingly, the affinity of Cd for most binding phases including Fe (hyr)oxides, organic matter and aluminosilicate clays, is low compared to other heavy metals and so it is considered as relatively labile (Shaheen 2009). Many reports on the reactivity of heavy metals, including Cd, have showed a higher lability of Cd compared to other metals (Gäbler *et al*., 2007; Römkens *et al*., 2009; Shaheen 2009; Izquierdo *et al*., 2017; Mao *et al*., 2017). Römkens *et al*. (2009) reported the lability of several heavy metals to reduce in the order Cd>Cu>Pb>Zn≈Ni, which is similar to the sequence reported by Izquierdo *et al*. (2012) (Cd>Pb>Zn). Mao *et al*. (2017) and Gäbler *et al*. (2007) also reported similar results (Cd≥Pb>Cu>Zn>Ni), although Pb was apparently more labile than reported in the two former studies. High lability of Cd has been observed even in soils with only background concentrations (Liu *et al*., 2013). The relatively labile nature of Cd has implications for its accessibility by a wider range of receptors. Unsurprisingly it has been reported to be in significantly high concentrations in most environmental media found in areas prone to Cd pollution (Yu *et al*., 2010; ATSDR 2012). Table 2-5 shows some (percentage) labilities of Ni, Cu, Zn, Cd and Pb that have been reported in the literature.

Table 2-5: Labilities of heavy metals in soils as reported in selected literature sources

Element	Average lability (%)	Reference
Cu	58	Baeyens *et al*., 1997
Zn	27	Marzouk *et al*., 2013a
	28	Marzouk *et al*., 2013b
	41	Baeyens *et al*, 1997
Cd	87	Marzouk *et al*., 2013a
	40	Marzouk *et al*., 2013b
	72	Baeyens *et al*., 1997
Pb	56	Marzouk *et al*., 2013a
	53	Marzouk *et al*., 2013b

When labile elements enter the solution-phase they become potentially phytoavailable (bioavailable) unless they are complexed with either organic or inorganic ligands and rendered non-accessible to plants (Siedlecka 1995). Although plant uptake of complexed ions has been reported in chelate-assisted phytoremediation studies (Liphadzi and Kirkham 2006; Dipu *et al*., 2012), the contribution of organically complexed heavy metal species to overall bioavailability is normally minimal. Labile concentrations of heavy metals in soil do not necessarily correlate with the total concentration (Topcuoğlu 2016) as this would depend on the soil properties and the nature and concentration of the soil binding phases. Zhang *et al*. (2006) concluded that the labile forms (exchangeable and carbonate-bound) in the soil depended on the soil reaction while the less mobile forms (organically-bound, oxide bound and residual) were controlled by their total concentrations in the soil. An element with a high total concentration in soil could be mainly in a non-labile form and, therefore, pose less risk of exposure to the environment and human health (Young *et al*., 2000; Ashraf *et al*., 2012; Topcuoğlu 2016). While it is likely that lability will correlate to bioavailability (Ogundiran and Osibanjo 2009), this may not always be true as labile elements in solution may still be complexed by organic ligands constituted in DOC and inorganic anions such as chloride and sulphate. For instance, Weng *et al*. (2002), reported reduced solubility of several heavy metals, especially Cu due to complexation by DOC. These concepts have formed the basis for *in-situ* stabilisation mechanisms utilised to remediate polluted soils which aim to reduce the mobility and bioavailability of PHEs in soils by enhancing their binding to the solid phase (Luo *et al.*, 2012; Zhai *et al.*, 2018). The labile portion of heavy metals therefore, provides a useful benchmark for bioavailable concentration (Zhang *et al.,* 2006). Consequently, its use as an input in speciation models for the prediction of the soil solution concentrations of heavy metal has improved prediction compared to use of total concentration (Marzouk *et al.*, 2013b; Degryse *et al*., 2003).

2.3.3. Soil solution concentration and speciation

Elements which are dissolved in the soil solution constitute the mobile portion and pose a risk to groundwater systems thereby affecting even offsite receptors. Moreover, plants absorb elements from the soil solution, thus dissolved metal ions also constitute the immediately bioavailable fraction. Thus, assessment of the solution concentration of heavy metals gives an indication of the immediate risk posed by the heavy metals either through uptake by biota or leaching down into groundwater systems.

Heavy metal ions in the soil solution occur in various species that are often analytically challenging to identify and quantify. They may exist as free ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}) or complexed by organic (humic acids, fulvic acids) and inorganic (NO_3^-, Cl^-, SO_4^{2-} etc) ligands. Moreover, heavy metals exist in multiple oxidation states and form several complex species with other ions. These species differ in solubility and hence in availability for uptake by biota. For example, hexavalent chromium (VI) is more soluble than trivalent chromium (III) and so may easily be taken up by plants (Hamilton *et al*., 2018). Thus, while dissolved elements in the soil solution are mobile, not all are available for uptake by biota such that the resultant bioavailability is a fraction of the total metal content. The free metal ions are the most reactive species; they control metal binding to complexing surfaces and are therefore primary determinants of heavy metal bioavailability. Although plants may take up complexed ions (Krishnamurti *et al*., 2004), free ions are the most readily bioavailable species in the soil solution such that the free ion activity is said to be the most accurate measure of bioavailability (Cancès *et al*., 2003).

Therefore, elements that exist in soil predominantly as free ions are the most bioavailable. According to Cancès *et al*. (2003), Cd and Zn tend to exist in soil solution as free aquo-ions (M^{2+} bound to water molecules only), hence their high bioavailability. Other authors have reported similar findings for Ni (Ren *et al*., 2015). However, Cu and Pb are usually found in complexes with organic or inorganic ligands. Dissolved organic matter, and particularly fulvic acid, is the dominant complexing ligand for Cu while both FA and inorganic ligands are important for Pb binding in the soil solution (Zhao *et al*., 2006; Ren *et al*., 2015). Thus, Wu *et al*. (2000) reported the following order of free ion activities; Cd>Zn>Cu>Pb with ranges 42-82 percent, 2-70 percent, 0.1-7.8 percent and 0.1-5.1 percent respectively. Similarly, Kwon-Rae and Owens (2009) reported activities in decreasing order: Zn>Cd>Cu>Pb.

Cadmium is highly soluble especially in acidic conditions (Marzouk *et al*., 2013a) and occurs predominantly in the soil solution as free ions (Ren *et al*., 2015; Sauvé *et al*., 2000; Wu *et al*., 2000), unless in saline soils where it may be complexed by chlorides and sulphates (Mclaughlin *et al*., 1996; Smolders *et al*., 1998). Plausibly, inorganic complexation of soluble Cd is more significant than organic complexation (Sauvé *et al*., 2000). Copper can be classified as slightly soluble and is widely reported to occur in the soil solution mostly complexed with organic ligands (Sauvé *et al*., 1997). However, inorganic ligands such as chloride, carbonates and hydroxides may significantly complex Cu if they are present in high concentrations in the soil. Contrastingly, the free ion activity of copper (Cu^{2+}) is a very small percentage of the overall soil solution concentration. For instance, Wu *et al*. (2000) reported only 0.1-7.8 percent of total dissolved Cu as free $Cu^{2+}$for 10 contaminated soils, while Kwon-Rae and Owens (2009) reported 0.2-30 percent. The chemical speciation of Ni in the soil solution is dominated by its free ion, Ni^{2+}, across a wide range of pH values, while its hydrated mono-ion ($NiOH^{+}$) predominates in highly alkaline conditions (Adriano 2001). Inorganic and organic complexation of Ni are equally important to its speciation depending on the concentration of these ligands in the soil solution. Lead is one of the least soluble heavy metals in soil as it easily precipitates (Degryse *et al*., 2009). Lead occurs in the soil solution, predominantly, complexed with both organic and inorganic ligands, and these species control its free ion activity. In the pH range 6 – 10, $PbOH^{+}$ is the most common species and Pb^{2+} predominates at lower pH (Wu *et al*., 2000; Ren *et al*., 2015). Therefore, the free ion activity of Pb is higher at lower soil pH than at higher pH. For instance, results reported by Ren *et al*. (2015) indicate that Pb^{2+} was closer to the total solution concentration at pH < 5 than at higher soil pH. Therefore, as with many other trace elements, solubility and speciation of Pb is influenced by soil properties. However, the source forms of Pb also have a significant bearing on the bioavailability of Pb. Lead halides, e.g chlorides, are said to be slightly soluble while its carbonates and hydroxides are essentially insoluble in water (Adriano 2001; Stanforth and Qiu 2001). Zinc is classified as highly soluble and occurs in its free ionic form (Zn^{2+}) at most pH ranges for arable land (Kwon-Rae and Owens 2009; Ren *et al*., 2015). Its hydroxylated ion, $ZnOH^{+}$, predominates in alkaline soil conditions (Wu *et al*., 2000). Other important Zn species in arable soils include a wide range of organic complexes, which apparently predominate in the rhizosphere (Hamon *et al*., 1995).

2.3.4. Plant uptake of heavy metals in tropical soils

Uptake of heavy metals by crops grown in the tropics is probably controlled by the same factors affecting uptake by crops grown in temperate soils. In both tropical and temperate regions, high concentrations of heavy metals have been reported in plants grown in contaminated soils. Table 2-6 shows heavy metal concentrations reported in some vegetables grown in contaminated tropical soil. Njagi *et al*. (2017) reported high heavy metal concentrations of several vegetables which were grown on contaminated soils around dumpsites in Nairobi Kenya. In Ghana's Korle Lagoon, high concentrations of Pb were found in vegetables grown in the area due to enrichment of soil with heavy metals originating from e-wastes (Fosu-Mensah *et al*., 2017). Felix-Henningsen *et al*. (2010) observed a high uptake of Cd, Cu and Zn in humus-rich soils from Georgia. Křībek *et al*. (2014) found high concentrations of As, Cu, Co and Zn in Cassava leaves grown in contaminated soils in the Zambian Copperbelt. Similar results have been reported from non-tropical regions including; Pakistan (Latif *et al*., 2018), Bingham Creek, America (Cobb *et al*., 2000), Austria (Lombi *et al*., 1998), Bulgaria (Angelova *et al*., 2010), United Kingdom (Intawongse and Dean 2006).

Table 2-6: Heavy metal accumulation in some vegetables grown in contaminated tropical soils

Crop	Heavy metals mg kg^{-1}					Reference
	Cd	**Cu**	**Ni**	**Pb**	**Zn**	
Spinach	NA	0.72±0.22	35.23±1.04	1.51±0.00	6.62±0.01	Njagi *et al*., 2017
Hairy nightshade	NA	1.52±0.01	22.54±1.57	1.56±0.03	1.73±0.07	Njagi *et al*., 2018
Cabbage	NA	1.72±0.11	24.89±0.97	1.59±0.03	2.43±0.15	Njagi *et al*., 2019
Lettuce	1.64±0.12	95.56±0.58	2.0±0.12	36.72±0.57	34.92±0.59	Fosu-Mensah *et al*., 2017
Tomato	0.4	6.5	NA	23.9	23.9	Felix-Henningsen *et al*., 2010
Spinach	2.65	19.5	NA	237.7	NA	Felix-Henningsen *et al*., 2010
Cabbage	0.32	19.9	NA	47.9	NA	Felix-Henningsen *et al*., 2010
Cassava leaves	NA	170.0	NA	2.5	113.7	Křībek *et al*., 2014
Cassava tubers	NA	14.4	NA	0.7	20.5	Křībek *et al*., 2014
Lettuce	0.5	117.2	16.5	6	218.4	Moreira *et al*., 2013
FAO/WHO permissible values in vegetables	0.02	10	10	0.1	0.6	FAO/WHO (1984)

**NA: not reported*

When heavy metals in soil occur predominantly in bioavailable forms, then plants grown in such soils can have high heavy metal concentrations in their tissues. Some studies suggest that the most important determining factor is the soil CEC. Factors which enhance the non reversible retention capacity of soils have a corresponding reducing effect on uptake of heavy metals by crops. Melo *et al*. (2014) studied transfer of Cd to crops grown on a Brazilian Entisol and Oxisol and reported more available Cd in the Entisol than the Oxisol which they attributed to higher CEC in the Oxisol than in the Entisol. Correspondingly, crops in the Entisol accumulated more Cd than those grown in the Oxisol. Moreira *et al*. (2013) studied absorption of a range of heavy metals by plants in Oxisols which were amended with sewage sludge applied at different rates (0, 60, 120, 180 tha^{-1}). They reported increased uptake of heavy metals by plants at sludge applictaions of 60 tonnes ha^{-1}, but a reduction at the higher rates due to increased CEC which caused immobilisation of heavy metals as a result of increased SOM.

Uptake of heavy metals by crops is also species specific, with leafy vegetables observed to accumulate the most compared to tubers and fruiting vegetables. Singh *et al*. (2012) evaluated the accumulation and distribution of heavy metals in several vegetables, grouped as leafy, root and fruiting, which were grown on contaminated soils in India. Singh *et al*. (2012) reported the highest and least heavy metals accumulation in leafy and fruiting vegetables respectively. Similar findings were reported by Latif *et al*. (2018), with spinach generally accumulating higher concentrations of heavy metals than melons and brinjal. The specificity of heavy metals (and micronutrients) uptake by plants was also observed by Melo *et al*. (2014), as they investigated the transfer of Cd and Ba by crops grown in tropical soils. They stressed that transfer coefficients (bioconcentration factor and transfer factor) are specific to soils and plants and, therefore, should not be applied universally in risk assessment models. Similarly, uptake of As, Cd, Pb and Zn by crops grown on and around the mine tailings at Bingham Creek in America was high in roots, but not in fruits of beans and tomato, in leaves and not roots of radish and in whole plants of lettuce (Cobb *et al*., 2000). Moreover, elements with specific functions (or known to substitute other elements with specific functions), are more readily translocated to the tissue where they are required. Lombi *et al*. (1998) observed that Cd, V and Zn were generally more concentrated in the leaves than in the seeds of sunflower grown in contaminated soils in Austria, whereas the opposite trend was observed for Cu and Ni. This observation could be attributed to importance of Cu and Ni in seed formation (Cataldo *et al*., 1978; Marshner 1995).

2.4. Determination of bioavailability of heavy metals

Although soil heavy metal bioavailability to plants can be estimated from solubility and speciation, it is usually preceded by measurements of lability. Studies have shown that labile concentrations as inputs in geochemical models for determining solubility give better estimates than when total metal concentrations are used as model inputs (Zhang *et al*., 2006; Izquierdo *et al*., 2013; Marzouk *et al*., 2013b). Moreover, the labile concentration is potentially bioavailable and so it is also useful for assessing the potential exposure to plants and other soil-borne organisms. Chemical extractions with weak neutral salt solutions have been used for a long time to determine the labile cations in soils. Other extractants include buffered salt solutions and chelating agents.

Recently, enriched stable isotopes have been used to help define the reactive heavy metal fraction in soils. Initially radioisotopes were used but the risks of handling radioactive materials limited their use. However, the development of spectroscopic equipment with high detection capabilities, such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) has made the use of stable isotopes possible (Beauchemin 2017). Similar approaches for determining labile concentrations are used to determine bioavailability in terms of solubility. These include chemical extractions, but usually of weaker concentrations (0.005 M DTPA, 0.01 M $CaCl_2$) than those used for determining lability (Groenenberg *et al*., 2017). Alternative methods to generate data to model bioavailability of heavy metals include physicochemical devices, such as Diffuse Gradient in Thin films (DGT) method, the Donnan Membrane Technique (DMT) and Anodic Stripping Voltammetry (ASV) which determine the free ion concentration in the soil solution. In most cases the results obtained by chemical extractions, isotopic dilution, physical soil analysis and modelling are compared to concentrations in plants which are grown in the medium under consideration. Mostly, chemical, physical and modelling assays have been validated against bioassays as the 'gold-standard' (Hodson *et al*., 2011).

2.4.1. Methods for determining heavy metal lability

2.4.1.1. Chemical extractions

Chemical extractions provide information on the solid-liquid partitioning of heavy metals in the soil. They may be multi-step sequential extractions or single extractions. Largely, however, chemical extraction regimes provide a metal-specific response to the particular extractant rather than a true reflection of the bioavailability or labile fraction of metals. Therefore, the results obtained by these extractions are variable and as Kabata-Pendias (2004) concluded, are element-specific and, depend on soil properties and the plant species under consideration. The choice of an extractant depends on the objective of the study but a good extractant is one which selectively solubilises only the labile fraction while minimising solubilisation of non-labile heavy metal fractions (Garforth *et al*., 2016). The extractants used to determine lability can be classified into three categories, namely: chelating agents, dilute acids and unbuffered salt solutions.

The mode of action for chelates is competition for heavy metal binding between the chelate molecule and the adsorption surface or competition with the metal ions for the adsorption surface binding sites (Hass and Fine 2010). This approach is useful for extracting the exchangeable and labile organically bound metals. However, they have been observed to extract non-labile metals bound to carbonates and (hydr)oxides of Fe and Mn when used in sequential extraction procedures (Marzouk *et al*., 2013a). Ethylenediaminetetraacetic acid (EDTA) is commonly employed as an extractant, usually in a disodium and diammonium form. The association of a chelating agent and a reactive monocation allows for a 'double action' on the mineral surface. Thus, the cation will compete with the adsorbed heavy metal cations for the adsorption surface while the chelating agent will compete with the adsorption surface to form soluble complexes with the heavy metal ions (Hass and Fine 2010). A standard concentration used to determine available trace elements is 0.05 M EDTA (Izquierdo *et al*., 2013), useful for estimating labile Ni, Cu, Zn, Cd and Pb for some peat/acidic soils but poor for alkaline mineral soils where it overestimates the labile fraction due to mobilisation and solubilisation of non-labile heavy metals (Garforth *et al*., 2016). Similar results were reported in earlier studies by Sabienë *et al*. (2004) and Marzouk *et al*. (2013a).

With dilute acids, extraction of metal cations is based on the competitive desorption by protons (H^+), whilst oxyanions are extracted due to protonation and partial dissolution of Fe, Al and Mn oxides to which they are adsorbed (Groenenberg *et al.*, 2017). Dilute acids are used to extract exchangeable ionss and those bound to Fe and Al (hydr)oxides. Assays in this category include extraction with 0.43 M nitric acid (HNO_3) and 0.43 M acetic acid (CH_3COOH). According to Hooda (2010), HNO_3 has the advantage that its counterion (NO_3^-) does not interfere in the mobilisation of heavy metals, whereas CH_3COOH and hydrochloric acid (HCl) have counterions which will likely complex cations in solution.

Using 0.43 M HNO_3, Groenenberg *et al.* (2017) reported recoveries ≥ 90 percent for several elements including Ni, Zn, Cd and Pb but concluded that the extractant was too weak to dissolve total reactive Cu at low Cu: SOM ratio, due to high surface binding affinity for Cu even at low pH during extraction. A similar observation was made by Garforth *et al.* (2016), who reported overestimation of labile Ni, Zn and Cd as a result of mobilising non-labile fractions. Dilute HNO_3 was reported to be more accurate in neutral to low pH soils than in calcareous soils (Marzouk *et al.*, 2013; Groenenberg *et al.*, 2017). Thus, the limitation of using dilute HNO_3 method is a possible overestimation of reactive heavy metals, especially for Ni and Zn in soils with high pH due to possible dissolution of Fe/Al (hydr)oxides and carbonates (Groenenberg *et al.*, 2017). Acetic acid works similarly to HNO_3 although theoretically could be expected to be a stronger extractant than the same concentration of HNO_3 owing to possible chelation by the acetate. However, its solubilisation of target heavy metals by complexation is negligible (Garforth *et al.*, 2016). Garforth used CH_3COOH on soils with variable pH from low to high and, using enriched stable isotope labelling, observed inconsistent results attributable to mobilisation of non-labile heavy metal and incomplete solubilisation of labile heavy metal concentrations. Therefore, based on the definition of a good extractant, both 0.43M HNO_3 and CH_3COOH fail to fully satisfy the requirements and their use must be carefully considered.

Other chemical extractants are neutral salts, usually nitrates and chlorides, of Ca, K, NH_4 and Mg. The mode of extraction by these cation salts is competitive adsorption between the cation in the electrolyte and the heavy metal ions held on the soil geocolloidal surfaces. Commonly, 1.0 M concentrations are used and nitrates are preferred to chlorides due to preferential solubilisation of Cd by chloride (Garforth *et al*., 2016). However, chloride salts could be a good predictor of labile Cd especially in low pH soils where $CaCl_2$ has been observed to quantitatively solubilise only the labile fraction (Garforth *et al*., 2016). Chloride salts are, however, too weak to completely solubilise the labile concentrations of other heavy metal such as Cu, Pb, Zn, Ni (Garforth *et al*., 2016)

Studies of bioavailability using these extractant have revealed the dependency of these methods on soil physical and chemical characteristics, leading to the conclusion that chemical extraction regimes provide a metal-specific response to the particular extractant rather than a real reflection of the bioavailability or labile fraction. Data from these extractions have been variable when compared to data from soil-to-plant transfer experiments. For instance, in a comparative study of soil–plant–animal transfer of Cd, Pb, Zn and Cu, Angelova *et al*. (2010) used 0.005 M DTPA to determine the labile fraction and although consistent with findings using sequential extraction procedures. The extraction data did not directly correlate with plant uptake. Similarly, Chenery *et al*. (2012) reported no direct correlation between 0.05 M EDTA extracted Pb and vegetation Pb concentrations. Kelepertzis and Argyraki (2015) subjected 45 soils to extraction with 0.005 M EDTA, 0.43 M HAc, and 0.43 M HNO_3, among others, and the results were variable with HNO_3 extracting the highest metal concentrations relative to the total and EDTA the least. However, they distinguished the extracted concentrations according to reagent used as: ‘potentially phytoavailable’, ‘mobilisable’ and ‘reactive’ for EDTA, HAc and HNO_3 respectively. Chemical extractions, however, are still widely used to estimate bioavailability owing to their simplicity compared to other techniques. Nevertheless, it is acknowledged that the results obtained may not be generalised for soil conditions outside the study and the data derived permit only a comparative analysis.

2.4.1.2. Isotopic dilutions

Isotopic dilution has been reported as the most accurate and reliable measure of elemental lability in soils, as it can provide an estimate of the fraction that immediately reacts with an added non-naturally occurring isotope, or enriched stable isotope, of the analyte heavy metal and thereby quantifies a reactive fraction commonly designated the 'E-value' (Young *et al*., 2005; Atkinson *et al*., 2011; Marzouk *et al*., 2013; Young 2013). A major advantage of isotopic dilution over chemical extraction is that there is minimal disturbance on the natural solid-liquid partitioning in the soil (Groenenberg *et al*., 2017). A weak electrolyte (e.g. 0.01 M $Ca(NO_3)_2$) or dilute chelating agent (e.g. 0.0001 - 0.0005 M EDTA), is used to partially solubilise labile heavy metal analyte but ensure non-mobilisation of non-labile heavy metal. Then an isotope of known isotopic abundance is added and the degree of dilution of the isotope is equated to the concentration of reactive analyte in the soil. Several authors have reported good prediction of bioavailability when E-values were used as inputs into geochemical speciation models to predict metal solubility (Hough *et al*., 2005; Marzouk *et al*., 2013b; Izquierdo *et al*., 2013).

2.4.1.3. Physical methods

These are direct methods of measuring heavy metal lability on solid soil samples without requiring extraction. They combine radiation with spectroscopy in exploring the structural molecules of the soils to reveal the nature of the bond between the heavy metals and the soil solids (Ehlers and Luthy 2003). They include: x-ray absorption fine structure (XAFS) analysis, X-ray diffraction (XRD), nuclear magnetic resonance (NMR) spectroscopy and scanning electron microscopy (SEM). The major disadvantage of these techniques is that they need a comparatively high metal concentration compared to levels often found in soil (Hooda 2010), which would make analysis of trace metal concentrations difficult. Such techniques can be useful in establishing a mechanistic understanding of bioavailability (Ehlers and Luthy 2003) and being non-destructive techniques means that they largely leave the soil physical and chemical characteristics of the soil intact during measurement (D'Amore *et al*., 2005).

2.4.2. Methods for determining heavy metal solubility/ or speciation

2.4.2.1. Chemical extractions

The principle of chemical extractions for determining solubility is the same as for determining lability. However, more dilute electrolyte solutions are used. Often these methods attempt to simulate soil pore water interactions within soil, thus only the soluble ions are targeted. Common extractants used include 0.01 M $CaCl_2$ (Houba *et al*., 1996), 0.005 M DTPA-TEA (Lindsay and Norvell 1978) and 1 M NH_4OAc (Zhang *et al*., 2006).

Calcium chloride (0.01 M $CaCl_2$) gives the advantage of being able to extract a wide range of cations and even dissolved organic matter at low concentration such that the soil chemistry is changed minimally during extraction (Houba *et al*., 1996). Moreover, being unbuffered ensures minimal changes in pH from the field conditions so the extraction occurs in more or less 'natural' conditions. Both the cation and anion play a role in the extraction process to some extent; while Ca^{2+} displaces other cations on the exchange surfaces of the soil, Cl^- ensures that the displaced cations are not re-adsorbed by weakly complexing them in solution (Gommy *et al*., 1998). However, excessive complexation of released cations by Cl^- may cause overestimation of bioavailable heavy metal. For example, Cd has a high affinity for Cl^-. Therefore, if it is the target ion, a nitrate salt solution may be a good alternative (Gommy *et al*., 1998). Houba *et al*. (1996) proposed 0.01 M $CaCl_2$ as a determinant of available plant nutrients and heavy metal bioavailability. However, heavy metal ions extracted by 0.01 M $CaCl_2$ would not necessarily represent the labile or bioavailable concentration since heavy metal ion activity is regulated by not only ion exchange, but also specific adsorption and precipitation (Houba *et al*., 1996).

DTPA-TEA here referred to as just DTPA is a standard method of extracting labile cations in soil. The method was originally developed to identify Zn, Cu, Mn and Fe deficiencies in neutral to calcareous soils (Lindsay and Norvell 1978). The extractant is a combination of 0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M triethanolamine and adjusted to pH 7.3 (Lindsay and Norvell 1978). The combination of reagents in this extractant ensures limited release of specifically bound and occluded cations which are not bioavailable while still employing chelation to effectively release target cations. Fonseca *et al*. (2010) found DTPA ineffective to predict the soil cationic micronutrients availability for a wheat crop after surface application of lime. However, the soils in this study had pH < 6, which is outside the range for which this method was developed.

Ammonium acetate (1 M NH_4OAc) equally has the advantage of weak chelation and has a monovalent cation in its molecule. It is buffered at pH 7.1 and at 4.8 depending on the chemical characteristics of the soil being extracted. In calcareous soils, NH_4OAc should be buffered at pH 7 to prevent release of non-soluble carbonate bound heavy metals (Gommy *et al*., 1998) while in acidic soils, it should be buffered at lower pH (Yu *et al*., 2004). Yu *et al*. (2004) reported that 1 M NH_4OAc was the best extractant for determining plant-available Cu in acidic variably charged soils. Ammonium acetate is normally used in sequential extractions to extract the exchangeable fraction, bound predominantly by electrostatic forces only.

In an evaluation of several single extractants, including DTPA, NH_4OAc and $CaCl_2$, for prediction of bioavailability of Cu, Zn, Cd and Pb to rice, Zhang *et al*. (2010) reported that the best correlation was with $CaCl_2$ and NH_4OAc. The bioavailable heavy metals were attributed to the exchangeable and water-soluble fractions. DTPA did not correlate well with plant uptake as chelates tend to extract organically bound fractions, exchangeable and water-soluble heavy metals. A satisfactory prediction of Cu and Pb in wheat roots was reported by Soriano-Disla *et al*. (2010). In contrast, Bakircioglu *et al*. (2011) reported a good correlation between DTPA-extracted heavy-metal and wheat grain heavy metal content while heavy metal concentrations in $CaCl_2$ extractions were relatively poorly correlated with bioavailability. However, Feng *et al*. (2005) reported poor, or no, correlation of both $CaCl_2$ and DTPA heavy metal concentrations with uptake by barley roots. Some of these methods are adopted as a standard procedure in specific study areas, such as soil fertility studies, but result in variable outcomes depending on soil to solution ratio, concentration of extractant, equilibration time and temperature (Leggett and Argyle 1983). To ensure that chemical extractions correlate with true bioavailable fractions, validation against plant uptake data is necessary and this is common practice for standards methods.

2.4.2.2. Physico-chemical methods

Diffusive Gradient in Thin-Films (DGT). The DGT tool works by allowing heavy metal ions to diffuse through a membrane filter and a layer of diffusional gel to a cation binding resin gel which represents a sink for heavy metals. The resin is then eluted in an appropriate extractant (HNO_3 for heavy metal) before analysis by spectroscopic detectors such as ICP-MS. As the analytes are absorbed, a concentration gradient is created at the soil-DGT interface thereby stimulating desorption from the exchange surface (Zhang and Davison 1995). This mechanism is similar to processes that occur during plant uptake where the ions in the soil solution that are taken up by plant roots are resupplied by the soil solid exchange surfaces. Thus, DGT have been reported to simulate heavy metal bioavailability more accurately than chemical extractions (Bade *et al*., 2012). According to Song *et al*. (2015), the DGT was superior to several extraction methods in estimating Cd bioavailability to rye grass. Similar findings were reported by Soriano-Disla *et al*. (2010) who evaluated the DGT and several chemical extractants for determination of bioavailability. Moreover, the DGT technique has been reported to be independent of soil properties that affect determination of bioavailability by chemical extractions (Dai *et al*., 2018; Song *et al*., 2015). However, the free ion activity in the soil solution is not the only factor affecting uptake by plants. Ion uptake by plants is said to also be regulated by internal controls. These include antagonistic mechanisms between ions (Degryse *et al*., 2012), for example, Song *et al*. (2018) observed a negative correlation between Ni content and that of Cr, Cu, Cd in the plant tissue. They postulated that Cr, Cu and Cd could be inhibiting Ni uptake by the plant. Thus, under such conditions, DGT may fail to predict heavy metal bioavailability (Song *et al*., 2018).

Donnan Membrane Technique (DMT). The DMT is a tool used to determine free metal ion concentration of cations which can be used for all divalent cations including heavy metals (Marang *et al*., 2006). This technique was first developed by Berggren (1990). It is a device that comprises two compartments, containing the sample solution and a synthetic solution called the donor and acceptor respectively. The two solutions are separated by a negatively charged cation exchange membrane which allows selective permeation of free cations from both solutions while repelling anions and metal complexes. An equilibrium is subsequently reached between the sample and acceptor solutions and the composition of both can then be determined by spectroscopic techniques such as ICP-MS. The technique allows for analysis of multi-species present in the sample (Wu *et al*., 2000; Li *et al*., 2005; Kalis *et al*., 2007; Ren *et al*., 2015). It is, however, not without limitations, the most highlighted being a long equilibration time, which can extend over several days (Lao *et al*., 2018; Marang *et al*., 2006). Another disadvantage is that it has a very small acceptor solution volume, which poses a challenge for analysis. However, the development of small sample volume introduction systems to the ICP-MS has helped to overcome this challenge (Hooda 2010). Wu *et al*. (2000), Li *et al*. (2005) and Ren *et al*. (2015) used DMT in their studies on the effect of DOM composition on heavy metals speciation and reported good correlations between DMT measured species and modelled speciation. The biggest advantage of DMT over other speciation techniques is the simultaneous speciation of several cations whilst maintaining the balance between the measured system and its surroundings. Kwon-Rae and Owens (2009) however, reported significant mass loss of Pb, Cu, Cd and Zn from the initial total concentration in the solution, which they attributed to adsorption by the ion exchange membrane especially in alkaline solutions.

Geochemical modelling. When the idea of geochemical modelling was first introduced, its only objective was to predict the solid-solution partitioning of inorganic species from established thermodynamic equilibrium data (Dudal and Gérard 2004). However, geochemical speciation modeling in aqueous systems has evolved from only containing data inputs of an inorganic nature (Dudal and Gérard 2004), to assemblages comprising various sub-models to describe metal ion binding to both inorganic and organic ligands and to a range of geocolloidal components (Lofts and Tipping 2011). Advances have been made towards consideration of reaction kinetics (Mayer *et al*., 2002) and uncertainty of stability constants in geochemical models (Hooda 2010) resulting in their wider applicability in soil geochemical studies. Applications have included toxic element behavior and fate in soils (Marzouk *et al*., 2013; Izquierdo *et al*., 2013), fixation processes (Buekers 2007), adsorption/desorption equilibria (Gustafsson 2003) and precipitation/dissolution reactions and their effect on chemical speciation (Lin and Singer 2006). Several geochemical models which predict speciation in aqueous phases and complexation to specific solid phases while assuming chemical equilibrium have been developed. Notable widely used examples include:

ORCHESTRA (Meeussen 2003), MINTEQ (Gustafsson and Schaik 2003; Tipping 1994), NICA-Donnan (Benedetti *et al.*, 1996) and WHAM (Tipping 1994). Most of these models were originally developed for water systems based on data from isolated 'purified' or synthesised solid phases. Whether these models work in complex systems, such as the soil, lies in how well they describe ion adsorption by important binding phases such as Fe/Al oxyhyroxides, aluminosilicate clays, natural organic substances and soluble ligands. However, these models inevitably have limitations. For example, Dudal and Gérard (2004) evaluated 6 geochemical speciation models, based on how they accounted for natural organic matter, and found that only the NICA-Donnan and WHAM models were able to reasonably describe most of the experimental results.

The Windermere humic aqueous model (WHAM) VII is a mechanistically based chemical equilibrium model which works by partitioning the free energy of metal-colloidal associations in aqueous systems into chemical bonding and electrostatic interactions (Tipping 1994). Model VII extends and improves the functionality of version VI which was also improved from the initial WHAM V version. The main feature which has been improved upon from the original version is binding to humic matter (Humic Ion Binding Model VI and VII) in which heavy metal binding was underestimated in the original version (Tipping 2002). The WHAM VII model is an assemblage of three sub-models which describe metal bonding to specific particulate and colloidal fractions (Lofts and Tipping 2011; Tipping 1998). These include: the Humic Ion Binding Model VII for metal binding to humic and fulvic acids, a cation exchange model for electrostatic metal binding to clay minerals and the SCAMP model for metal binding to Fe and Mn oxyhydroxides. The concentration of particulate fractions is externally inputted as part of input parameters and the model assumes that partitioning to these fractions is additive. Default parameters, based on extensive laboratory studies and validated for field conditions (Lofts and Tipping 2011), for many metals have been inputted into the model. The WHAM VII model needs inputs of: soil (solution) temperature, pH, clay content, Fe/Mn oxyhydroxides, particulate FA and HA, colloidal FA, major dissolved cations and anions and the reactive concentration of the heavy metal of interest, to output solution speciation and solid phase fractionation. The output by WHAM VII therefore, should help with understanding of the dominant sorption phases for specific metals in particular soils and, potentially, the effect of soil managerial changes on heavy metal bioavailability in the soil.

The WHAM VII model has been used in a few studies on temperate soils (Buekers 2007; Izquierdo *et al*., 2013; Marzouk *et al*., 2013b) but there are no substantial investigations on mineral soils of tropical nature with low pH values, low organic matter contents and high metal oxides concentrations. The WHAM VII model has been tested against field experiment data for speciation efficiency and it was generally found to predict metal speciation reasonably well - especially for Ni, Zn and Cd among others (Lofts and Tipping 2011). Izquierdo *et al*., 2013 and Marzouk *et al*. (2013b) reported good prediction of Cd, Zn and Pb speciation using isotopically exchangeable concentrations (E-values) as inputs for the metals. However, Buekers *et al*. (2008) reported overestimation of the concentration of free Cu and Pb in high alkaline soils when default particulate concentrations were used. Otherwise, good prediction was observed in acidic organic soils (Tipping *et al*., 2003). Ensuring efficient speciation by WHAM VII (or any geochemical model) requires accurate inputs. Since there are no 'WHAM specified' methods to obtain these parameters, variable methods are used according to the preferences of the investigators. For example, HA and FA can be estimated from total organic matter content (LOI) or from measurements of total organic carbon. Furthermore, it is assumed in the model that all humic bound metals are labile, but recent studies have shown otherwise (Mao *et al*., 2015). Various potential heavy metal adsorbents are not yet considered in WHAM VII, including Ca carbonate, Ca phosphate and hydroxy-interlayered clays. There is a need therefore to improve these assumptions to optimise the model's performance. If properly validated and input variables measured with standard methods, WHAM VII may be a valuable tool for risk assessment of soils affected by heavy metals as it outputs variables which are directly linked to bioavailability.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Overview

In this chapter are presented all the materials and methods used in this study. The materials and methods are grouped into ten thematic sections as follows: i) Description of the study site; ii) Conduct of the field experiment; iii) Conduct the pot experiment; iv) Soil characterisation; v) Determination of heavy metal fractions in soil samples; vi) Determination of heavy metal concentrations in plant samples; vii) Determination of soil sorption phases; viii) Prediction of solubility, speciation and fractionation using WHAM VII; ix) Statistical analysis; x) Quality control.

3.2. Description of study site

The study was conducted in the Mugala area (12°47’20’’S and 28°06’10’’E) of Kitwe district in Copperbelt province Zambia (Figure 3-1). The site was located on agricultural fields adjacent to a 711 ha mine tailings dam along the Kalulushi – Mufulira road. The site was chosen because of the expected enrichment of the soils with mine tailing materials, which have been documented to have high concentrations of heavy metals (García-Giménez and Jiménez-Ballesta, 2017; Gevorgyan *et al.*, 2015; Li and Yang, 2008). Mugala area is in agro-ecological region III of Zambia, which is characterised by a high rainfall of 1000 -1500 mm per annum. The area has an average minimum temperature of 11.5 ⁰C and a maximum of 28.2 ⁰C. The predominant wind direction is from the North-west to South-east. The mean wind speed ranges from 2.4 m s^{-1} to 2.9 m s^{-1}, although winds gusts can reach 30 m s^{-1}.

The full pedological description of the soils was conducted and the results are presented in Appendix 1. The area is characterised by deep, well drained, highly weathered soils. The topsoils are dark reddish brown, dark red to red in the mid profile, and reddish yellow in the base of the profile. The texture is predominantly sandy loam to sandy clay loam for the topsoil, while the subsoils are sandy clay to clay. The soils have a well-developed structure with a weak subangular morphology in the top, a strong structure in the mid horizons and becoming weak again in the subsoil, which is a common characteristic of highly weathered soils. The topsoils tend to be loose when dry, and are non-sticky when wet. According to the FAO-UNESCO system, the soils in this area are classified as orthi-rhodic Ferrasols (Soil Survey Unit 1991).

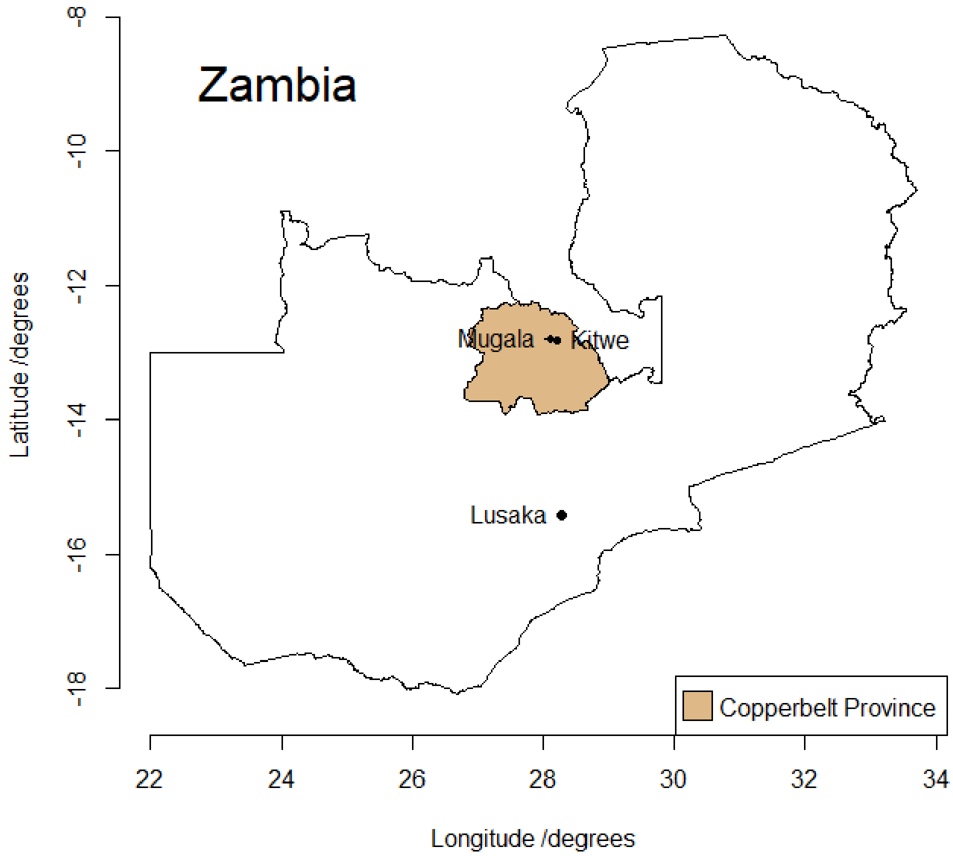


Figure 3-1: Location of the study site.

3.3. Field experiment set-up and sampling

A field experiment was set up across two seasons commencing in the 2016/17 cropping season and re-cropped in the 2017/18 season. The objective of the experiment was to determine the effects of liming and manuring treatments on the bioavailability of Cd, Cu, Ni, Pb and Zn, and to compare their (amendments) effects in settings close to the tailings dam, and further from the dam.

The experiment consisted of sixteen sub-plots measuring 4.5 × 10 m (four treatments and four replicates) which were set up in a Randomised Complete Block Design

(RCBD). The treatments included: lime applied with no manure (L1M0); chicken manure applied with no lime (L0M1); lime and manure both applied (L1M1); a control treatment with no amendments (L0M0). Two sites (B1 and B2 in Figure 3-) were selected, each about 300 - 400 m from the tailings dam, and these were designated as 'far' (Dam1 or D1). Another two sites were selected which were about 100 – 200 m from the tailings dam (B3 and B4 in Figure 3-), designated as 'near' (Dam2 or D2). The sites were selected on the basis of previous reconnaissance sampling of the land around the dam (Lark *et al*., 2017). These sites were the main-plots in this experiment. Within each of these main-plots, each of the four agronomic treatments were allocated independently and at random to the sub-plots. Soil samples were collected from each plot within the 0 – 20 cm soil depth, just before the treatments were applied and during crop sampling at specific periods in each of the two consecutive cropping seasons.

Figure 3-2: Aerial view of the study area showing location of the fields and their proximity to the tailings dam.

Note: Locations B1 and B2 were considered as 'far' from the dam (D1), while B3 and B4 were considered 'near' to the dam (D2).

Land preparation was done according to farmer practice in this area where crops are planted on ridges. Each plot had six 10 m long ridges, spaced 90 cm apart. Calcitic lime and chicken manure (will be referred to as manure) treatments were only applied in the first season (2016/17) and their residual effect was observed in the subsequent season. Both amendments were applied only within the ridge. Lime was applied on 23 November 2016 at an equivalent of 2 ton ha^{-1}, while manure was applied a month later (21 December, 2016) at a dry weight equivalent of 5 ton ha^{-1}. The rates of lime and manure were based on the Ministry of Agriculture recommendation for the area. Maize and pumpkins were intercropped within the plots. Maize was planted at an equivalent of 44,444 plants ha^{-1} translating into 200 plants $plot^{-1}$. The plant population for pumpkins was 18 plants $plot^{-1}$. In the first season, maize and pumpkins were planted on 23rd December 2016 and on 9th December 2017 in the subsequent season.

Six weeks after planting, paired soils and pumpkin leaf samples were collected within a 'harvest plot' located within each sub-plot. A harvest plot was created by eliminating one metre from both ends of each ridge (row) and the two border rows in each plot. Two random soil samples were collected per plot from vertices of a square (side length 90 cm) then composited into one sample before being transported to the laboratory where the soil was air dried, sieved to <2 mm and stored in paper bags. A sub-sample of each soil was ground using an agate mortar and pestle, and stored in paper bags pending organic matter and total metal analysis. About 200 g of pumpkin leaves were picked with their stalks at about 5 cm from the ground. Plant samples were washed with tap water three times and rinsed once with distilled water. The leaf samples were then oven-dried at 55 ^{o}C, cooled in a desiccator and finely ground using stainless steel coffee grinders before being stored in paper bags, pending analysis.

Figure 3-3: Field experiment

Five months after planting, soil, maize cobs and stover were sampled from the same harvest plot marked for pumpkin. A sample of stover was collected and prepared as described for pumpkin leaves. The cobs were then shelled and about 100 g of grain was oven-dried overnight at 55 ^{o}C, cooled, and finely ground in coffee grinders before being stored in paper bags pending analysis. All elemental analyses were conducted at the British Geological Survey, Inorganic Geochemistry Laboratory, Keyworth, UK.

3.4. Pot experiment set-up and sampling

A pot experiment was set up in a glasshouse at ZARI Chilanga to evaluate the bioavailability of Cu in the soils from the study site and assess the performance of the WHAM VII geochemical model in predicting solubility, speciation and fractionation. Soil samples were collected at 90 (+10) locations selected to give good spatial coverage of the area with the strGrid function from the spcosa package for the R platform (R Core Team 2017) (Walvoort *et al*., 2010). The soils were sampled at 0-20 cm depth and from outside the areas for the field experiments. At 10 of the 90 locations, one additional sample was collected at 1 m distance in a random direction to support statistical modelling of the data (Lark and Marchant, 2018). At each sample location a composite sample was formed by combining soils taken from the vertices and centre of a square (side length 30 cm). Sampling was done using a bucket auger and 4 kg of soil was placed into plastic sample bags after sieving to < 4 mm. The soil was air dried and about 100 g of the sample was sieved to < 2 mm and stored in paper bags pending laboratory analysis of labile Cu (using DTPA extraction and isotopic dilution methods) and soluble Cu (extraction in 0.01 M $Ca(NO_3)_2$). A sub-sample of the <2 mm sieved soils was ground to <53 µm for total Cu analysis.

One kg of each of the soil samples were put in separate labelled planting pots each pot represented a geo-referenced location in the field. Three replicates of each soil sample were prepared and arranged in a completely randomised design. Five grams of N, P, K (10:20:10) fertiliser was mixed with the soil and two weeks old Chinese cabbage (*Brassica rapa*) plants, germinated and grown on vermiculite, were transplanted into the pots (one plant per pot). The moisture content in the pots was maintained around field capacity. The plants were harvested after eight weeks of growth.

Figure 3-4: Chinese cabbage plants during the pot experiment

Using a pair of scissors, plants were cut at 1 cm above the soil level. The plant tissue was then thoroughly washed with tap water (three times) and then immediately rinsed in distilled water. Samples were then air-dried until visibly dry, then placed in the oven at 55 ºC until a consistent weight was attained. The plant samples were then finely milled in stainless steel coffee grinders, after cooling in desiccators, and stored in paper bags pending elemental analysis for total Cu concentrations.

3.5. Soil characterisation

All soils used for soil characterisation were sieved to < 2 mm, except for those used for determination of (i) organic matter content by loss on ignition (LOI) and (ii) total metal concentration, which were milled to <53 µm particle size.

3.5.1. Determination of soil pH

Soil pH was measured using an Orion 720A+ pH meter, in 0.01 M $CaCl_2$ soil suspensions with a soil-to-solution ratio of 1:2.5. The pH meter was calibrated prior to making measurements, using pH buffers at 4.0 and 9.0, and checked with a pH 7 buffer solution. The soil suspensions were put on a magnetic stirrer for 15 min and allowed to equilibrate for a further 5 min before determination of pH.

3.5.2. Determination of particle size fractions

Five ml H_2O_2 was added to 0.35-0.4 g of soil and left overnight to oxidise the soil organic matter. Then a further 5 ml of H_2O_2 was added to the soil suspension which was shaken before adding 5 ml of the dispersing agent Calgon (0.057 M sodium hexametaphosphate and 0.66 M calcium carbonate) and left overnight on a hot water bath at 70 ºC to fully disperse the soil inorganic particles. Particle size analysis was done using a laser diffraction particle size analyser model LS 13 320.

3.5.3. Determination of organic carbon

Soil samples for the field experiment were characterised for organic carbon content by the Walkley and Black method (Nelson and Sommers, 1982). Approximately 1g ± 0.1 was weighed into a conical flask and 10 ml of 1 N Potassium dichromate solution ($K_2Cr_2O_7$) was added. Then 20 ml of concentrated sulphuric acid was promptly added and the mixture stirred gently at first then more vigorously, for one minute. The suspension was allowed to react for 30 min, to allow for complete oxidation of organic matter before 150 ml of distilled water and 10 ml of concentrated phosphoric acid were added. Then 1 ml diphenylamine indicator was added to the suspension before titrating with iron (II) sulphate solution until the solution turned green from an initial dark blue colour. The same procedure was done for blank solutions. The amount of ferrous sulphate used to titrate the residual dichromate was used to compute the organic carbon content, and the soil organic matter content was determined by assuming that it is 1.774 times more than organic carbon in mass (i.e. SOM is assumed to be 56.4percent carbon). Therefore, SOM was calculated using Eq. 2.

$$\% SOM = \%C\ X\ 1.774 \qquad \text{Eq. 2}$$

Determination of organic matter by loss on ignition (LOI)

Loss on ignition was used to estimate the soil organic matter content in soils from the pot experiment, and in the field experiment before and after application of amendments to assess the treatment effects. About 1 g (1 ± 0.9000 g) of each air-dried soil was put in the drying oven at 105 ± 4 °C for four hours before cooling in the desiccator. Their weights were then recorded before being ignited in the furnace at 450 ± 30 ºC for four hours. The samples were then re-weighed after cooling in a desiccator and LOI was determined gravimetrically using Eq. 3

$$\%\mathrm{SOM} = \frac{\mathrm{b-c}}{\mathrm{b-a}} \mathrm{X100} \qquad \text{Eq. 3}$$

where a is the weight of the container, b-i is the weight of the container and sample before ignition, c is the weight of the container and sample after ignition.

3.5.4. Determination of total nitrogen content

Total nitrogen was determined using the Kjeldahl method. To 1 g of soil 10 ml of concentrated sulphuric acid and 3 g of catalyst mixture (Se powder, Cu sulphate, K sulphate) were added. The suspensions were then digested on block digester set at 410 ºC until samples cleared. The digests were allowed to cool then the end of the condenser of the distillation apparatus was dipped in 25 ml boric acid before commencing the distillation process. Then the distillate was titrated with 0.01 M hydrochloric acid until a colour change from green to pink was observed. The same procedure was done for a blank and the total N content was computed using Eq. 4.

$$\% \text{ N} = \frac{(S_T - B_T) \times \text{molarity of acid (M)} \times 1.401}{\text{weight of soil used (g)}} \qquad \text{Eq. 4}$$

where S_T is the titre volume of standard acid for sample and B_T is the titre volume of standard acid for blank in ml, 1.401 is the molar mass of nitrogen divided by 10 to convert the value to percentage.

3.5.5. Determination of available phosphorus

Available phosphorus was determined by the Bray 1 method. Soil and extracting solution containing 1 M NH_4F and 0.5 M HCl, was mixed at a 1:7 ratio. A 5 ml aliquot of supernatant was collected into a flask after shaking for one minute and then filtering. Distilled water and 4 ml of reagent containing ammonium molybdate, potassium antimony tartrate, 2.5 M sulphuric acid and ascorbic acid were added. The solution was then allowed to react for 15 minutes to develop colour. A blank and a set of standards were also prepared to derive the calibration curve on which the amount of P in the sample was extrapolated. Using a Jenway 6305 UV spectrophotometer transmittance at 882 nm wavelength for blanks and standards were plotted into a calibration curve relating transmittance to P concentration. The P concentration of samples were then obtained from the standard curve for respective transmittance of the samples.

3.5.6. Determination of exchangeable cations, exchangeable acidity and ECEC

Exchangeable cations were determined following extraction of soil in a 1:5 (w/v) suspension of 1M ammonium acetate buffered at pH 7. After equilibration on a shaker for 30 minutes, the suspension was filtered to < 0.45 µm before analysing the filtrate for K^+ and Na^+ on the Analyst 400 Perkin Elmer flame atomic absorption spectrophotometer (FAAS) and Ca^{2+} and Mg^{2+} by Atomic Absorption Spectrometry.

Exchangeable acidity was determined by a titration method following extraction of 4 g of soil in 40 ml of 1 M KCl. Twenty-five ml of filtrate was collected and 5 drops of phenolphthalein indicator was added before titrating with 0.01 M NaOH solution to a permanent pink endpoint. The volume of NaOH used was used to compute exchangeable acidity. The effective cation exchange capacity (ECEC) in cmol(+) kg^{-1} was computed by the summation of the exchangeable cations and exchangeable acidity.

3.6. Determination of heavy metal fractions in soil samples

3.6.1. Acid digestion for total soil metal concentration (M-tot)

Total concentrations of Cd, Cu, Ni, Pb, Zn were determined in all soil samples from the field experiment, and Cu only in samples from the pot experiment. Soil samples (0.2500g±0.0025) were pre-digested with 3 ml of 5 percent and 3 ml 50 percent of nitric acid. Then the suspension was allowed to dry down for 16 hours at 80 °C. After cooling, 2 ml of concentrated nitric acid, 2.5 ml hydrogen fluoride and 1 ml perchloric acid were added. The vials were placed in a programmed hot block: 8 hrs @ 80 °C, 2 hrs @ 100 °C, 1 hr @ 120 °C, 3 hrs @ 140 °C, 4 hrs @ 160 °C. The samples were then cooled to 50 °C, after which 2.5 ml 50 percent nitric acid was added. The mixtures were then allowed to react for 30 minutes at 50 °C and then later cooled to 30 °C. Then 10 ml MilliQ water (18.2 MΩ cm^{-1}) was added together with 2.5 ml of hydrogen peroxide. The suspensions were allowed to settle for 15 min before being transferred into 30 ml bottles and the volume made up to 25 ml with 10 ml of MilliQ water. The bottles were loosely capped overnight and then tightened for storage pending elemental analysis.

Subsequent total elemental analyses of the acid digests were carried out using ICP-QQQ-MS (Agilent 8900 ICP-QQQ-MS). A collision cell gas (He) was used to reduce polyatomic interferences of Ni, Cu, Zn, Cd, and Pb; internal standards employed to correct for signal drift included Sc, Ge, Rh, In, Te and Ir. An ISIS-3 sample introduction loop (Agilent Technologies, USA) was used to minimise the volume of sample (500 µL) aspirated to the ICP-MS to reduce the risk of carryover between samples (Watts *et al.*,2019). Then M-tot was calculated using Eq. 5.

$$M - tot = R \times DF \times \frac{V}{W} \quad \text{Eq. 5}$$

where M-tot is the total heavy metal concentration in mg kg^{-1}, R is the instrument reading i.e. the concentration of the heavy metal in the analysed solution in mg L^{-1}, DF is the dilution factor of the solution prior to analysis, V is the volume in L of the extracting solution and W is the weight of the soil in kg

3.6.2. Labile heavy metals by DTPA extractions (M-dtpa)

Five grams of soil were extracted with 10 ml of DTPA solution (0.005 M DTPA, 0.01 M $CaCl_2$, 0.1 M TEA adjusted to pH 7.3 with HCl) according to the method of Lindsay and Norvell, (1978). The suspensions were centrifuged, filtered to <0.2 µm and diluted five-fold prior to analysis. For quality control purposes, duplicate samples from every batch of ten samples and reagent blanks were included. Copper concentrations (Cu-dtpa) were determined by ICP-QQQ-MS with matrix-matched calibration and quality control standards. Then M-dtpa was calculated using Eq. 6.

$$M - dtpa = R \times DF \times \frac{V}{W} \quad \text{Eq. 6}$$

where M-dtpa is the concentration of heavy metal extracted using DTPA extractant, R is the instrument reading i.e. the concentration of the heavy metal in the analysed solution in mg L^{-1}, DF is the dilution factor of the solution, V is the volume in L of the extracting solution and W is the weight of the soil in kg.

3.6.2.1. Optimisation of ICP-QQQ instrumentation for analysis of DTPA extracts

The analysis of bioavailable metals in DTPA extracts is commonly undertaken using either mono- or multi-elemental spectroscopic techniques with high matrix tolerance, such as flame atomic absorption spectroscopy (FAAS) and/or inductively coupled plasma atomic emission spectroscopy (ICP-AES) (de Abreu *et al.*, 1997). The high concentration of magnesium (Mg) in the extracted samples present as a component of the extraction solution matrix, can cause significant analytical signal drift and an overall reduction in the number of samples that can be analysed in a single batch (Tan and Horlick, 1987). One way to overcome this matrix effect is to apply a physical dilution to the samples prior to analysis. However, this can be time-consuming if a large number of samples require processing, introduces a potential source of error if the density of the sample is significant (Higgins *et al*., 1998) and can result in increased detection limits for analytes of interest (Godshaw *et al*., 2017).

In this study, DTPA extracts were analysed using an Agilent 8900 ICP-QQQ inductively coupled plasma triple quadrupole mass spectrometer (Agilent Technologies, Tokyo, Japan) equipped with an Ultra High Matrix Introduction (UHMI) accessory. This system introduces a flow of argon (Ar) gas between the spray chamber and the torch, diluting the aerosol produced by the nebuliser and improving the tolerance of the plasma to matrix suppression and/or enhancement.
The use of ICP-QQQ for analysis of DTPA extracts resulted in similar detection limits (Table. 1) when compared to previously published values for ICP-OES (Kulikov, 2016b), and superior detection limits when compared to microwave plasma atomic emission spectroscopy (MP-AES) (Kulikov, 2016a).

Table 3-1: Comparison of detection limits for determination of selected metals in DTPA extracts

Element	DL ICP-QQQ (mg kg^{-1})	DL ICP-OES (mg kg^{-1}) (Kulikov, 2016b)	DL MP-AES (mg kg^{-1}) (Kulikov, 2016a)
Cu	0.003	0.003	0.06
Fe	0.007	0.012	0.03
Mn	0.006	0.005	0.03
Zn	0.005	0.002	0.05

3.6.3. Labile copper by isotopic dilutions (Cu-E)

Isotopically exchangeable Cu (Cu-E) was determined according to the method described by Marzouk *et al*. (2013). Two g of <2 mm soil was suspended in 25 ml of 0.00001 M EDTA-NH_4 on a shaker for 3 days. Each sample was replicated five times and three blanks, with 0.00001 M EDTA-NH_4 alone, were included in each batch. An enriched stable isotope of Cu (^{65}Cu) with a certified isotopic abundance (IA) of 99.0 percent was purchased from ISOFLEX USA as a metal foil, dissolved in HNO_3, and diluted with Milli-Q water to give a primary stock solution with a ^{65}Cu concentration of 3000 mg L^{-1} in 5 percent HNO_3. The soil samples were grouped into two according to the total Cu concentration in the soil and a fresh spike solution (480 µg ml^{-1} and 900 µg ml^{-1}) was made from the primary stock for each group. The concentration of the spike used was estimated to cause, at least, a 50 percent increase in natural abundance of ^{65}Cu in the soils. Three out of the five soil replicates were spiked with 0.4 ml of the spike solution, while two samples were not spiked to act as controls from which to derive the natural abundances of Cu isotopes in the individual soils. After spiking, the soil suspensions were equilibrated further for 2 days before being centrifuged and filtered (<0.2 µm). The filtered supernatant in all spiked, and two unspiked, solutions were acidified to 1 percent HNO_3 in order to stabilise the elements prior to determination of Cu concentration by ICP-MS.

Values of Cu-E (mg kg^{-1}) were calculated using Eq. 7 (Marzouk *et al*., 2013) from the isotopic abundance ratio of the spike isotope (^{65}Cu) to the selected background isotope (^{63}Cu).

$$Cu\ E\ =\ \left(\frac{Am_{Cu\ soil}}{W}\right)\ \left(\frac{C_{spk}V_{spk}}{Am_{Cu\ spk}}\right)\ \left(\frac{{}^{spk}IA_{spike} - {}^{bg}IA_{spike}\,R_{ss}}{{}^{bg}IA_{soil}R_{ss} - {}^{spk}IA_{soil}}\right) \qquad \text{Eq. 7}$$

where $Am_{Cu\text{-}soil}$ and $Am_{Cu\text{-}spk}$ are the average atomic masses of Cu in the soils and the spike isotope solution respectively, W is the weight of the soil (kg), C_{spk} is the gravimetric concentration of the Cu in the spike solution, V_{spk} is the volume of spike added (L), IA is the isotopic abundance and R_{ss} is the ratio of isotopic abundances, spiked (spk): background (bg), for the two isotopes in the spiked soil solution.

3.6.4. Determination of soluble heavy metals (M-soln) by extraction with 0.01 M $Ca(NO_3)_2$

Two grams of soil were suspended in 25 ml of 0.01 M $Ca(NO_3)_2$ solution and equilibrated on a shaker for 3 days. The suspensions were centrifuged and filtered to <0.2 µm. Filtrate was diluted 10-fold and Cu, Cd, Ni, Pb and Zn concentrations were determined by ICP-QQQ-MS with matrix-matched calibration and quality control standards. Then concentration in plant samples was calculated using Eq. 8.

$$M - soln =\ R \times DF \qquad \text{Eq. 8}$$

where M-soln is the concentration of heavy metal extracted in 0.01M $Ca(NO_3)_2$ in mg L^{-1}, R is the instrument reading i.e. the concentration of the heavy metal in the analysed solution in mg L^{-1}, DF is the dilution factor of the solution.

3.7. Determination of total heavy metal concentrations in plant samples

Vegetation samples (pumpkin leaves, maize stover, maize grain and Chinese cabbage) were analysed for elemental composition using an ICP-QQQ-MS (Agilent 8900 ICP-QQQ-MS) following acid-digestion in a microwave digestion system as described by Watts *et al*. (2019). About 0.5000g ± 0.0050 of ground vegetative materials was digested in 10 ml HNO_3:1 ml H_2O_2 in a closed vessel microwave heating system (Mars Xpress, CEM Corporation, Mathews, USA). The solutions were then cooled and vented before adding 30 ml MilliQ water and the contents transferred into a 60 ml Nalgene bottle. Nine ml of MilliQ water was added to the vial in order to rinse off any residue and poured into the bottle containing the solution. The bottles were then kept in a cool place pending elemental analysis. A 10-fold dilution was done prior to analysis and concentrations of heavy metals in crop sample were obtained using Eq. 9.

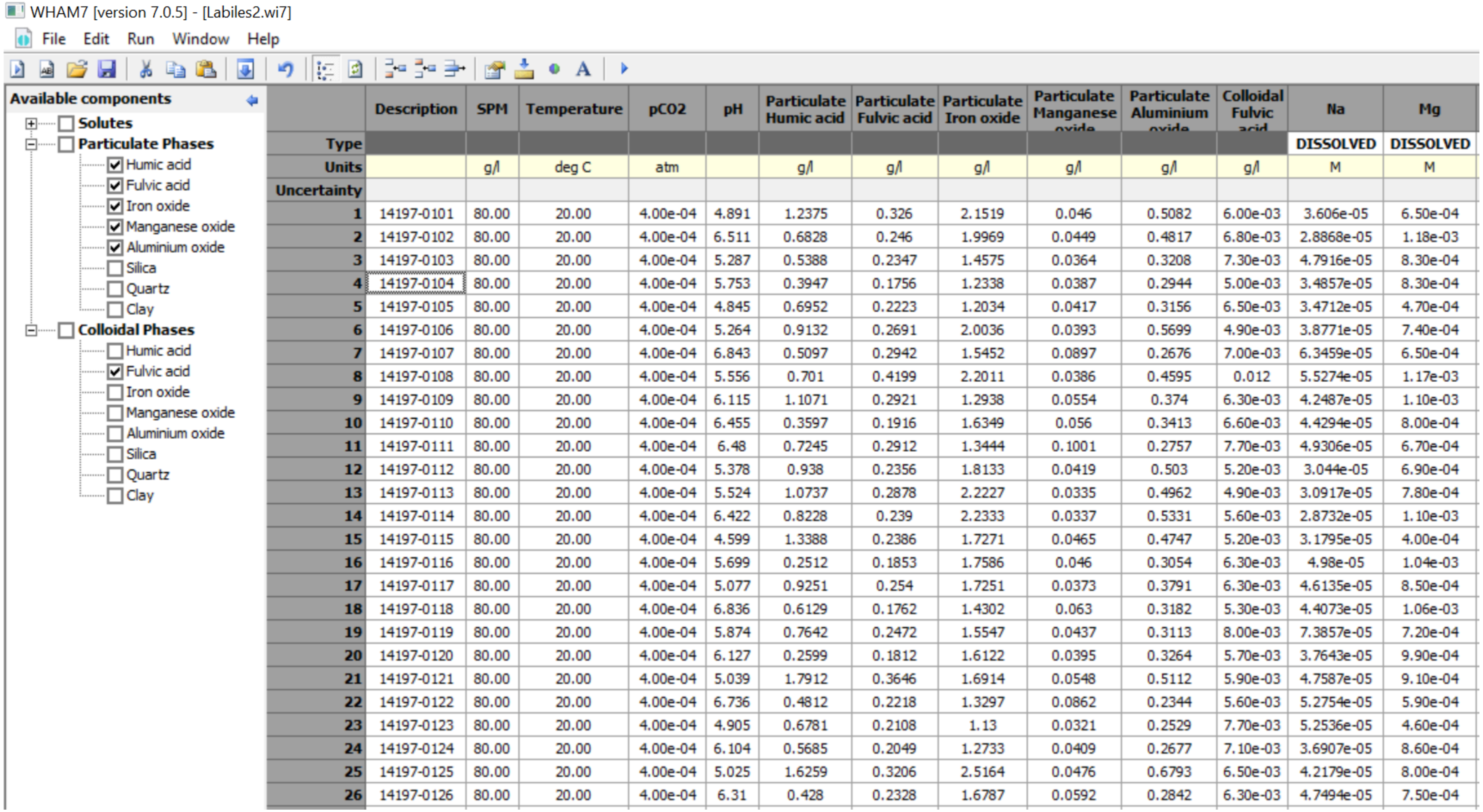
$$Conc = R \times DF \times \frac{V}{W} \qquad \text{Eq. 9}$$

where Conc is the concentration of heavy metal in the plant sample in mg kg^{-1}, R is the instrument reading i.e. the concentration of the heavy metal in the analysed solution in mg L^{-1}, DF is the dilution factor of the solution, V is the volume in L of the extracting solution and W is the weight of the soil in kg.

3.8. Modelling solubility, speciation and fractionation of copper by WHAM VII

Total soluble concentration and speciation in the solution phase of soil suspensions, and the solid phase fractionation of Cu, were predicted using the geochemical speciation model WHAM-VII (Windermere Humic Aqueous Model, version 7).

Values of Cu-E and Cu-dtpa representing alternative reactive heavy metal concentrations, and Cu-tot were used as inputs to WHAM for whole soil fractionation and prediction of Cu concentration in solution. The modelled metal concentration in solution was compared with measured values of Cu-soln to test the model performance in predicting Cu solubility. Measured values of Cu-soln were then used as model input for speciation of Cu in the solution. Linear mixed models were fitted to examine evidence of a relationship between measured and predicted soil solution concentration. Measured input parameters included: i) solution pH; ii) particulate fractions including oxides of Fe, Al and Mn (FeOx, AlOx and MnOx respectively) and HA and FA, and iii) colloidal FA estimated from DOC and iv) solution concentrations of Cu as shown in Figure 3-5.



	Description	SPM	Temperature	pCO2	pH	Particulate Humic acid	Particulate Fulvic acid	Particulate Iron oxide	Particulate Manganese oxide	Particulate Aluminium oxide	Colloidal Fulvic acid	Na	Mg
Type												DISSOLVED	DISSOLVED
Units		g/l	deg C	atm		g/l	g/l	g/l	g/l	g/l	g/l	M	M
Uncertainty													
1	14197-0101	80.00	20.00	4.00e-04	4.891	1.2375	0.326	2.1519	0.046	0.5082	6.00e-03	3.606e-05	6.50e-04
2	14197-0102	80.00	20.00	4.00e-04	6.511	0.6828	0.246	1.9969	0.0449	0.4817	6.80e-03	2.8868e-05	1.18e-03
3	14197-0103	80.00	20.00	4.00e-04	5.287	0.5388	0.2347	1.4575	0.0364	0.3208	7.30e-03	4.7916e-05	8.30e-04
4	14197-0104	80.00	20.00	4.00e-04	5.753	0.3947	0.1756	1.2338	0.0387	0.2944	5.00e-03	3.4857e-05	8.30e-04
5	14197-0105	80.00	20.00	4.00e-04	4.845	0.6952	0.2223	1.2034	0.0417	0.3156	6.50e-03	3.4712e-05	4.70e-04
6	14197-0106	80.00	20.00	4.00e-04	5.264	0.9132	0.2691	2.0036	0.0393	0.5699	4.90e-03	3.8771e-05	7.40e-04
7	14197-0107	80.00	20.00	4.00e-04	6.843	0.5097	0.2942	1.5452	0.0897	0.2676	7.00e-03	6.3459e-05	6.50e-04
8	14197-0108	80.00	20.00	4.00e-04	5.556	0.701	0.4199	2.2011	0.0386	0.4595	0.012	5.5274e-05	1.17e-03
9	14197-0109	80.00	20.00	4.00e-04	6.115	1.1071	0.2921	1.2938	0.0554	0.374	6.30e-03	4.2487e-05	1.10e-03
10	14197-0110	80.00	20.00	4.00e-04	6.455	0.3597	0.1916	1.6349	0.056	0.3413	6.60e-03	4.4294e-05	8.00e-04
11	14197-0111	80.00	20.00	4.00e-04	6.48	0.7245	0.2912	1.3444	0.1001	0.2757	7.70e-03	4.9306e-05	6.70e-04
12	14197-0112	80.00	20.00	4.00e-04	5.378	0.938	0.2356	1.8133	0.0419	0.503	5.20e-03	3.044e-05	6.90e-04
13	14197-0113	80.00	20.00	4.00e-04	5.524	1.0737	0.2878	2.2227	0.0335	0.4962	4.90e-03	3.0917e-05	7.80e-04
14	14197-0114	80.00	20.00	4.00e-04	6.422	0.8228	0.239	2.2333	0.0337	0.5331	5.60e-03	2.8732e-05	1.10e-03
15	14197-0115	80.00	20.00	4.00e-04	4.599	1.3388	0.2386	1.7271	0.0465	0.4747	5.20e-03	3.1795e-05	4.00e-04
16	14197-0116	80.00	20.00	4.00e-04	5.699	0.2512	0.1853	1.7586	0.046	0.3054	6.30e-03	4.98e-05	1.04e-03
17	14197-0117	80.00	20.00	4.00e-04	5.077	0.9251	0.254	1.7251	0.0373	0.3791	6.30e-03	4.6135e-05	8.50e-04
18	14197-0118	80.00	20.00	4.00e-04	6.836	0.6129	0.1762	1.4302	0.063	0.3182	5.30e-03	4.4073e-05	1.06e-03
19	14197-0119	80.00	20.00	4.00e-04	5.874	0.7642	0.2472	1.5547	0.0437	0.3113	8.00e-03	7.3857e-05	7.20e-04
20	14197-0120	80.00	20.00	4.00e-04	6.127	0.2599	0.1812	1.6122	0.0395	0.3264	5.70e-03	3.7643e-05	9.90e-04
21	14197-0121	80.00	20.00	4.00e-04	5.039	1.7912	0.3646	1.6914	0.0548	0.5112	5.90e-03	4.7587e-05	9.10e-04
22	14197-0122	80.00	20.00	4.00e-04	6.736	0.4812	0.2218	1.3297	0.0862	0.2344	5.60e-03	5.2754e-05	5.90e-04
23	14197-0123	80.00	20.00	4.00e-04	4.905	0.6781	0.2108	1.13	0.0321	0.2529	7.70e-03	5.2536e-05	4.60e-04
24	14197-0124	80.00	20.00	4.00e-04	6.104	0.5685	0.2049	1.2733	0.0409	0.2677	7.10e-03	3.6907e-05	8.60e-04
25	14197-0125	80.00	20.00	4.00e-04	5.025	1.6259	0.3206	2.5164	0.0476	0.6793	6.50e-03	4.2179e-05	8.00e-04
26	14197-0126	80.00	20.00	4.00e-04	6.31	0.428	0.2328	1.6787	0.0592	0.2842	6.30e-03	4.7494e-05	7.50e-04

Figure 3-5: Input sheet of WHAM VII

3.8.1. Determination of particulate Fe, Al and Mn oxides

Iron, aluminium and manganese were determined by ICP-AES (Perkin Elmer Optima) following extraction with a dithionite-citrate-bicarbonate (DCB) reagent containing 0.07 M sodium dithionite, 0.3 M sodium citrate and 1 M sodium bicarbonate. The combination of the sodium compounds ensures reduction of Fe (III) to Fe (II) by dithionite while citrate buffered the pH to prevent decomposition of dithionite to hydrogen sulphide and complexed Fe^{2+} to prevent precipitation of iron and bicarbonate raised the pH. Twenty ml of DCB was added to 1 g ± 0.1 of <2 mm sieved soil and left overnight on a shaker, to solubilise the soil oxides. The suspensions were then centrifuged at 2500 rpm for 20 min before filtering (<0.2 µm) and collecting an aliquot for analysis. The aliquot was diluted 200-fold prior to analysis. The concentrations of the oxides; Fe_2O_3, Al_2O_3 and MnO_2 were determined proportionally from concentrations of the Fe, Al and Mn as shown in Eq. 11, 12, 13 respectively.

$$y = R \times DF \times \frac{V}{W} \qquad \text{Eq. 10}$$

Therefore,

$$Fe_2O_3 = \frac{1.597 \times yFe}{111.7} \qquad \text{Eq. 11}$$

$$Al_2O_3 = \frac{86.9 \times yAl}{54.9} \qquad \text{Eq. 12}$$

$$MnO_2 = \frac{102 \times yMn}{54} \qquad \text{Eq. 13}$$

where y is the concentration of either Fe or Al or Mn in mg kg^{-1}, R is the instrument reading i.e. the concentration in the analysed solution in mg L^{-1}, DF is the dilution factor of the solution, V is the volume of the extracting solution in L and W is the weight of the soil in kg.

3.8.2. Determination of particulate HA and FA

Approximately 2 g of sieved soil (< 2 mm) was suspended in 20 ml of 0.1 M sodium hydroxide and equilibrated on a shaker for 1 day. Following centrifugation at 2000 rpm for 10 min, 5 ml aliquot was collected into ICP tubes for determination of HAFA concentrations. The soltion was diluted 20-fold prior to analysis. A further 10 ml aliquot was acidified with 1 ml of 1.5 M HNO_3 and left for 16 hours for HA to precipitate out; the suspensions were then centrifuged at 2000 rpm for 10 min and an aliquot of supernatant collected for FA determination after a six-fold dilution. Both HAFA and FA were analysed on a Shimadzu TOC-L total organic carbon analyser. Humic acid concentrations were then determined by difference (Eq. 14 and 15).

$$HAFA/FA\ (mg\ kg^{-1}) = R \times DF \times \frac{V}{W}$$

$$\mathrm{HAFA\ (mg\ kg^{-1})} = 200 \times \mathrm{R_1}$$

$$FA\ (mg\ kg^{-1}) = 60 \times R_2 \qquad \text{Eq. 14}$$

$$HA\ (mg\ kg^{-1}) = HAFA - FA \qquad \text{Eq. 15}$$

where R_1 and R_2 are the instrument readings for HA+FA and FA concentration respectively i.e. the concentration of the humic acids in the analysed solution in mg L^{-1}, DF is the dilution factor of the solution, V is the volume of the extracting solution in L and W is the weight of the soil in kg.

3.8.3. Determination of colloidal and solution phase WHAM VII input parameters

Two grams of soil were suspended in 25 ml of 0.01 M $Ca(NO_3)_2$ solution and equilibrated on a shaker for 3 days. Solution pH was determined in the soil-solution suspension using an Orion 720A+ pH meter and temperature was set at 20 °C. The suspensions were centrifuged and filtered to <0.2 µm and diluted 6-fold before analysis. Copper and Al, Fe, Mn, Na, Mg, K, Ca and NO_3 concentrations were determined by ICP-QQQ-MS with matrix-matched calibration and quality control standards. Dissolved organic carbon (DOC) was measured in the solutions using a Shimadzu TOC-L Total organic carbon analyser. Colloidal FA was determined from DOC by assuming that FA contains 50 percent carbon, but also that only 65 percent of the measured carbon is ‘active’ FA (Buekers *et al*., 2008) (Eq. 16).

$$\text{Colloidal FA (mg L}^{-1}) = \text{DOC} \times 2 \times 0.65 \qquad \text{Eq. 16}$$

3.9. Statistical analysis of data

3.9.1. Field experiment

The data were analysed on the R platform (R Core Team, 2017). Because of the hierarchical structure of the experiment, with the treatments randomised within each field, and replication for the investigation of the Dam effect at the level of field, the data were analysed with a linear mixed model (LMM) using the lme function in the nlme library for the R platform (Pinheiro *et al*, 2017). Using a LMM allowed for the analysis of repeated measures taken at different times within other groups (plots within fields and fields far or near to the tailing dam). Simple linear modeling cannot incorporate the extra grouping of already grouped subjects and so a LMM was applied to explore both the between and within groups differences. Random effects were Field and Plot within Field, and the fixed effects were proximity to Dam (close or far), lime, manure and their interactions. The effect of lime, manure, their interaction and their residual effect on soil pH, organic matter and the different soil-metal fractions were determined from the model output using the anova() function. Differences between each season and the baseline were computed for each variable to determine the change (either reduction or increase). Bar graphs were generated in excel, for each variable, using the actual values (not the change) for each treatment, in order to show the progression from the baseline to season S2.

3.9.2. Pot experiment

The spatial coverage sampling described in Section 3-3 was undertaken to support spatial mapping from the resulting data. The analyses of interest were: i) the prediction of soil solution Cu from soil Cu-tot, Cu-E and Cu-dtpa and ii) the prediction of the concentration of Cu in the crop from soil solution Cu, Cu-tot, Cu-E and Cu-dtpa

A linear mixed model was used to predict soil solution Cu from Cu-tot, in which Cu-tot is the single fixed effect, and in which the random effects comprise a spatially correlated Gaussian random field and an independent and identically distributed residual term. This was then repeated for the other predictors (Cu-E, Cu-dtpa) and then models were fitted to predict the concentration of Cu in the crop.

The variation between sites from which the soil samples were selected was assumed to be a normal random variable (variance C1), and with the correlation between any two values dependent only on the separation distance between them, according to a Matérn correlation function (Stein, 1999) with a distance parameter ϕ and a smoothness parameter κ which together determine how the correlation decays with distance. In addition, an uncorrelated random variable of variance C0 accounts for variation which is not spatially dependent at scales resolved by sampling. The parameters C1, C0 and ϕ were estimated by residual maximum likelihood (REML) conditional on a value of κ, which was fixed in turn at a set of discrete values to find the best value to specify by profile maximum likelihood (Diggle and Ribeiro, 2007).

Once these parameters were obtained, generalised least squares estimates of the fixed effect parameters were computed, in effect the intercept (b_0) and slope (b_1) of the regression of the soil solution Cu on Cu-Tot, and the standard errors of these parameters. The significance of the relationship was tested by comparing the model with a "null" equivalent in which the only fixed effect is an unknown constant mean. This was done by means of a log-likelihood ratio test following Welham and Thompson (1997). An approximate adjusted R^2 statistic to measure the amount of variation accounted for by the model was computed as the difference between the sum of variances of the random effects for the null model and the fitted alternative, expressed as a proportion of the sum of variances for the null model.

Because the linear mixed model assumes normal random effects, a simple exploratory linear model was first fitted, and the summary statistics of the residuals were examined. As a result of this, for all models, it was decided to transform both the dependent and independent variables to natural logarithms in order to make the assumption plausible.

3.10. Quality control

All reagents used in the laboratory analysis of the samples were analytical grade or better (e.g., Primar Plus™ acids), and were used as received without any further purification. Reagent blanks, duplicate samples, reference materials and certified reference materials (CRMs), where possible, were included with every batch of samples. The CRMs used for total heavy metal analysis in soils were BGS 102, BCR-2, 2711a (Montana soil), and for plant samples, CRMs included NIST 1573a (tomato leaves, NIST 1570a (spinach) and NIST 1567b (wheat flour). Solutions were prepared using milli-Q water (resistivity ≤18.2 MΩ cm^{-1}).

CHAPTER FOUR

4. EFFECT OF LIME AND MANURE ON BIOAVAILABLE HEAVY METALS IN SOILS AT VARYING DISTANCES FROM THE MINE TAILINGS DAMS

4.1. Overview

The objectives of this study were to determine the concentrations of heavy metals at varying distances from the mine tailings dam and to determine the effect of lime and manure on the bioavailable concentration of heavy metals. Diethylenetriaminepentaaceticacid (DTPA) and $Ca(NO_3)_2$ extractants were used to determine the concentrations of the heavy metals. The individual and interactive effect of lime and manure were assessed by determining the available concentrations of Cd, Cu, Ni, Pb and Zn before and after their (amendments) application. The determination of solid (M-dtpa) and solution (M-sol) phase metal concentrations enabled the assessment of the distribution coefficient (K_d), an index widely used to predict the fate of heavy metals in contaminated soils.

The study was conducted through a field experiment, with treatments replicated at varying locations relative to the tailings dam. This set-up and subsequent statistical analysis allowed for the investigation of how responses to agronomic practices might differ in contrasting micro-environments. In this study setting, proximity to mine tailings is important because along with spatial variations in the input of heavy metals via wind or rain driven transfer, previous observation shows that the pH of soils closer to the tailings is substantially higher (Lark *et al*., 2017). The materials and methods used are described in Chapter three.

4.2. Results and discussion

4.2.1. General soil characteristics

Table 4-1 shows the range of some initial chemical and physical characteristics of the soils in each of the four fields B1, B2, B3, B4. According to the classification by Hazelton and Murphy (2007), the organic carbon and total nitrogen contents of the soil ranged from moderate to high, while available phosphorus concentration was generally low, cation exchange capacity (CEC) was low to moderate, and the pH (5.1 – 7.2) was moderately acidic to neutral. The soils textures were clay loam (B1, B2, B3) and loam (B4) with clay content ranging from 24 to 30 percent. Regardless of the heavy metal concentrations, the soils in this area are moderately fertile and suitable for growing field crops.

Table 4-1: Soil chemical and physical parameters prior to addition of the amendments

Proximity to the tailings dam	Far (300-400 m) D1		Close (100-200 m) D2	
Field/ block	**B1**	**B2**	**B3**	**B4**
Organic carbon (%)	1.42–1.83	1.55–1.92	1.1–2.11	1.55–1.96
SOM (% LOI)	2.6–3.5	3.1–3.7	2.4–3.2	3.1–3.6
pH (0.01 M $CaCl_2$)	5.2–5.6	5.1–5.3	6.4–6.7	6.3–7.2
Total N (%)	0.29-0.45	0.13-0.34	0.20-0.34	0.20-0.31
Available P (mg kg^{-1})	6.60-24.05	4.49-20.66	12.38-19.46	14.06-30.31
ECEC (cmol(+)kg^{-1})	8.2–13.5	10.1–13.3	9.0–13.6	12.1–21.0
Sand (%)	39.4	37.6	43.9	42.6
Silt (%)	30.8	35.2	27.4	33.0
Clay (%)	29.8	27.2	28.7	24.4
USDA textural class	Clay loam	Clay loam	Clay loam	Loam

Fields B1 and B2, which were further from the tailings dam were in the moderately acidic pH range (pH 5.1 – 5.6), which is expected in unamended Ferralsols. The fields which were closer to the tailings dam (B3 and B4) had higher pH classified as slightly acid to neutral pH range (6.3 – 7.2), contrary to expectation for this soil type. This is because of proximity to the tailings, which are treated with lime prior to disposal. Soil organic carbon content did not show a systematic trend but displayed minimal variation across the blocks.

4.2.2. Soil heavy metal concentrations before application of lime and manure

Table 4-2 shows the average concentrations of metals extracted by acid digestion (M-tot), DTPA (M-dtpa), and 0.01 M $Ca(NO_3)_2$ (M-sol), representing total, exchangeable and soluble fractions, respectively. The background values shown indicate that significant contamination of soils with Cu and Ni has occurred, but not with Cd, Pb and Zn. According to the FAO maximum permissible limits for agricultural soils (FAO/WHO, 1984; WHO, 1993), all values of M-tot, except for Cu-tot, were within the acceptable thresholds.

When expressed as a proportion of M-tot, percent M-dtpa decreased in the order Cd>Cu>Pb≥Zn>Ni. Thus, Cd was the most extractable metal with DTPA, ranging from 20-36 percent of Cd-tot, while Ni was the least extractable (Ni-dtpa < 1percent). A similar trend was also reported by Römkens *et al*. (2009) and Izquierdo *et al*. (2012), who used the isotopic dilution method to determine 'labile' heavy metal concentrations. Cadmium is reported to have a relatively low affinity for most binding phases in the soil compared to other heavy metals (Shaheen, 2009). It is, therefore, the most bioavailable even in soils with only background concentrations (Liu *et al*., 2013). Contrastingly, Ni has been reported to bind strongly to amorphous iron oxides (Malinowska, 2017). Considering that the soils for this study are rich in Fe oxides, this could explain the observed low Ni-dtpa. Copper-dtpa was 13-22 percent of Cu-tot, while Pb-dtpa and Zn-dtpa were 4-7 and 3-7 percent, respectively. The proportion extracted by 0.01 M $Ca(NO_3)_2$ solution (M-sol) decreased in the order: Cd-sol > Zn-sol> Cu-sol> Pb-sol≥ Ni-sol. The clear difference between the concentrations and order for M-dtpa and M-soil show that the two protocols extract different fractions of soil metals.

Table 4-2: Average values of M-tot, M-dtpa, and M-sol metal fractions in the soil prior to addition of soil amendments of lime and manure

Soil fraction	Block	Metal concentrations (four replications±standard error)				
		Cd	Cu	Ni	Pb	Zn
M-tot (mg kg-1)	B1	0.12±0.01	874±84	36±1.6	9.4±0.5	32±2
	B2	0.11±0.01	935±31	44±1.6	10.2±0.2	32±0.6
	B3	0.11±0.01	947±66	41±0.9	9.6±0.5	26±1.2
	B4	0.09±0.01	979±85	40±1.5	8.1±0.3	24±1.5
	Mean	0.11	934	40.9	9.3	28.5
	Manure	0.04	56	17.5	2.44	484
	Background values for Kitwe (Křibek et al., 2010)	0.1	289	<5	<10	10
	FAO/WHO max. allowable	3	100	50	100	300
M-dtpa (mg kg-1)	B1	0.04±0.003	128±10	0.04±0.002	0.6±0.02	1.7±0.1
	B2	0.03±0.003	140±12	0.03±0.005	0.6±0.04	1.4±0.1
	B3	0.04±0.002	173±13	0.06±0.008	0.5±0.02	1.0±0.2
	B4	0.03±0.002	226±6	0.03±0.011	0.4±0.1	1.3±0.2
	Mean	0.03	167	0.04	0.5	1.4
M-sol (µg L-1)	B1	0.32±0.1	72±13	1.54±0.43	0.05±0.01	19±3.8
	B2	0.45±0.1	68±9	1.79±0.44	0.07±0.01	17±4.5
	B3	0.11±0.1	37±7	0.60±0.1	0.06±0.02	27±5.5
	B4	0.37±0.1	41±15	2.92±0.5	0.59±0.11	18±6.5
	Mean	0.31	55	1.71	0.19	20.3
	Manure	0.002	2.09	1.03	0.048	17.8

4.2.3. Effect of lime and manure on bioavailable heavy metal fractions

4.2.3.1. Effect of lime and manure on M-dtpa

Figure 4-1(a-e) shows values of M-dtpa for Cd, Cu, Ni, Pb, and Zn in soil subject to the various treatments. Lime alone only had a significant effect on Cu. Manure alone had a significant effect on Cd, Pb Zn, while a significant interactive effect of the two amendments was observed for all except Zn.

Detailed results of the effect of lime and manure on the M-dtpa per season are shown in Table 4-3. When compared to the baseline, Cd-dtpa was significantly reduced by manure ($p=0.0062$) and the interaction of lime and manure ($p= 0.0032$). Thus, Manure with and without lime immobilised Cd. For Cu, lime significantly reduced ($p=0.0003$) Cu-dtpa and a significant interaction of lime and manure ($p=0.0001$) was also observed. This suggest that Cu was immobilised by lime and the combined lime and manure. However, Ni-dtpa was not significantly affected by sole treatments of lime or manure but their interaction increased ($p=0.0067$) it in S1 and reduced it in S2. Contrasting results to those of Cd were observed for Pb which was significantly increased by manure ($p=0.05$) in both seasons. The effect of lime alone was not significant while the interaction of lime and manure immobilised ($p=0.0008$). Zinc was also significantly increased by manure ($p=0.0334$) while no significant effect of lime alone and lime+ manure was observed. These results suggest that Zn in the soil was associated with SOM. Otherwise, the manure applied in this study had a large total concentration of Zn (434 mg kg^{-1}) relative to the heavy metal concentration in the soil. Therefore, it is plausible that manure could account for some of the increase in Zn-dtpa.

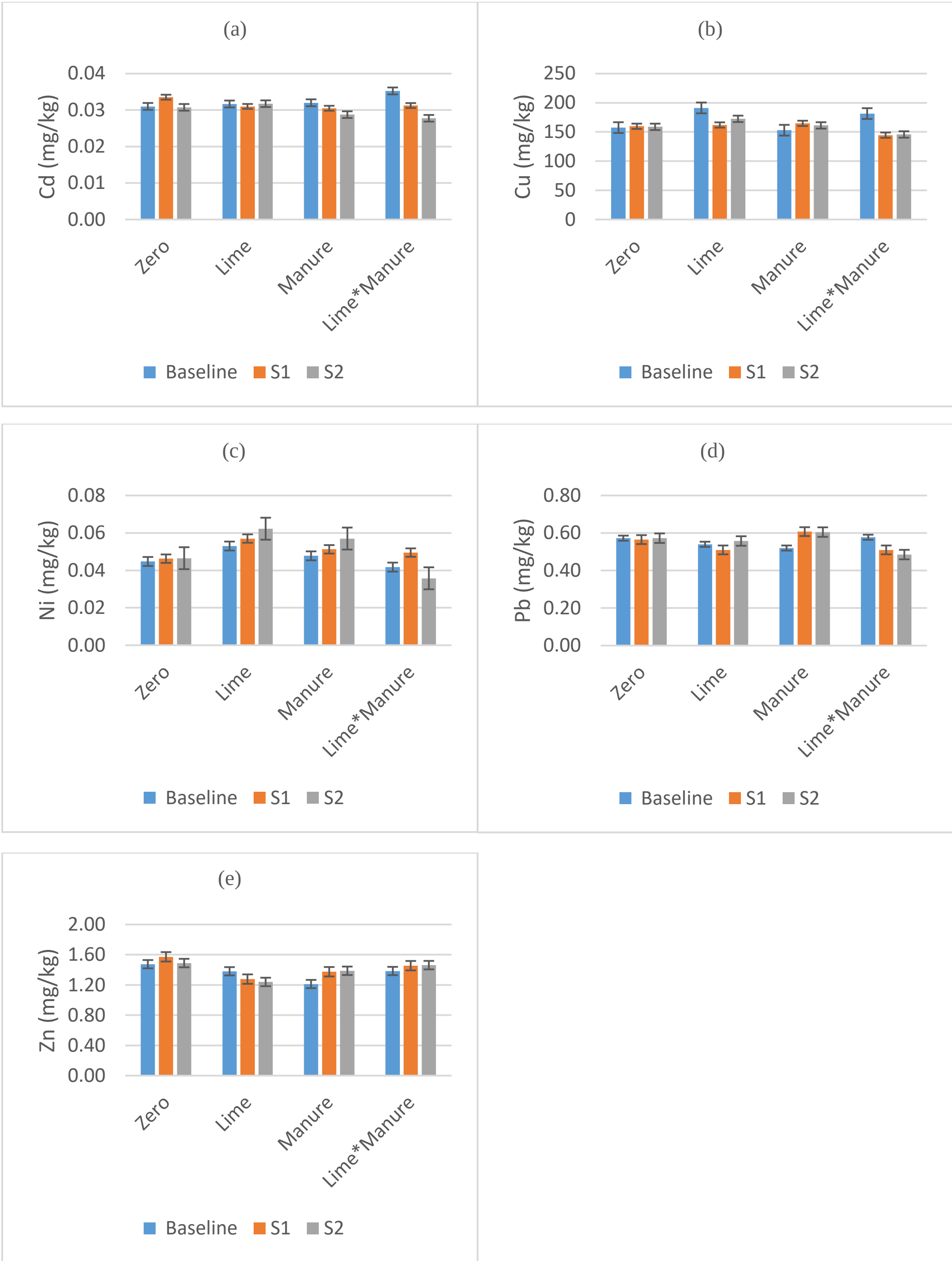


Figure 4-1 (a-e): M-dtpa concentration in each treatment.

S1 and S2 represent seasons 1 and 2, respectively. Treatment effects are inferred from comparing the concentration obtained in a season under treatment to its baseline concentration.

Table 4-3: Effect of lime and manure on M-dtpa

Variable	Proximity to tailings	Treatment	Growing season		
			Baseline	S1	S2
Cd-dtpa (mg kg^{-1})	D1	Control	0.033±0.002	0.033±0.001	0.033±0.001
		Lime	0.033±0.003	0.034±0.004	0.036±0.004
		Manure*	0.033^a±0.003	0.031^a±0.001	0.030^b±0.001
		Lime+Manur*	0.043^a±0.002	0.039^b±0.003	0.036^b±0.003
	D2	Control	0.036±0.003	0.040±0.004	0.039±0.004
		Lime	0.036±0.004	0.038±0.002	0.037±0.002
		Manure*	0.029^a±0.002	0.028^a±0.003	0.024^b±0.003
		Lime+Manur*	0.027^a±0.002	0.024^b±0.002	0.020^b±0.002
Cu-dtpa (mg kg^{-1})	D1	Control	115±1.49	123±1.4	121±1.09
		Lime*	111^a±13.2	100^b±23.50	101^b±23.50
		Manure	117±2.3	119±1.80	115±1.80
		Lime+Manur*	157^a±18.1	126^b±13.80	125^b±13.80
	D2	Control	210±24.9	206±26.0	212±26.0
		Lime	40^a±36.9	37^a±35.8	38^a±38.4
		Manure	157±11.5	147±17.8	156±12.8
		Lime+Manur*	188^a±14.9	150^b±10.5	146.0^b±12.5
Ni-dtpa (mg kg^{-1})	D1	Control	0.033±0.006	0.035±0.006	0.033±0.006
		Lime	0.030±0.009	0.028±0.018	0.28±0.018
		Manure	0.034±0.006	0.033±0.004	0.034±0.004
		Lime+Manur*	0.039^a±0.006	0.034^b±0.01	0.044^c±0.01
	D2	Control	0.056±0.013	0.05±0.015	0.058±0.012
		Lime	0.020±0.032	0.022±0.007	0.017±0.01
		Manure	0.048±0.012	0.045±0.002	0.052±0.00
		Lime+Manure	0.038±0.012	0.038±0.01	0.036 ±0.01
Pb-dtpa (mg kg^{-1})	D1	Control	0.579±0.012	0.622±0.018	0.583±0.018
		Lime	0.540±0.012	0.493±0.04	0.535±0.034
		Manure*	0.582^a±0.022	0.605^b±0.02	0.611^b±0.01
		Lime+Manur*	0.747^a±0.032	0.695^b±0.047	0.429^b±0.074
	D2	Control	0.562±0.032	0.566±0.033	0.555±0.033
		Lime	0.269^a±0.11	0.285^a±0.046	0.315^b±0.041
		Manure*	0.456^a±0.012	0.483^b±0.024	0.479^b±0.02
		Lime+Manur*	0.504^a±0.02	0.468^b±0.026	0.403^c±0.025
Zn-dtpa (mg kg^{-1})	D1	Control	1.29±0.311	1.29±0.332	1.35±0.292
		Lime	1.25±0.521	1.45±0.311	1.15±0.209
		Manure*	1.55^a±0.243	1.79^b±0.181	1.85^b±0.118
		Lime+Manure	2.01±0.311	1.680±0.404	1.880±0.101
	D2	Control	1.81±0.281	1.85±0.199	1.570±0.213
		Lime	0.17±0.077	0.21±0.326	0.14±0.312
		Manure*	0.87^a±0.187	1.06^b±0.307	1.04^b ±0.350
		Lime+Manure	0.858±0.199	0.720±0.45	0.935±0.29

*Significant effect, means having the same superscript within a row were not significantly different

The immobilisation of Cu by lime can be attributed to increased sorption capacity as pH increased. In tropical soils dominated by sesquioxides, increasing pH also increases the surface charge resulting in increased sorption capacity of the soil. Table 4-4 shows that lime increased the pH while manure increased the SOM content, which increases the CEC of the soil. An increase in pH favours adsorption and precipitation of metal hydroxides and carbonates (Lombi *et al*., 2003), and this explains the reduction in Cd-dtpa by lime. The reduction of Cd-dtpa by manure was greater when lime was present. Kumarpandit *et al*. (2017) also reported immobilisation of Cd by combining lime and manure, which translated into reduced uptake by spinach grown in contaminated soils. The immobilisation of Cd-dtpa by organic amendments was also reported by Angelova *et al*. (2013), who attributed it to an increase in the cation exchange capacity (CEC) of the soil as the organic matter content increased.

Table 4-4: Soil pH and organic matter content (means of four fields±standard errors) at baseline and the end of each season (S1 and S2)

*Treatment	pH			SOM (% LOI)		
	Baseline	S1	S2	Baseline	S1	S2
L0M0 (control)	6.0±0.48	6±0.47	6±0.50	5.39±0.32	5.44±0.32	5.01±0.49
L1M0 (lime)	5.6±0.30	6.6±0.18	6.4±0.26	5.43±0.44	5.73±0.26	5.03±0.67
L0M1 (manure)	6.0±0.47	6.1±0.41	6.1±0.48	4.9±0.25	6.12±0.42	6.18±0.41
L1M1 (lime/manure)	6.0±0.40	6.5±0.27	6.9±0.18	5.4±0.44	6.28±0.32	6.21±0.63

**Treatments do not apply to the baseline data*

The immobilisation of metals by lime in the presence of manure which was also observed for Cu, Ni, and Pb, is likely an indication of more stable metal-organic complexes. Angelova *et al*. (2013) reported a reduction in Cu-dtpa, in compost and vermicompost-treated soils, attributed to the formation of stable complexes with HA, which are less soluble than FA. Immobilisation of Ni by increasing the pH through liming and complexation with organic matter, has been reported (de Macedo *et al*., 2016; Malinowska, 2017; Ren *et al*., 2015; Wallace *et al*., 1977; Weng *et al*., 2002). However, Pb is preferentially complexed by DOM, which may explain the increase in Pb-dtpa with the manure treatment (Figure 4-2). Weng *et al*. (2002) noted that complexation with DOM was more significant for Cu and Pb than for Cd, Ni, and Zn. The combination of lime and manure immobilised all the heavy metals except Zn. For Cd and Cu which were also immobilised by applying the single amendments, the combination of lime and manure had a much larger effect than with either lime or manure alone. This shows a synergistic effect of lime and manure in reducing the availability of heavy metals. The average reduction in M-dtpa, for seasons S1 and S2, by combined lime and manure followed the order Cu (20percent)> Cd (13percent)>Pb (11percent)>Ni (7percent).

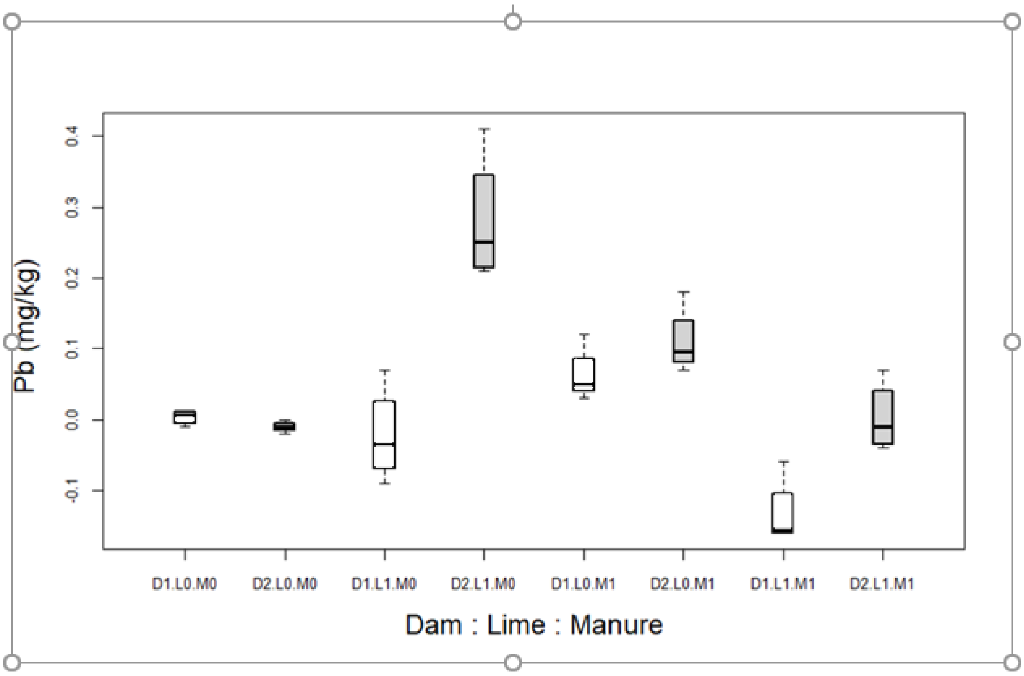


Figure 4-2: Effect of the interaction of the proximity to the tailings and lime+manure on Pb-dtpa

D1 and D2 represent far and close blocks to the tailings, respectively, L0 and L1, and M0 and M1 represent plots without and with lime (L)/ manure (M) treatment respectively. The change was calculated as the difference from baseline values; therefore, values above zero signify an increase and vice versa.

Significant effects of the distance from the dam with lime treatment were observed for Cd, Ni, Pb, where their M-dtpa were low in D1 and higher at D2 as shown in Figure 4-3(a-c). In the acidic soils of D1, the increase in pH due to liming led to increased adsorption of the heavy metals, thereby reducing their availability. In the neutral to alkaline soils in D2, lime increased the solubility of SOM i.e., increasing formation of DOC (Figure 4-4), resulting in the release of organically bound heavy metals. The release of DOM due to increased soil pH was also reported by Andersson *et al*. (2000)

and Curtin *et al*. (2016). The results showed that the effect of lime and manure on M-dtpa were dependent on soil pH and were metal specific.

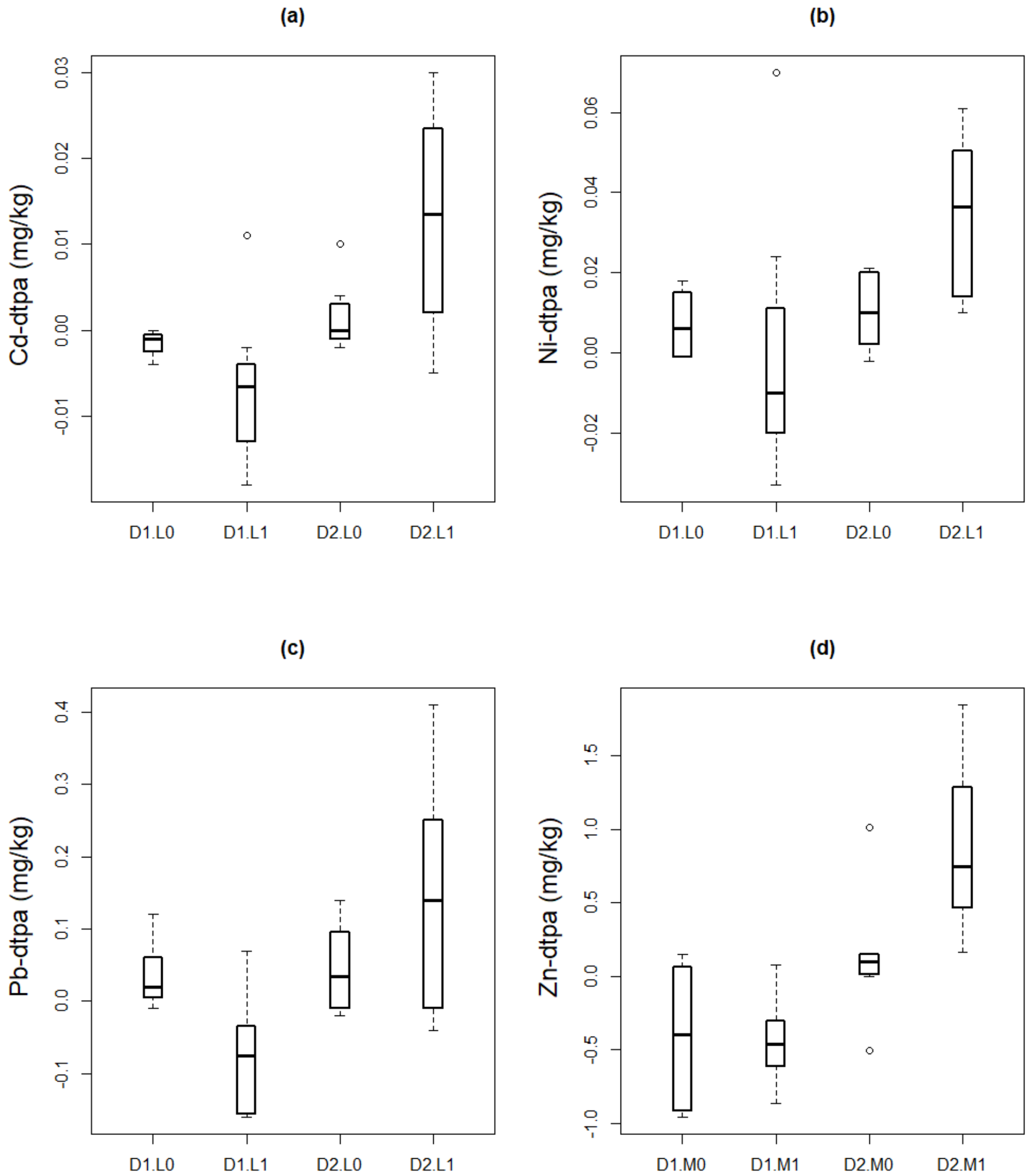


Figure 4-3: Effect of the interaction of the proximity to the tailings with lime on (a) Cd-dtpa, (b), Ni-dtpa, (c) Pb-dtpa, and with manure (d) Zn-dtpa

D1 and D2 represent far and close blocks to the tailings, respectively, L0 and L1, and M0 and M1 represent plots without and with lime (L)/ manure (M) treatment, respectively. The change was calculated as the difference from baseline values; therefore, values above zero signify an increase and vice versa.

A similar explanation can be rendered to the observed significant interaction of the distance to the tailings with manure on Zn-dtpa where an increase was seen in D2 while no change was seen in D1 (Figure 4-3d). These results suggest that pH was the predominant immobilising factor in acidic soils, while at pH>6, DOM was more critical.

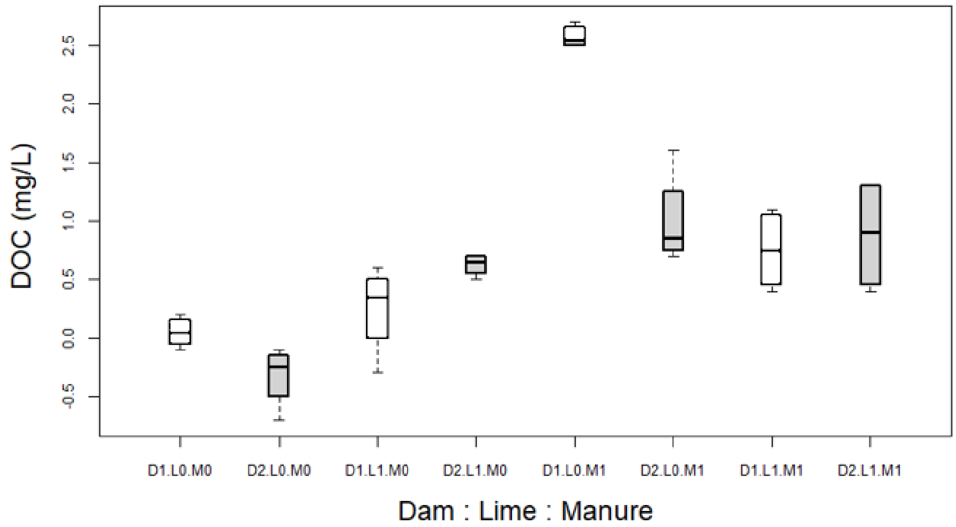


Figure 4-4: Response of DOC to the interaction of lime, manure and the distance to the tailings (Dam).

D1 and D2 represent far and close blocks to the tailings; respectively, L0 and L1 represent plots without and with lime treatment. The change was calculated as the difference from baseline values. Therefore, values above zero signify an increase and vice versa.

4.2.3.2. Effect of lime and manure on M-sol

The effect of lime and manure on the soil fraction extracted with 0.01 M $Ca(NO_3)_2$ is shown in Table 4.5. Treatment effects were deduced by comparing results obtained under the treatment with the baseline. There were no significant main effects of individual lime and manure on Cd-sol but their interaction led to a reduction. A significant interactive effect of lime with the season on Cu-sol was observed, where a larger reduction was observed in S2 than in S1. For Cu-sol, significant effects of individual lime, manure and their interaction were observed. The effect of individual lime and manure on Cu-sol varied significantly with distance to the tailings, where concentrations in plots further from the tailings (D1) were reduced while an increase was observed closer to the tailings (D2). For Ni-sol, lime significantly reduced it while other factors had no significant effect. Both lime and manure had significant reducing effects on Pb-sol while a significant interaction of the distance to the tailings with lime similar to Cu-sol was observed. Lime had a significant reducing effect on Zn-sol, while manure and the interaction of lime and manure did not.

Similarly, to the effect on M-dtpa, lime and manure had a reducing effect on M-sol. However, their contrasting effect on Cu and Pb when associated with the distance to the tailings suggest an association with DOM. It is plausible that both Cu and Pb were complexed with SOM but they were released in D2 with lime or manure due to solubilisation of SOM at alkaline conditions. Manure or organic amendments affect the solubility of heavy metal by changing their speciation, through the action of DOC (Bolan et al., 2011). The results of this study are consistent with findings by Malinowska (2017) who observed that increasing rates of sewage sludge applied to soils increased the soluble fraction of Pb in soils, but a reduction was observed in acidic soils where lime was also applied. The fraction of heavy metals extracted with 0.01 M Ca salts such as $CaCl_2$ or $Ca(NO_3)_2$ is said to be soluble (Houba *et al*., 1996), therefore, any treatment which causes dissolution of soil constituents is important for this fraction.

Table 4-5: Effect of lime and manure on M-sol

Variable	Proximity to tailings	Treatment	Growing season		
			Baseline	S1	S2
Cd-sol $(mg\ L^{-1})$	D1	Control	0.30±0.1	0.26±0.17	0.31±0.22
		Lime	0.22±0.0	0.22±0.05	0.19±0.02
		Manure	0.35±0.1	0.31±0.10	0.33±0.12
		Lime+Manur*	$0.35^{a}\pm0.2$	$0.15^{c}\pm0.03$	$0.26^{b}\pm0.02$
	D2	Control	0.42±0.1	0.39±0.15	0.41±0.12
		Lime	0.19±0.1	0.19±0.15	0.21±0.12
		Manure	0.30±0.2	0.28±0.15	0.31±0.12
		Lime+Manur*	$0.35^{a}\pm0.1$	$0.19^{b}\pm0.15$	$0.26^{b}\pm0.12$
Cu-sol $(mg\ L^{-1})$	D1	Control	66±13	69±17	72±29
		Lime*	$37^{a}\pm10$	$33^{b}\pm4.1$	$22^{c}\pm2.3$
		Manure*	74±15	50±8	50±15
		Lime+Manur*	$88^{a}\pm17$	$35^{b}\pm2.8$	$23^{c}\pm0.98$
	D2	Control	54±6	49±6.7	52±4.4
		Lime*	$27^{a}\pm11$	$35^{b}\pm7.4$	$36^{b}\pm9.7$
		Manure*	$27^{a}\pm3.2$	$29^{a}\pm2.8$	$34^{b}\pm4.3$
		Lime+Manur*	$37^{a}\pm7$	$30^{ab}\pm15$	$25^{b}\pm11$
Ni-sol $(mg\ L^{-1})$	D1	Control	1.16±0.1	1.89±0.4	1.59±0.4
		Lime*	$0.78^{a}\pm0.3$	$0.61^{b}\pm0.1$	$0.29^{c}\pm0.0$
		Manure	1.30±0.4	1.13±0.38	1.44±0.63
		Lime+Manure	1.32±0.4	1.44±0.12	1.18±0.04
	D2	Control	0.31±0.1	0.29±0.09	0.49±0.23
		Lime*	$0.23^{a}\pm0.1$	$0.19^{b}\pm0.09$	$0.18^{b}\pm0.04$
		Manure	0.25±0.2	0.29±0.09	0.49±0.23
		Lime+Manure	0.28±0.1	0.33±0.1	0.29±0.13
Pb-sol $(mg\ L^{-1})$	D1	Control	0.061±0.	0.07±0.01	0.12±0.02
		Lime*	$0.033^{a}\pm0.$	$0.008^{b}\pm0.00$	$0.014^{b}\pm0.01$
		Manure*	$0.044^{a}\pm0.$	$0.01^{b}\pm0.01$	$0.01^{b}\pm0.04$
		Lime+Manure	0.053±0.	0.052±0.01	0.049±0.03
	D2	Control	0.027±0.	0.025±0.01	0.027±0.05
		Lime*	$0.021^{a}\pm0.$	$0.026^{ab}\pm0.01$	$0.029^{b}\pm0.01$
		Manure*	$0.022^{a}\pm0.$	$0.037^{b}\pm0.01$	$0.021^{a}\pm0.00$
		Lime+Manure	0.038±0.	0.037±0.01	0.032±0.05
Zn-sol $(mg\ L^{-1})$	D1	Control	13±4.2	12.9±5.8	13±7.5
		Lime*	$7^{a}\pm5.2$	$2.86^{b}\pm1.7$	$2.15^{b}\pm1.03$
		Manure	21±3.5	24.2±4.28	18.9±4.74
		Lime+Manure	16±1.8	10.6±1.42	$18.^{a}\pm6.3$
	D2	Control	2.1±0.8	2.6±0.9	2.1±1.3
		Lime*	$1.6^{a}\pm0.5$	$0.67^{b}\pm0.02$	$1.5^{a}\pm0.3$
		Manure	1.0±0.1	0.86±0.7	1.5±0.4
		Lime+Manure	1.4±0.2	0.99±0.09	0.78±0.61

*Significant effect, means having the same superscript within a row were not significantly different

4.2.4. Distribution coefficient (Kd)

The distribution coefficient (Kd) is an index describing the distribution of metal ions between the solid and the solution phase in soil. It is a measure of sorption and defined as the ratio of the quantity of the adsorbate adsorbed per unit mass of solid to the quantity of the adsorbate remaining in solution at equilibrium (Kruka et al., 1999). Metal elements with larger Kd values have more ions adsorbed onto the soil solid-phase than in the soil solution. In comparison, smaller Kd values imply more ions in the solution phase than on the adsorption surfaces. Thus, Kd indicates the relative mobility of ion in the soil and is therefore, widely applied in determining the fate of heavy metals in the soil environment (Carlon et al., 2004; Yuan, 2003).

Figure 4-5 shows the seasonal Kd of Cd, Cu, Ni, Pb, and Zn for each soil amendment determined according to Eq. 17. The values for the various metals varied over two orders of magnitude and were approximately 30-90 L kg^{-1} for Cd, 800-7000 L kg^{-1} Cu, 15-150 L kg^{-1} Ni, 1500-4000 L kg^{-1} Pb, and 50-300 L kg^{-1} Zn.

$$Kd = \frac{M\ dtpa}{M\ sol} \qquad \text{Eq. 17}$$

Where Kd is the distribution coefficient in L kg^{-1}, M-dtpa (mg kg^{-1}) and M-sol (mg L^{-1}) are heavy metal concentrations determined by DTPA and $Ca(NO_3)_2$ respectively.

In unamended soils, the order of Kd values of the studied metals was; Pb>Cu>Zn>Cd>Ni. The amendments applied led to an increase in the Kd of Cd, Cu, Ni, and Zn. This showed that the amendments resulted in an increase in the adsorption of the metals, which would reduce their mobility and availability for uptake by plant. Conversely, the Kd for Pb was reduced in amended soils. This shows that more Pb was released into the solution, which would then be available for plant uptake and/or leaching down to groundwater.

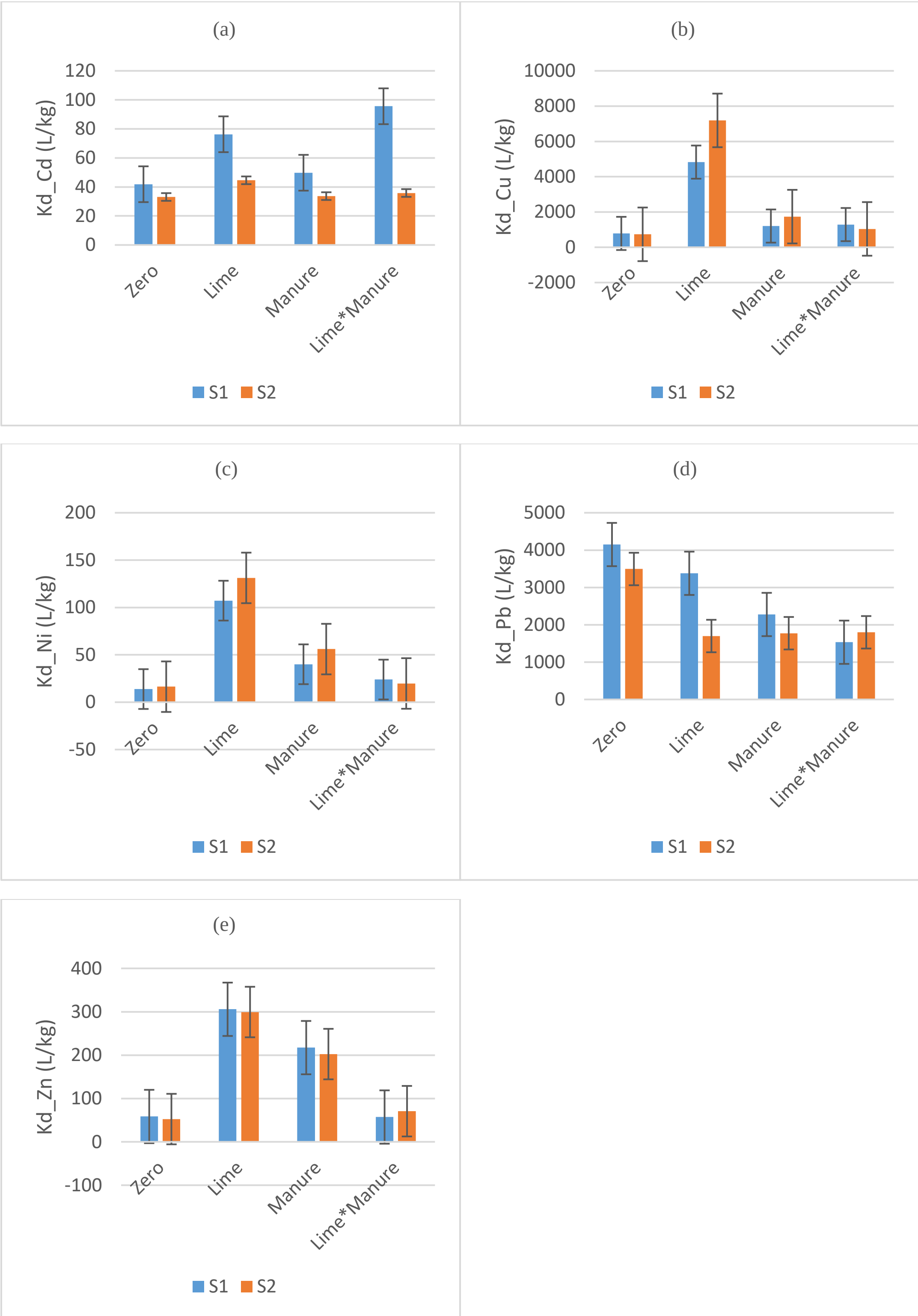


Figure 4-5: The effect of lime and manure amendments on distribution coefficients (Kd) of Cd (a), Cu (b), Ni (c), Pb (d), Zn per season.

Note: S1 and S2 represent season 1 and 2, respectively

4.3. Conclusion

The results of the study show that lime and manure had a reducing effect on solid and liquid- phase bioavailable heavy metals. However, soil pH affected the solubility of SOM such that Cu and Pb were increased under alkaline conditions. This increase may increase their availability to crops. A synergistic reducing effect of lime and manure was seen for dtpa extracted Cd, Cu, Ni and Pb. The amendments had a positive residual effect on both solid and liquid – phase heavy metals and were more effective in reducing extractable metals in soils with $pH \leq 5.6$.

CHAPTER FIVE

5. EFFECT OF LIME AND MANURE AMENDMENTS ON CROP UPTAKE OF HEAVY METALS IN SOILS AFFECTED BY MINE TAILINGS

5.1. Overview

The objective of this study was to determine the effect of lime and manure amendments on the uptake of Cd, Cu, Ni, Pb and Zn by maize and pumpkin. Maize and pumpkin were grown in the field experiments described in Chapter four. The uptake by crops was determined from the concentrations of the heavy metals in pumpkin leaves and in maize (grain and stover). Treatment effects were evaluated by comparing concentrations in crops grown on unamended (control) plots with the crops grown on amended plots. The materials and methods used are described in Chapter three.

5.2. Results and discussion

5.2.1. Concentration heavy metal in the crops

Across all treatments, pumpkin leaves` accumulated concentrations of Cu and Pb above the FAO/WHO (2011, 2001) maximum permissible limit for vegetables, while Cd, Ni, and Zn were below the limit (Figure 5-1). Maize grain accumulated much lower concentrations of all heavy metals compared to pumpkin leaves. Concentrations in maize stover were similar to those found in pumpkin leaves. Leafy samples tend to accumulate more of the essential nutrients such as Cu, Zn, Ni in their chloroplast where they aid in photosynthesis. However, in contaminated soils, even non-essential elements like Cd and Pb tend to be more in leafy tissue than in fruits due to similar uptake mechanisms with essential element.

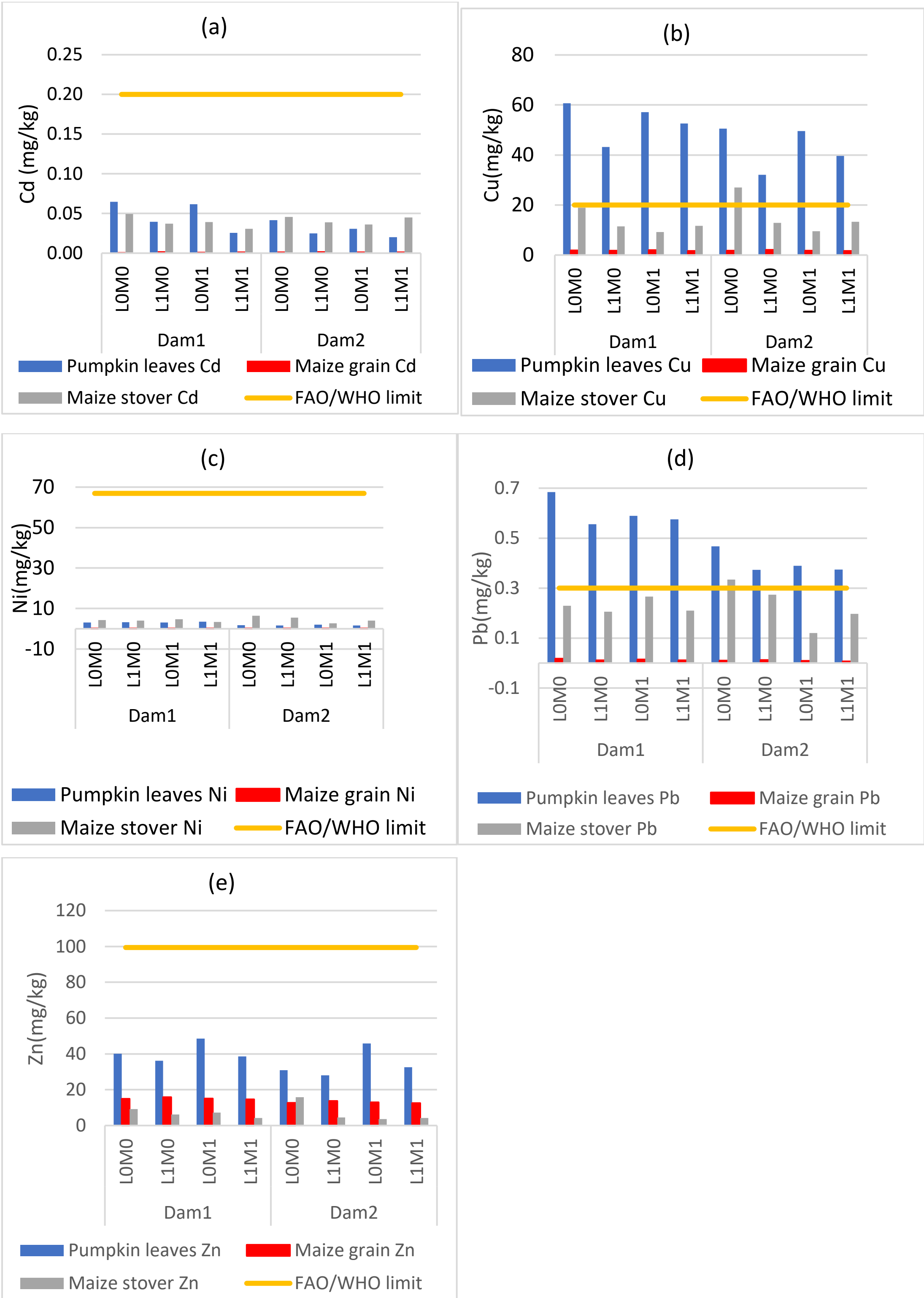


Figure 5-1: Effect of lime and manure on accumulation of Cd, Cu, Ni, Pb, Zn in pumpkin leaves, maize grain, maize stover per distance from the tailings (Dam), compared to the FAO/WHO maximum permissible limits

L0M0, L1M0, L0M1, and L1M1 refer to zero, lime, manure, and lime and manure treatments.

Concentrations found in maize grain were below the limits set by FAO/WHO (2011, 2001) for all five heavy metals. The heavy metal concentrations of Cd, Cu, Pb, and Zn obtained in this study for maize grain are similar to observations (0.01, 2.72, 0.06, and 8.25 mg $kg^{-1,}$ respectively), reported by Mirecki and Agi (2015) for maize grown on soils contaminated with Pb and Zn in Kosovo. However, Mirecki and Agi (2015) reported that these values did not vary significantly compared with values obtained in uncontaminated control soils. Conversely, leafy vegetables grown in the same contaminated soil accumulated greater concentrations of heavy metals than those grown in the control soil, indicating that plant physiological factors were the determinants of uptake (Baker 1981; Mirecki and Agi 2015). According to Puschenreiter *et al*. (2011), maize is classified as a low accumulator while leafy vegetables like lettuce and spinach are high accumulators. The results obtained in this study imply that the consumption of pumpkin leaves grown in this area increases the risk of exposure to Cu and Pb. In contrast, the risk is minimal from maize, despite its considerable dietary contribution to daily food consumption.

Although humans do not consume maize stover, its elemental content was of interest in order to determine the accumulation pattern of the heavy metals by the maize plant and as an indicator of plant uptake. In Zambia, maize stover is fed to livestock on mixed farming systems or left as surface cover in the field, especially in conservation farming systems. In both cases, the heavy metal content is of concern. The heavy metals may be transferred into the food chain, either through ingestion by livestock or through decomposition of SOM, and a resulting contribution to high bioavailable metal concentrations. Analysis of the stover and grain revealed that appreciable concentrations of the heavy metals were taken-up by the maize plant but remained in the vegetative tissue. Out of the total heavy metal concentrations found in whole maize, only about 5 percent of Cd and Pb, 2 percent of Ni and 11 percent of Cu were found in the grain. Interestingly for Zn, more was assimilated in the grain than in the stover. Copper and Zn are essential plant nutrients while Cd and Pb are not. Therefore, it is expected that the plant would have physiological barriers to uptake of non-essential elements. These results support the idea that plant physiological factors are an important consideration for the uptake of elements from soil and their assimilation into plant tissue.

5.2.2. Effect of lime and manure on accumulation of heavy metals in Pumpkin leaves

The results of the analysis of variance show a significant effect of lime on the accumulation of Cd (p=0.0004), Cu(p=0.0001), Pb (p=0.048) and Zn(p=004). Compared to the control treatment, the application of lime led to a reduction of 40 percent in concentration for Cd in pumpkin leaves, 33 percent for Cu, 19 percent for Pb and 10 percent for Zn (Appendix 4). The lower concentrations of heavy metals in pumpkin leaves from limed plots can be attributed to immobilisation of the heavy metals due to liming. There was also a significant effect of manure application and accumulation of Cd (p=0.038) and Zn (p=0.004). Compared to the control treatment, the application of manure reduced Cd concentration in pumpkin leaves by an average of 16 percent, while Zn concentration increased by 35 percent. Manure equally immobilised soil Cd resulting in reduced uptake by the crop while Zn was higher in the pumpkin leaves due to addition from the manure.

No evidence of an interaction between lime and manure for Cd (p=0.711), Ni (p=0.733), and Pb (p=0.281) was found, while Cu was significantly reduced (p=0.008) and Zn increased (p=0.048). The decrease of Cd, Cu, Ni, Pb, Zn in pumpkin leaves due to lime, either applied solely or in combination with manure was as a result of the reduction of the bioavailable form in the soil. Additionally, the reduction of Cd-dtpa and increase of Zn-dtpa in the soil by manure led to low and high concentrations respectively in pumpkin leaves.

There was no evidence of a relationship between the season and concentration of Cd, Cu, Ni, and Pb in pumpkin leaves, but a highly significant (p=<0.0001) relationship with Zn was observed, where S2 had more Zn than in S1. More Zn in S2 than in S1 is likely due to a dilution effect in S1 which had a higher dry matter yield (Table 5-1). A significant effect of the distance to the tailings on accumulation of Cd (p=0.0298), Ni (p=0.043), and Pb (p=0.0463) was found, with pumpkin leaves further from the tailings (D1) having a higher concentration than closer to the tailings (D2). There was a significant interactive effect of the season with the distance to the tailings for the uptake of Cd (p=0.0216), Cu (p=0038), Ni (p=0054), and Zn (p=0.004) with combinations involving D1 having higher heavy metal concentrations compared to D2 (Figure 5-2).

Table 5-1: Pumpkin leaf sample weight per treatment

Season	Treatment	Leaf weight [a] (g per 20 leaves)
S1	L0M0	368 ± 26
	L1M0	399 ± 12
	L0M1	575 ± 25
	L1M1	524 ± 39
S2	L0M0	349 ±8
	L1M0	363 ± 17
	L0M1	316 ± 73
	L1M1	380 ± 51

[a]average of four replications
L0M0, L1M0, L0M1, and L1M1 refer to zero, lime, manure, and lime and manure treatments respectively.

Note: S1 and S2 refer to seasons 1 and 2; D1 and D2 represent plots that were further and closer to the tailings dam, respectively

This observation indicated the resultant effect of immobilisation of heavy metals in the soil at higher pH (Gadepalle *et al*., 2007) because plots closer to the tailings had a higher pH than those further away. Furthermore, there were significant interactions of lime with the season for accumulation of Cu (p=0.0305) and with the distance from the tailings for uptake of Cd (p=0.0333), where pumpkin leaves under treatments with lime had lower Cu concentrations than those without lime. Lower accumulation of heavy metals in limed treatments was attributed to immobilisation of the heavy metals by lime. Pumpkin leaves in D1 had more Cd than those in D2, but within the same location relative to the tailings, those under lime treatment had lower Cd concentrations than those without lime (Figure 5-2).

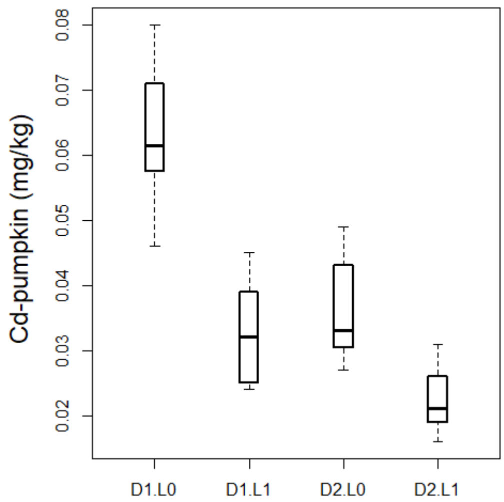


Figure 5-2: Heavy metal concentration in pumpkin leaves as affected by the interaction of lime and proximity to the tailings

Note: D1 and D2 represent plots denoted as 'far' and 'near' from the tailings dam.

The lower concentrations of Cd, Cu, Pb and Zn measured in pumpkin leaves implies that liming soils for the purpose of ameliorating acidity, can inadvertently also reduce uptake of metals in these contaminated soils, even at agronomic application rates. This observation indicated the resultant effect of immobilisation of heavy metals in the soil at higher pH (Gadepalle *et al*., 2007) because plots closer to the tailings had a higher pH than those further away. However, before soils are limed, it must be ascertained that they are acidic as liming un acidic soils may induce solubilisation of SOM thereby releasing organically bound metals.

Several studies have shown that amending heavy metals contaminated soils with lime at much higher application rates reduced extractable concentrations of heavy metals. For example, Vondráčková *et al*. (2013) reported a decrease in $CaCl_2$-extractable Cd and Zn following soil amendment with quicklime and dolomite. Similar results were reported for Cd and Pb by Abd El-Azeem *et al*. (2013) after amending soils with calcitic lime. Manure reduced the uptake of Cd but led to an increase in Zn uptake. The soil metal immobilising ability of organic amendments have also been reported (Conder *et al*., 2001; Angelova *et al*., 2010; Angelova *et al*., 2013). Angelova *et al*. (2010) found that organic amendments were especially effective for reducing Cd uptake by potatoes. However, Zn uptake by pumpkin leaves was increased because the manure used had a large concentration of Zn (434 mg kg^{-1} total Zn). This result agrees with findings by Manzeke *et al*. (2020) that manure can improve the supply of Zn from the soil for crop uptake. Similar trends were observed in all interactions involving the proximity to the tailings, i.e., with the season for Cd, Cu, Ni, and Zn, and with lime for Cd.

There was no significant interactive effect of lime and manure for Cd, Ni, and Pb. At the same time, a reduction and an increase were observed for Cu and Zn, respectively Although the amendments of lime and manure led to a significant reduction in uptake of some heavy metals, the low concentrations were not below the maximum permissible limit for Cu and Pb for pumpkin leaves, therefore the risk still exists.

These results show that the initial soil chemical characteristics influence the effectiveness of the amendments in reducing heavy metal uptake by crops and should be considered when devising in-situ immobilisation strategies. Precisely for this area, this implies that liming of the tailings prior to disposal ensures the fixation/immobilisation of the heavy metals as they are deposited via wind-blown dust onto surrounding soils.

5.2.3. Effect of lime and manure on uptake of heavy metals by Maize

There was no evidence of an effect of both lime and manure and their interaction on accumulation of Cd, Cu, Ni, Pb, and Zn in maize grain. This could be because very little of the heavy metals studied were assimilated into the grain, except for Zn, an essential nutrient and particularly important for grain filling (Xue et al., 2019). There was, however, evidence of a significant seasonal effect ($p=0.048$) on Pb accumulation in maize grain, with its interaction with lime ($p=0.0375$), where the concentrations in S1 was greater than in S2 (Figure 5-3a). Higher concentrations of heavy metals in season S1 than in S2 can be attributed to dilution effect resulting from higher yield in S1 than in S2 as shown in Table 5-2.

Table 5-2: Maize grain yield (Mean ± standard deviation) under each treatment

Season	**Treatment**	**Grain yield a (kg ha-1)**
S1	L0M0	1279 ± 385
	L1M0	1953 ± 635
	L0M1	1980 ± 496
	L1M1	2243 ± 396
S2	L0M0	1033 ± 214
	L1M0	1708 ± 476
	L0M1	1629 ± 171
	L1M1	1615 ± 122

aaverage of four replications

L0M0, L1M0, L0M1, and L1M1 refer to zero, lime, manure, and lime and manure treatments respectively.

A significant interactive effect of the season and the distance to the tailings was observed for Zn concentration in grain (p=0.0127), with interactions involving D1 having higher Zn concentration than those with D2 (Figure 5-3b). A similar explanation as with the pumpkin leaves can be rendered for the grain in D1 having more Zn than in D2.

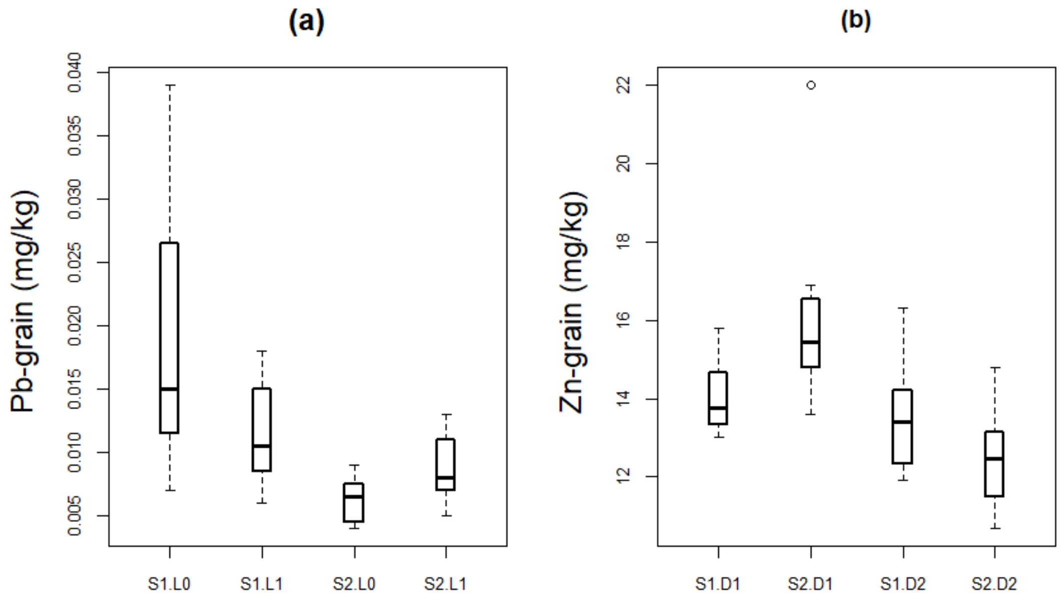


Figure 5-3: Lead and Zn concentrations in maize grain as affected by the interaction of season with lime (a) and with the distance to the tailings (b), respectively

S1 and S2 represent seasons 1 and 2, respectively, and D1 and D2 represent D1 and D2.

Contrastingly to the results observed for maize grain, significant effects lime and manure amendments on maize stover were observed. In comparison to the control treatment, lime reduced the Cu concentration in maize stover by an average of 46 percent. Manure also reduced the Cu concentration in stover by 58 percent and the interaction of lime and manure reduced it by 45 percent. Concentrations of Cu in stover were significantly greater ($p=0.0002$) in season S2 than S1 and this was observed even with its interaction with lime ($p=0.0195$) and manure ($p=0.0111$). Similar trends between the seasons were also observed for Ni ($p=0.0117$), Pb ($p=0.0287$) and Zn ($p=0.0004$). There was a significant effect of lime ($p=0.0069$) and manure ($p=0.0072$) on stover Zn concentration. Lime reduced maize stover Zn concentration by 48 percent, while manure reduced it by 46 percent. As seen for Cu, a significant interaction of season and lime ($p=0.0419$) was observed for maize stover Zn, where interaction with season S2 had higher maize stover Zn concentrations than those involving S1.

Lime and manure both led to a reduction in stover Cu and Zn concentration due to their immobilisation in soil. Despite having a large Zn concentration, manure did not result in increased Zn concentration in maize. Manzeke *et al*. (2020) reported reduced Zn concentration in maize grown in soils treated with manure which they attributed to limited N availability. The significantly greater stover- Cu, Ni, Pb and Zn in season S2 than S1 could be due to a dilution effect in S1 (Cakmak, 2008), as the yield was larger in S1 than S2.

5.2.4. Risk of exposure

The risk of exposure to humans of the heavy metals was assessed using the hazard quotient (HQ). An HQ is the ratio of the potential exposure to a substance and the level at which no adverse health effects are expected. When HQ<1, no adverse health effects are expected and when HQ >1, the risk to health is high. Equation 4 was used to compute HQ for Cd, Cu, Ni, Pb, Zn for an average person weighing 60 kg. Values of HQ for Cd, Cu, Ni, Pb, Zn calculated according to Eq. 4 for an average person weighing 60 kg are shown in Table 5-3.

$$\mathrm{HQ} = \frac{C \times ADI \times FWC}{RfD \times BW} \qquad \text{Eq. 18}$$

where C is the concentration of a heavy metal in the food material on a dry weight basis in mg kg^{-1}, ADI is the fresh weight average per capita daily intake of foods, RfD is the reference dose (mg kg^{-1} day^{-1}) which is an estimate of the daily oral exposure below which no adverse health effects are likely in one's lifetime (see Table 5-3 for values). The FWC is the Fresh weight conversion (moisture) factor; BW is the average adult body weight of the population in kg, which was taken as 60 kg for the study area (Nakaona *et al*., 2019).

Table 5-3: Hazard quotients associated with consumption of pumpkin leaves and maize grain at Mugala village

Heavy metal	Median conc. in pumpkin leaves (mg kg^{-1})	Median conc. maize grain (mg kg^{-1})	FAO/WHO max. allowable limit (mg kg^{-1})	RfD[a] (mg kg^{-1} day^{-1})	HQ for pumpkin leaves FWC=0.078	HQ for maize grain FWC=0.896
Cd	0.033	0.002	0.2	0.001	0.002	0.004
Cu	49.0	1.8	20	0.04	0.091	0.093
Ni	2.34	0.073	67	0.02	0.009	0.008
Pb	0.478	0.011	0.3	0.0035	0.01	0.007
Zn	39.8	14.0	99	0.3	0.01	0.096

[a] US EPA Iris database (2015)

For both pumpkin leaves and maize grain, Cd presented the lowest HQ, while Cu was highest. For maize grain, Zn presented similar HQ as Cu. However, values of HQ for Cd, Cu, Ni, Pb, Zn for both crops were all less than 1, implying that there were no potential adverse health effects expected from the consumption of pumpkin leaves and maize grain grown in this area. This result confirms the interpretation made according to the concentrations found in the grain, which were below the FAO/WHO (2011, 2001) maximum permissible limits. However, there is a discrepancy between the interpretation of the associated risk for Cu and Pb in pumpkin leaves between FAO/WHO limits and the HQ values. According to the FAO/WHO (2011, 2001) limits, the median values of Cu and Pb found in pumpkin leaves were above the allowable thresholds and could harm consumers. On the other hand, their associated HQs are at least an order of magnitude less than one, implying that they pose a minimal threat to consumers. This discrepancy suggests the need for standardizing risk assessment tools to ensure consistency in findings. Although pumpkin leaves had larger concentrations of heavy metals than maize grain, their HQs were within the same order of magnitude with grain because of their smaller per capita consumption than maize grain.

5.3. Conclusions

The results show that there was higher accumulation of heavy metals in pumpkin leaves than in maize grain. Similar concentrations to those in pumpkin leaves were found in maize stover, indicating accumulation in photosynthetic tissue. Copper and Pb were above the FAO/WHO prescribed safety limit even in amended soils, implying that the risk still exists under the conditions of this study. Lime reduced the concentrations of Cd, Cu, Pb and Zn in pumpkin leaves, while its effect on the concentrations in maize grain was non-significant because only low concentrations were assimilated into the grain. Manure reduced the concentration of Cd in pumpkin leaves while Zn was increased. Higher concentrations of some heavy metal were observed in the second season than in the first for both pumpkin leaves and maize grain and this was attributed to the yield dilution in the prior season where dry matter yield where higher in S1. Crops grown farther from the tailings dam had more Cd, Cu, Ni, Zn (pumpkin leaves) and Zn (maize grain), than those near the dam. This observation was attributed to leaching losses of DOC-bound metals in soil closer to the dam and to immobilisation of bioavailable heavy metals in lime amended acidic soils.

CHAPTER SIX

6. PREDICTION OF COPPER CONCENTRATION FROM SELECTED SOIL FRACTIONS USING THE WHAM VII MODEL

6.1. Overview

The objective of this study was to evaluate the performance of the Windermere Humic Aqueous Model version seven (WHAM VII) for prediction of Cu fractions in tropical soils. The fractions of soil Cu originating from the mine tailings was assessed through direct measurement of soluble concentration and prediction with Windermere Humic Aqueous Model version seven (WHAM VII). Soil samples were collected from 98 locations around the study area. A pot experiment in the glasshouse was conducted and the relationship between bioavailable Cu in soil and plant uptake was studied using Chinese cabbage (*Brassica rapa*) as a test crop. Total Cu concentration, isotope dilution determined labile Cu and DTPA determined labile Cu were used as inputs for the solid phase Cu concentration to test the performance of WHAM VII. The partitioning of soil Cu into solid and liquid phases was studied in detail in order to understand the drivers of its bioavailability in the soil.

6.2. Results and Discussion

6.2.1. Soil characterisation

Soil properties, including pH and concentrations of SOM, FA, HA, DOC, FeOx, MnOx and AlOx are shown in Table 6-1. Soil pH spanned a wide range of values, from 4.1 to 7.1, with 66 percent of the samples in the acidic range (pH<5) and the rest in the neutral to slightly alkaline range (PH>5.5). Soil organic matter, estimated from LOI, ranged from 3.3-11.6 percent. Humic acid concentrations were higher than the FA concentrations, although within the same order of magnitude. Dissolved organic matter concentration ranged from 2.9-34.3 mg kg^{-1}, with most values around 5 mg kg^{-1}. Oxides of Fe, Mn and Al (FeOx, MnOx and AlOx respectively) occurred within narrow ranges as 12-29 g kg^{-1}, 0.35-1.25 g kg^{-1} and 1.7-6.4 g kg^{-1}, respectively. Iron oxides were the most abundant, being greater than AlOx and MnOx by one and two orders of magnitude respectively. The soils had a sandy loam texture with clay content ranging from 10-28 percent.

Table 6-1. Summary statistics (n=98) of the soil characteristics of the study area

Soil property	Min.	Max.	Mean	Median	Standard deviation
pH	4.1	7.1	5.5	5.4	0.79
% LOI	3.3	11.6	5.7	5.6	1.23
Cu-tot (mg kg^{-1})	136	1462	863	874	293
FeOx (g kg^{-1})	12	29	20	19	4
MnOx (g kg^{-1})	0.35	1.25	0.58	0.55	0.17
AlOx (g kg^{-1})	1.74	6.38	3.98	4.00	1.03
DOC (mg L^{-1})	2.95	9.23	4.66	4.54	0.96
FA (g kg^{-1})	0.99	2.62	1.63	1.62	0.32
HA (g kg^{-1})	1.57	12.01	5.67	5.49	2.29
% Clay	10	28	18	18	4

Values of Cu-tot in the soil samples covered a wide range of values from 135-1460 mg kg^{-1}, reflecting the variability in the dispersal of the tailings material. All Cu-tot values exceeded the FAO/WHO maximum limit for Cu in agricultural soils, given as100 mg kg^{-1} (WHO, 1993).

6.2.2. Evaluation of extraction method for bioavailable Cu in soil

Copper was extracted from 98 soil samples collected from various locations in the study area. Values of Cu-tot, Cu-E, Cu-dtpa, and Cu-soln are shown in Figure 6-1. Concentrations of Cu-E exceeded those of Cu-dtpa; the concentrations of Cu-soln were much lower than both estimates of labile Cu. Different single extraction methods determine varying concentrations of bioavailable fractions of heavy metals in soil due to the influence of soil properties on the extraction ability of the reagents used (Ayoub *et al*., 2003; Nazarpour *et al*., 2019). Marzouk *et al*. (2013a), also reported that lability for Zn, Cd and Pb determined by DTPA extraction was much lower than their E-values (M-E). The extraction using Ca-nitrate is not intended to extract the reservoir of labile Cu but rather approximate the soil pore water Cu concentration (Houba *et al*., 2000).

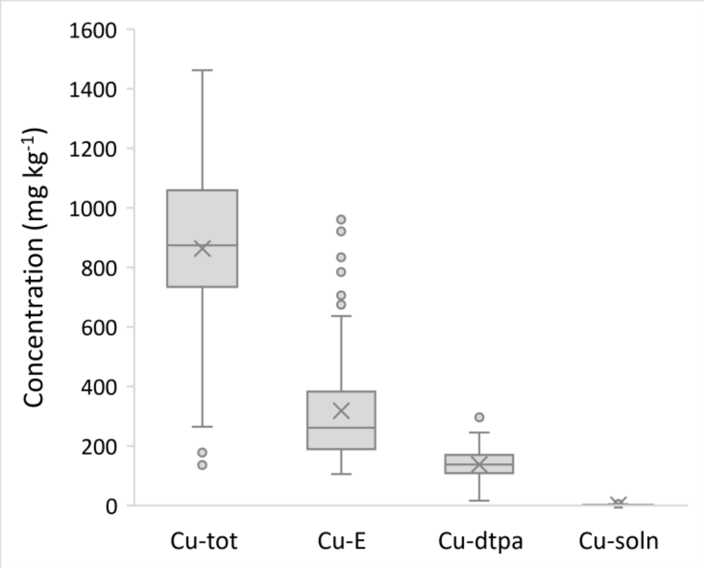
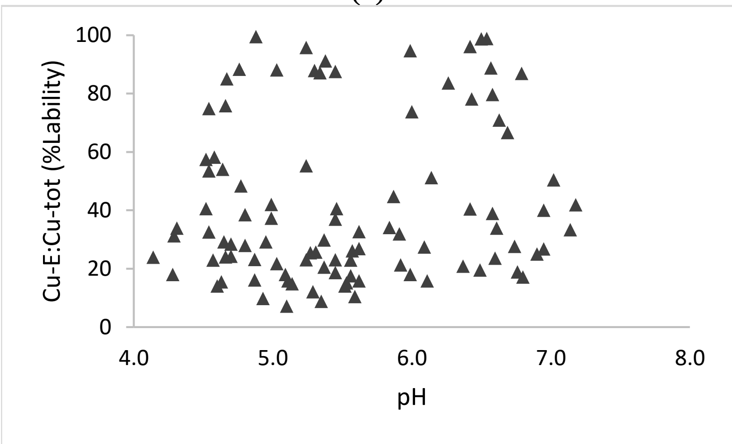


Figure 6-1. Cu-tot, Cu-E, Cu-dtpa, and Cu-soln.

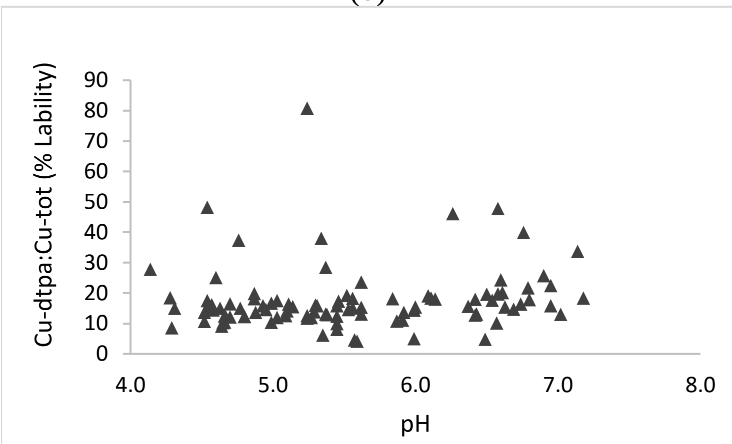
The median is shown by the horizontal line and the mean by the cross in the box; outliers are shown by closed circles. The upper and lower limits of the box correspond, respectively, to the third and first quartile of each data set.

Figure 6-2 shows how proportions of Cu-E, Cu-dtpa and Cu-soln are affected by soil reaction. There was no consistent trend between pH and ratios of Cu-E, and Cu-dtpa to Cu-tot, while Cu-soln:Cu-tot was larger at pH values lower than 5 and decreased to almost zero above pH 5. These results suggest that soil reaction affects the exchange of Cu between the colloidal surfaces and the solution but does not readily change its reactivity. Calcium salts (nitrate and chloride) at 0.01 M extract a readily available fraction which approximates the soil 'soluble' fraction (Houba *et al*., 2000). This soluble fraction is pH-dependent with higher concentrations expected at low pH and lower concentrations at high soil pH (Gray *et al*., 1999) as shown by findings from this study.

(a)



(b)



(c)

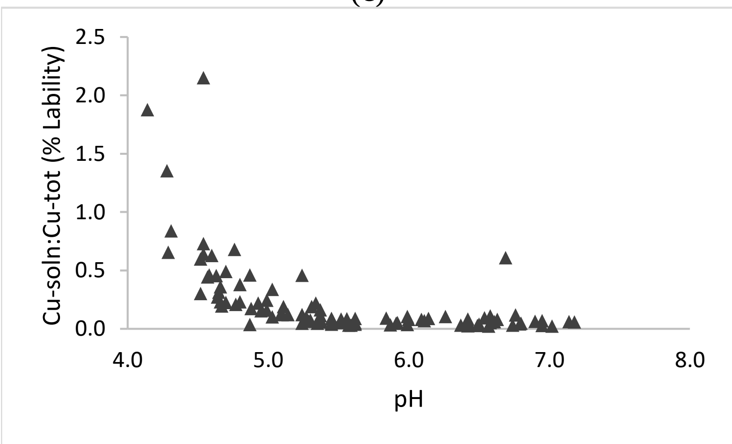


Figure 6-2. Ratio of (a) Cu-E, (b) Cu-dtpa and (c) Cu-soln, to Cu-tot, as a function of pH

6.2.3. Validation of the WHAM VII model for prediction of Cu in solution

The WHAM VII model is an assemblage of three sub-models which model binding of metals to major particulate and colloidal binding phases namely; Humic Ion Binding Model VII, Cation Exchange Model and Surface Chemistry Assemblage Model for Particles (SCAMP). The Humic Ion Binding Model VII models binding to humic substances. The humic substances are Fulvic acids (HA) and humic acids (HA) which are assumed to have hydrophilic ion-binding carboxyl and phenolic hydroxyl groups on the surface. The Cation Exchange Model is based on Donnan principles and predicts electrostatic binding of ions to aluminosilicate clays. The SCAMP model predicts ion binding to soil mineral oxides.

6.2.3.1. Prediction of soil solution Cu concentration

It is widely accepted that the solid-liquid partitioning and speciation in the solution phase of soil determine the bioavailability of metals. Therefore, the concentration of soluble Cu in the soils were of interest to the overall objective of the study. Concentrations of soil solution Cu were predicted by WHAM VII using the three different capacity indices: Cu-tot, Cu-E and Cu-dtpa as model inputs. Model predictions of the soil solution Cu were compared with measured Cu-soln values (Figure 6-3), and linear mixed models were fitted to examine evidence of a relationship between the indices and the measured soil solution Cu concentration. In all cases there was a positive relationship between the index and the solution concentration, indicating that soil solution Cu concentration depended on the concentration of the input capacity indices.

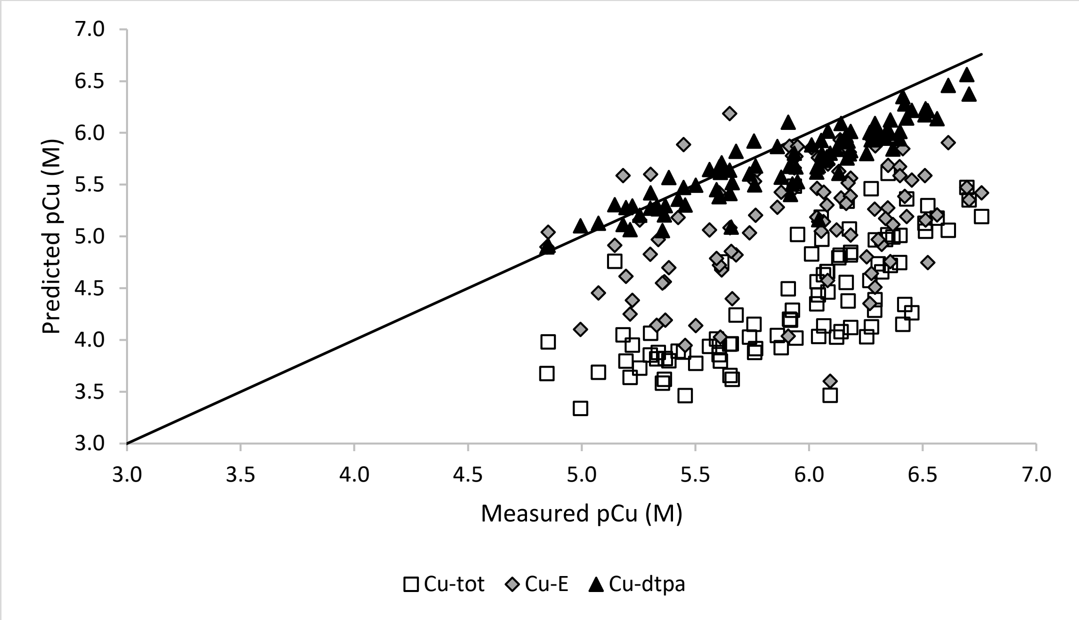


Figure 6-3. Goodness of fit of Cu concentrations (pCu-soln=-Log_{10}[Cu-soln]) predicted with WHAM VII

Measured concentrations were determined in a 0.01 M $Ca(NO_3)_2$ soil suspension. WHAM(VII), with either Cu-tot or Cu-E or Cu-dtpa as input variables, was used to predict Cu-soln. The solid lines represent the 1:1 relation

Table 6-2 shows the model (Eq. 19) fitting. In the first row is a "null model" with the only fixed effect a constant mean, and then the three models with each predictor follow in the subsequent rows. The first four parameters are for the random effects in the model and are the uncorrelated variance (c0), the spatially correlated variance (c1), the distance parameter and a spatial smoothness parameter. b_0 is the intercept, followed by its standard error, b_1 is the regression coefficient, also with a standard error. L is a log-likelihood ratio statistic for the null hypothesis that the regression coefficient is zero, followed by the p-value. The final column is an adjusted R-sq (R^2 adj) which is the proportion of the variation in the response which is explained by the model. A large R^2 adj (0.82) value was realised from the model with Cu-dtpa as predictor. These results imply that labile concentrations determined from DTPA extraction data are the best inputs to the WHAM VII for predicting soil solution Cu in these soils. This is useful in practical terms because the DTPA protocol is the most commonly used method of extracting trace elements in Zambia and many other countries.

$$y = b_0 + b_1 x_0 + \eta + \varepsilon \qquad \text{Eq. 19}$$

where y is the dependent variable (Cu-soln), x is the independent variable (Cu-dtpa), b_0 is the intercept of the linear model and b_1 is the coefficient for the independent variable η is a spatially correlated random effect with a normal distribution of mean zero, variance C_1 and a Matérn spatial autocorrelation function with smoothness parameter *k* and distance parameter *f*, ε is an independent and identically distributed random effect with mean zero and variance C_0.

Table 6-2. Fitted model parameters for measured Cu-soln (log transformed)

Predictor	C0	C1	η	κ	b_0	S.E b_0	b_1	S.E. b_1	L	P value	R^2_{adj}
Null model	1.10	0	−	−	−13.63	0.11	−	−	−	−	−
log Pred Cu-tot	0.52	0	−	−	−7.72	0.57	0.587	0.056	72.3	<0.0001	0.53
log Pred Cu-E	0.93	0	−	−	−9.72	0.92	0.329	0.077	16.6	<0.0001	0.15
log Pred Cu-dtpa	0.00	0.2	9.7	0.1	0.946	0.69	1.105	0.052	165.6	<0.0001	0.82

6.2.3.2. Properties of the soil solution

The properties of the soil solution were determined in order to evaluate the major drivers of Cu bioavailability in the soil. Soil solution properties varied over a wide range (Table 6-3). Soil pH ranged from 4.3-6.8, with 75 percent of the values in the acid range. The solution concentration and free ion activity of Cu (Cu^{2+}) predicted from WHAM VII, spanned four orders of magnitude, indicating substantial variation in Cu speciation and consequently in its bioavailability across the study area. Colloidal FA-bound Cu concentrations all fell within one order of magnitude.

Table 6-3. Properties of soil pore water

Variable	Min.	1st Quartile	Median	Mean	3rd Quartile	Max.	Standard deviation
Soil solution pH	4.3	4.9	5.4	5.5	6.1	6.8	0.66
Free Cu^{2+} activity (M) x10^{-9}	3	40	170	840	1080	7130	3
Solution Cu (M) x10^{-9}	5	80	320	1570	2020	13500	5
FA bound Cu (M)	0.04	0.23	0.58	0.54	0.84	0.99	0.31

6.2.3.3. Speciation of Cu in the soil solution

Speciation of Cu in the soil solution (Cu-soln) showed a dependency on the soil pH with the most predominant species being Cu^{2+} (free ion) and FA-Cu (Figure 6-5). In acidic soils (pH ≤ 5) Cu in the soil solution occurred predominantly (>80 percent) as Cu^{2+}, while FA-Cu predominated at around pH 5.5 and above. The free ion of a metal is regarded as the most bioavailable species (Golui *et al.*, 2020). Thus, although competition from the hydrogen ion for uptake is expected at pH < 5.5, the bioavailability of Cu to plants which are grown in these soils may be at its highest, as evidenced by results reported in Chapter five where pumpkin leaves in acidic soils further from the tailings accumulated more heavy metals that those in neutral-alkaline soils closer to the tailings. At pH 6, Cu^{2+} declined to about 20 percent, while FA-Cu increased correspondingly. Thus, a reduction in soil acidity favours carbonate and hydroxide complex formation in the soil solution. Organically complexed; Cu (FA-Cu) is unlikely to be bioavailable due to the large molecular weights of the complexing molecules. These results showed that even within the agronomic pH range (5-7), the plant roots are in contact with a range of different Cu species, potentially with differing levels of bioavailability in solution.

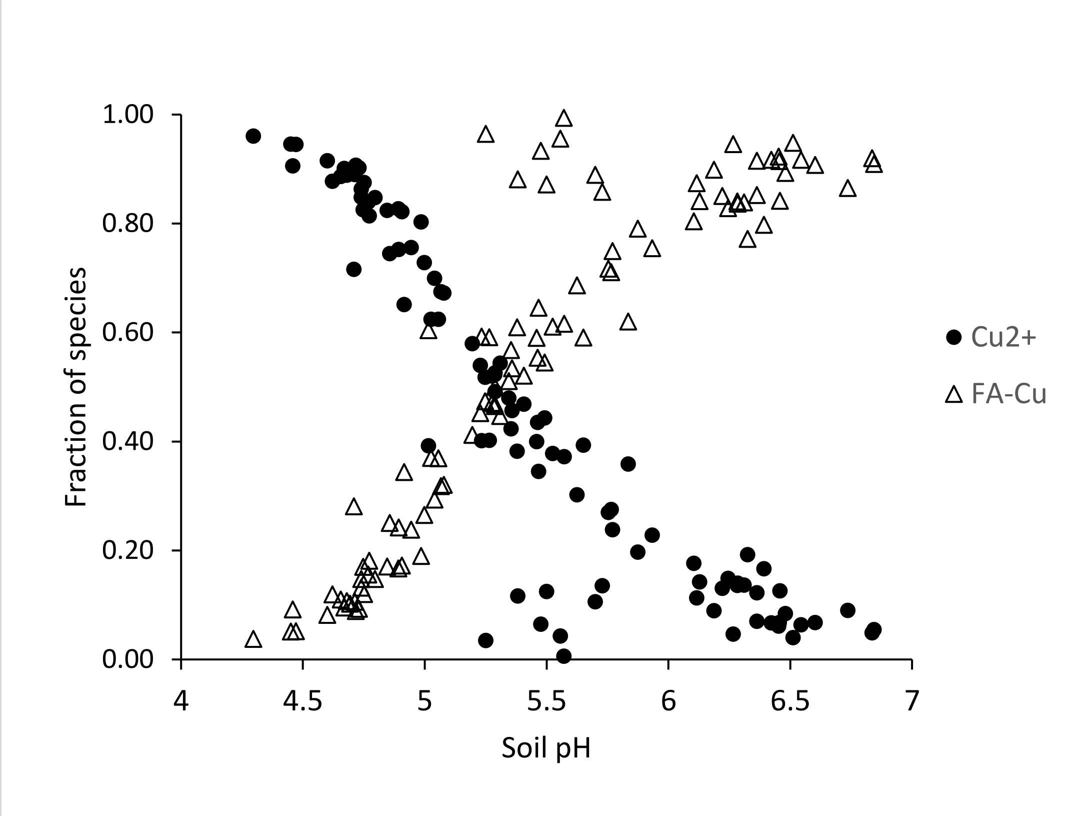


Figure 6-4. Speciation of Cu in the soil solution (0.01 M $Ca(NO_3)_2$ suspensions).

6.2.4. Prediction of crop Cu concentration from Cu in the soil solution

Table 6-4 shows the results of the model (Eq. 20) fitting for Cu concentration in the crop. Although some evidence can be seen for a relationship between Cu concentration in the crop, and both Cu-soln and the predicted solution Cu from DTPA, the largest proportion of the variance accounted for by the predictor was only 0.05. Furthermore, the fixed effects coefficients for these relationships were negative, which is clearly against expectation. These results show that factors other than soil solution concentration influenced the uptake of Cu from the soil. On the other hand, it could be that within the range of soil conditions investigated in this study (pH, Cu concentration etc), there was sufficient control over Cu uptake exercised by the plant to negate any effect of the soil solution Cu concentration. The lack of correlation between concentrations in the soil and in the plant has been reported in other studies conducted in other places. Feng *et al*., (2005) reported a poor correlation between barley shoot concentrations of Cu (also Cr, Zn, Ni, Pb) and various soil extraction methods including 0.01 M $CaCl_2$, but good correlations were observed with Cu in the roots. Based on their findings they concluded that shoot concentrations were not a good indicator of bioavailability. This was partly attributed to the physiological factors affecting the movement of metals from roots to shoots through the xylem to storage in the vacuole of leaf cells. Chenery *et al*. (2012) also reported no direct correlation between paired soil and vegetation Pb in a study of a historical mining area, however, it was established that access by the plant was through root uptake.

$$y = b_0 + b_1 x_0 + \eta + \varepsilon \qquad \text{Eq. 20}$$

where y is the dependent variable (Cu concentration in crop), x is the independent variable (measured Cu-dtpa), b_0 is the intercept of the linear model and b_1 is the coefficient for the independent variable η is a spatially correlated random effect with a normal distribution of mean zero, variance C_1 and a Matérn spatial autocorrelation function with smoothness parameter *k* and distance parameter *f*, ε is an independent and identically distributed random effect with mean zero and variance C_0.

Table 6-4. Fitted model parameters for Cu concentration in crop (log-transformed)

Predictor	C0	C1	η	κ	b_0	S.E b_0	b_1	S.E. b_1	L	P value	R^2_{adj}
Null model	0.00	0.100	2.8	1	3.86	0.03	−	−	−	−	−
log Cu-soln	0.00	0.095	1.8	2	2.88	0.41	−0.07	0.03	5.55	0.018	0.05
log Predicted using Cu-tot	0.00	0.098	4.6	0.5	3.44	0.25	−0.04	0.02	2.76	0.097	0.01
log Predicted using Cu-E	0.00	0.101	2.2	1	3.51	0.30	−0.03	0.03	1.17	0.28	0.00
Predicted using Cu-dtpa	0.00	0.096	1.8	2	2.75	0.48	−0.08	0.04	5.27	0.02	0.04

6.2.5. Prediction of Cu fractions from WHAM VII using Cu-dtpa to proxy labile Cu

Along with predicting the solution phase speciation, WHAM produces solid phase partitioning (fractionation) of the metal ion (Tipping *et al*., 2003). Solid-phase fractionation of metals in soil has been shown to influence the soil solution metal concentration (Mao *et al*., 2017; Marzouk *et al*., 2013b). Therefore, knowledge of metal fractionation is important in the management of contaminated soils. Soil reaction (pH) is the most important determinant of solid-liquid partitioning of the metals in soils (Buekers *et al*., 2007). In this study, a good correlation was found between pH and particulate Cu fractions including: HA (r=86), FeOx (r=90) and AlOx (r=83). Figure 6-5[shows the fractionation of Cu as a function of pH. Particulate HA was the major binding surface for Cu, while MnOx and AlOx were the least important. Copper binding to particulate HA was greater at lower pH and decreased with increasing pH, while binding to particulate FA was almost constant across all pH ranges. Binding to FeOx and AlOx increased with pH, although AlOx was only evident at pH values above 5.5. Humic substances (HA and FA) were the major binding phases for Cu in these soils, therefore confirming the high affinity of Cu for organic ligands which is reported in the literature (Bonten *et al*., 2008; Mao *et al*., 2017).

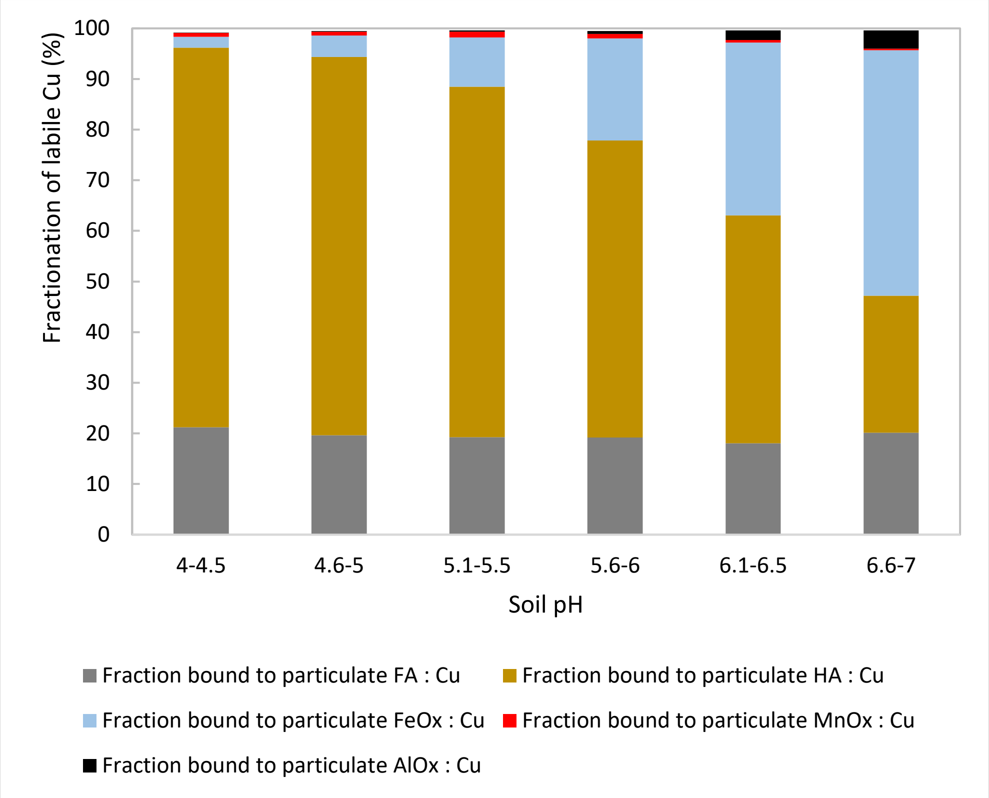


Figure 6-5. Relationship of soil reaction to the particulate Cu fractions in the soil

WHAM VII was used to predict fractionation, with Cu-dtpa as the input variable representing labile soil Cu.

6.2.3.4. Effect of pH and Cu fractionation on WHAM VII prediction of solubility of Cu

Since the solubility of cations in soils is influenced by the predominant particulate binding phases, the sensitivity of the prediction of Cu concentration in solution to variation in the concentrations of the different geocolloidal adsorbents (and pH) was examined. Figure 6-6 shows the effect of soil reaction and particulate sorption parameters on prediction of soil solution Cu by WHAM VII. At pH above 5.5, the model overestimated the prediction for Cu-soln. Particulate FA and MnOx did not seem to affect the prediction of Cu-soln. The prediction was improved with increasing particulate HA concentration, while an opposite trend was seen for Fe and Al oxides. Although HA is the most important sorption phase for Cu, the effect of FeOx, which is a major geocolloidal adsorbent in these soils, could significantly influence the model results, especially at higher pH (>6) where iron oxides are a significant sorption phase for Cu.

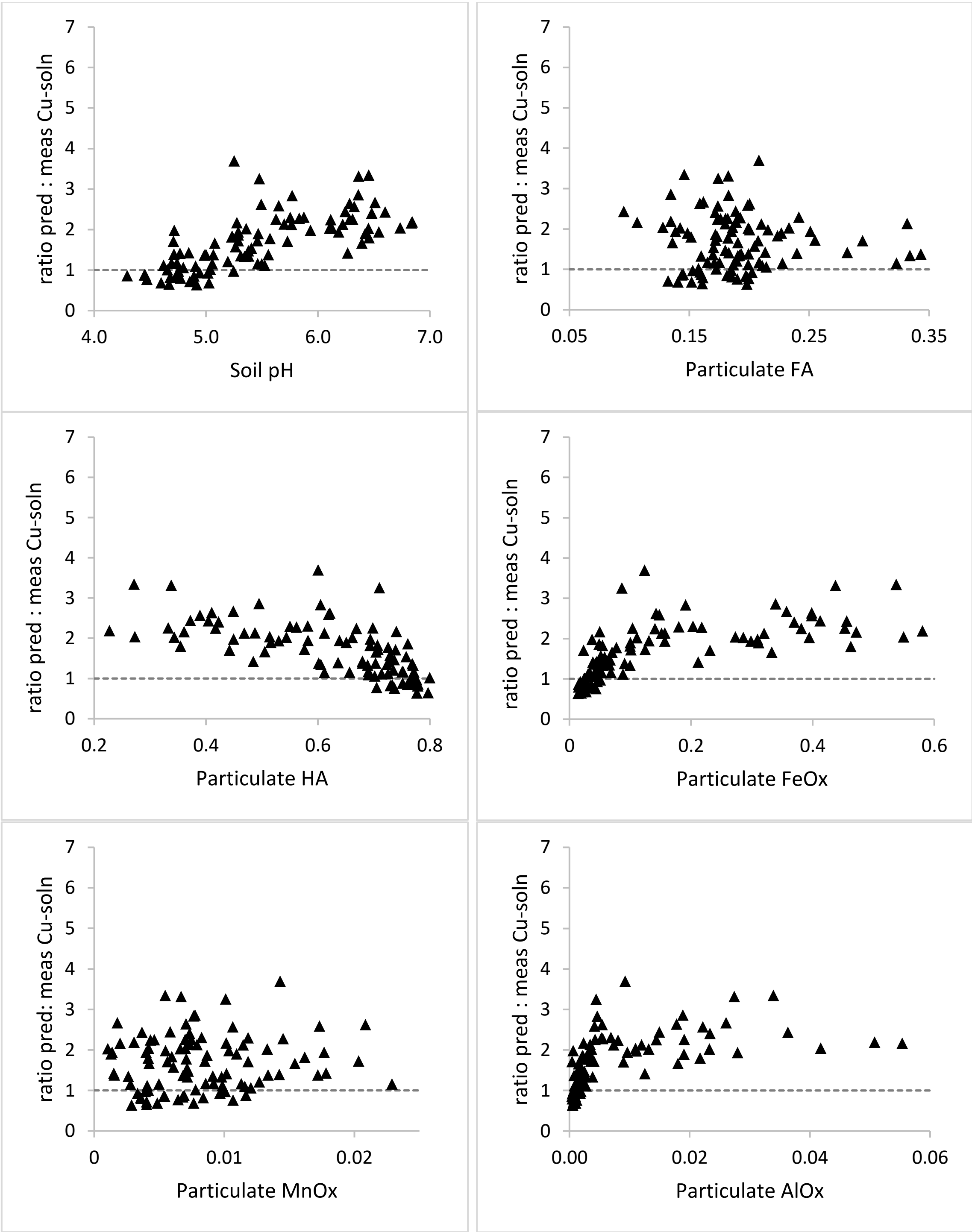


Figure 6-6. Effect of soil reaction and particulate sorption parameters on prediction of soil solution Cu by WHAM VII

Dotted line shows the 1:1 relation of the predicted and measured soil solution Cu

6.3. Conclusion

The lability of the total soil Cu concentration as determined by isotopic dilution ranged from around 10-100 percent. Lability determined by DTPA extraction was predominantly between 10-30 percent, and it gave the best predictor of soil solution Cu concentration. Free ion Cu was the most predominant species at pH below 5. Above pH 5.5, Cu speciation in solution was dominated by complexation with colloidal FA. Humic substances (HA and FA) were the dominant solid sorption phases, with HAs being more dominant at pH<6. Fulvic acids were constant across the pH range in the soil. Iron oxides were more important at pH above 5.5 and predominated at around neutral pH. The WHAM VII model's prediction of soil solution Cu was overestimated at higher pH and lower particulate HA content, while particulate FeOx and AlOx had an opposite effect. The concentration of Cu in the Chinese Cabbage did not correlate well with either the measured or the predicted soil solution concentrations as shown in Section 6.2.4.

CHAPTER SEVEN

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. Overview

Mine tailings usually contain large concentrations of various heavy metals, including those which may not be purposely mined. In the Zambian Copperbelt, most tailings are from Cu mining, with undoubtedly higher Cu concentrations than other heavy metals. However, studies in Zambia and within the region have shown that tailings from Cu mining contain significant concentrations of other heavy metals, including Pb, Ni, Zn, and to a lesser extent, Cd (Chileshe *et al*., 2020; Mileusnić *et al*., 2014), which are potentially transferable to surrounding land. Therefore, the studies presented in this thesis were aimed at evaluating the concentrations of Cd, Cu, Ni, Pb, and Zn in; i) the soils close to a large Cu mine tailings dam and ii) accumulation in some commonly grown crops in the contaminated area. The effect of lime and manure on the available indices and subsequent uptake by crops was investigated through a field experiment (Chapters four and five). A green-house pot experiment was also conducted to characterise the bioavailable Cu fraction and to validate the ability of the Windermere Humic aqueous Model VII (WHAM VII) geochemical speciation model to predict Cu fractionation, solubility, and free ion activity in the soils under study (Chapter six). The study shows the importance of the bioavailability index in risk assessment of soils potentially contaminated with heavy metals. The weaknesses of some methods commonly used to determine bioavailable metal concentrations are also presented.

7.2. Main findings from the studies

7.2.1. Effect of lime and manure on metal lability and uptake by crops

From the results of the total heavy metal concentrations obtained in Chapter four, the soils in the study area appear to be heavily contaminated with Cu, but not Cd, Ni, Pb, and Zn. The lability of Ni, Pb, and Zn was below 10 percent of their total concentration, which translates into minor potentially available concentrations. The lability of Cd and Cu were higher at about 30 and 20 percent of their total concentrations, respectively.

Despite having the highest lability, cadmium's potentially bioavailable concentrations are insignificant because the total concentration was low. For Cu, this lability translates into potentially available concentrations greater than the maximum allowable total concentration in agricultural soils of 100 mg kg^{-1} (WHO, 1993). Such Cu levels should be concerning as they could lead to excessive uptake and accumulation by crops grown in these soils as was observed for pumpkin in Chapter four.

Different liming materials and manures have been used to immobilise heavy metals in soil to avert the risk to crops, animals and humans. However, for land used for growing crops, the quantities used must be within tolerable agronomic considerations. Applications of lime and animal manure are recommended to improve the productivity of the highly-weathered acidic soils in agro-ecological region III of Zambia. Ability of these amendments to reduce the bioavailability of soil contaminants such as heavy metals would be an added advantage as this would prevent the risk of potentially harmful elements (PHE) entering the food chain.

The results obtained in this study have shown that both lime and manure affect the lability of heavy metals. Lime had an immobilising effect on heavy metals, thereby reducing the bioavailability. The results in Chapter four show that Cd, Cu, Ni, Pb were immobilised in limed soils especially when lime and manure were applied together. At high pH or alkaline conditions, the soil surfaces become more electronegative, favouring the adsorption of cations (Tlustoš *et al*., 2006). The greater immobilisation of heavy metals in the presence of manure suggests that stable organic complexes formed as soil pH increased. The response to the amendments was greater in the more acidic soils farther from the tailings dam than the soils closer to the tailings, presumably because of the effect of lime pre-applied to the tailings which had spread to the nearby soil. Therefore, lime amendments should only be applied to acidic soils to avoid inducing solubilisation of SOM which can result in increased bioavailability of PHE. Soil pH was the dominant immobilising factor in acidic soils, and the influence of organic matter was more important at higher pH.

The immobilisation of Cd, Cu, Ni, Pb in the soil led to a reduction in uptake by pumpkin and maize (Chapter five). Although lime and manure did not affect the accumulation of the heavy metals in maize grain, the observedaccumulation in stover suggest that there are strong physiological controls on the translocation of these elements to the grain. Thus, the findings reported in this study suggest that maize grown in this area poses minimal risk from heavy metal ingestion by consumers because only low concentrations accumulated in the grain which is directly consumed. This is an important consideration for the affected community and many similar settlements across Zambia and regionally, particularly given the dominance of maize in the diet.

However, while the grain accumulated minimal concentrations of Cd, Cu, Ni, Pb and Zn, the stover accumulated substantial amounts which could warrant further investigation given the importance of maize stover as an inexpensive and accessible livestock feed in Zambia and across Africa. Livestock may be at risk of ingesting hazardous amounts of heavy metals, therefore, this study area may not be suited for livestock grazing. On the other hand, pumpkin leaves accumulated large concentrations of the heavy metals. Copper and Pb in pumpkin leaves were above the FAO/WHO prescribed safety limit which could pose a threat to consumers. However, when per capita consumption is considered, there is a minimal risk of exposure to all of the five heavy metals.

Amendment of contaminated soils with lime and manure at agronomic rates reduced the uptake of Cd, Cu and Zn by both maize (stover) and pumpkin. This is important because using these amendments improves soil quality for agricultural production, but they also have a secondary benefit of reducing the potential uptake of heavy metals by crops in contaminated soils. However, under conditions of this study, the reduction in concentrations of Cu and Pb in crops was insufficient to place them below the FAO/WHO prescribed safety limits. Thus, at the rates of lime and manure equal to or less than 2 ton ha^{-1} and 5 ton ha^{-1} respectively, pumpkin leaves may still accumulate Cu and Pb concentrations which exceed safe limits, and therefore, pumpkin should not be grown in this area. Liming of the tailings prior to disposal effectively fixed the heavy metals in the soil, reducing their bioavailability to crop plants. Interventions for reducing uptake of heavy metals in areas with contrasting micro-environments, such as mining sites, should consider the spatial variation in contamination for effective planning of the application of agronomic amendments.

7.2.2. Bioavailability of Cu in soils

The most bioavailable concentration of any element in the soil is the soluble fraction, which is simply the 'dissolved' concentration in the soil solution. Copper was highly bioavailable at typical soil pH ranges (pH = 4-5) found in agro-ecological region III of Zambia, where the soils are highly weathered. At pH values higher than 5, Cu in solution occurred as a complex with organic ligands, a form which is not taken up by plants.

The soluble fraction is of environmental significance as it is mobile and therefore, there are potential hazards from uptake by crops and leaching to groundwater systems. Therefore, to assess the risk posed by heavy metals such as Cu, the soluble fraction should be determined. However, many laboratories in developing countries cannot measure this fraction as it requires analytical equipment with high detection capacities such as ICP-MS. Thus, solid-phase labile fractions continue to be used to indicate potential bioavailability. The results in Chapter six have shown that there are large differences between the soluble and solid-phase labile fractions and that they are influenced differently by soil properties. The soluble fraction was seen to have an inverse relationship with the pH, while no association was observed for the labile fraction.

No direct relationship between the Cu concentration in the plants and the soil solution was found. Although it is widely accepted that the primary route by which plants access the ions is via uptake through roots, direct relationships between concentrations in soils and the plant are rarely found (Chenery *et al*., 2012; Feng *et al*., 2005).

Apart from elemental concentrations in the soil solution, there are probably many other factors, including physiological growth factors which affect crop uptake of ions from the soil. The expectation of establishing uptake relationships from measurements of concentrations in the soil and the crop is an oversimplification of the soil-plant interaction. The results obtained in this study highlight the need for more holistic approaches to soil-plant uptake studies. As Erel *et al*. (2017) observed for the uptake of phosphorus by maize plants, environmental factors that affect root characteristics are critical for soil-plant relations. Another approach that can aid the study of uptake relationships between the soil and the plant is use of stable isotopes as tracers of uptake.

7.2.3. Validation of WHAM VII for predicting the soil solution concentration

The difficulty of determining soluble soil concentrations of heavy metals demands that alternative means are found to estimate this fraction. The WHAM VII model has been evaluated in a number of studies, and its strengths and limitations have been identified. For example, Mao *et al*. (2017) attributed the overestimation of the soluble concentration by WHAM VII to poorly determined input parameters and sorption surfaces. Instead of using default values of parameters for binding phases, including clay content, humic (HA) and fulvic acids (FA), and Fe, Al, and Mn oxides, these parameters were determined in the soils used in this study. Additionally, it was observed in this study that the DTPA protocol gave the best estimate of the reactive concentrations compared to the isotope dilution method. The isotope dilution method, in principle, provides the best mechanistic description of the reactive fraction in the soil (Izquierdo *et al*., 2017), and this has been observed in a number of studies conducted on soils from temperate regions (Degryse *et al*., 2007; Marzouk *et al*., 2013; Izquierdo *et al*., 2017; Mao *et al*., 2017). Therefore, there is need to investigate the possible interferences of the soil chemical properties on the metal analysis before the method can be applied in tropical soils.

The fractionation of the solid-phase using WHAM VII allows for identifying the cause of the model's poor predictions of the soluble concentration. This is because the 'variation in the solid-phase fractionation has a significant impact on the soils 'soluble concentration' *(*Mao *et al*., 2017). The prediction of the soluble concentration was reasonable, with some over-prediction at pH>5.5. Over-predictions were also observed at larger FeOx and AlOx concentrations, which could be a problem in these soils where these oxides are in abundance. However, the study showed that with a reasonable estimate of the labile/ reactive fraction, the WHAM VII model could predict the Cu bioavailability reasonably well in these soils. The WHAM VII model is, therefore, a promising tool for risk assessment of Cu uptake by crops grown on Cu contaminated tropical soils.

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APPENDICES

Appendix 1: Profile pits description

GENERAL SITE INFORMATION – PIT 1 (near to the dam – D1)									
Geo reference		**Elevation**	**Parent material**	**Topography**	**land use**	**vegetation**	**human influence**	**Drainage**	**Soil Mapping Unit**
35L 619737	8585632	1240	Limestone	Simple Straight slope(0-1%) with cutans on the surface	Crop agriculture with Maize	Mixed vegetation with Acacia, woodland and herbaceous species	Disturbed vegetation,, regular burning, ploughing and bunding	Moderately drained area near a mine drain and the pit is slightly moist through out	Konkola soil series
SOIL PROFILE DESCRIPTION									
Depth	**Colour moist**	**Colour dry**	**structure**	**Texture**	**Consistenc e**	**Porosity**	**Mottles**	**Roots development**	**Biological Activity**
0-22	7.5YR3/4	7.5YR4/4	Weak medium sized subangular blocky	Sandy loam	Loose when dry, Friable when moist, Non sticky and slightly plastic	Many fine sized pores with a very high total porosity		Many fine sized roots	Common
22-39	5YR4/6	5YR5/8	Strong course sized subangular blocky	Sandy clay	Very hard when dry, firm when moist, very	Many fine and coarse sized pores		Few very fine roots	Common

					sticky and very plastic when wet	with a high total porosity			
39-70	2.5YR5/6	5YR5/8	Strong course sized subangular blocky	Sandy clay	Hard when dry, friable when moist and very sticky and very plastic when wet	Common, very fine pores with high total porocity		Few very fine roots	Common
70-100	2.5YR5/6		Weak fine sized crumbs	Clay	Loose when dry, Friable when moist, very sticky and very plastic	Many, very fine pores with high total porocity	Common, distinct fine sized yellowish mottles	Few very fine roots	Common
100-155	2.5YR5/6		Weak fine sized crumbs	Clay	Loose when dry, Friable when moist, very sticky and very plastic	Many, very fine pores with high total porocity	Many, distinct Fine and couase sized Mottles	Few very fine roots	Common
GENERAL SITE INFORMATION- PIT 2 (far from the dam – D2)									
Geo reference		**Elevation**	**Parent material**	**Topography**	**Land use**	**vegetation**	**human influence**	**Drainage**	**Soil Mapping Unit**

35L 6190640	8585966	1240	Limestone	Simple Straight slope (0-1%)	Crop agriculture with Maize	Mixed vegetation with woodland and herbaceous species	Disturbed vegetation,, regular burning and ploughing	Well drained soils	Konkola soil series
SOIL PROFILE DESCRIPTION									
Depth	**Colour moist**	**Colour dry**	**structure**	**Texture**	**Consistence**	**Porosity**		**Roots development**	**Biological Activity**
0-9	7.5YR4/4	7.5YR6/4	Weak fine sized sub angular blocky and crumb	Sandy loam	Loose when dry, Friable when moist, Non sticky and slightly plastic	Many very fine sized pores with a very high total porosity Non		Common very fine sized roots	Common
9_26	5YR3/6	7.5YR5/8	Strong medium sized sub angular blocky	Sandy clay	slightly hard when dry, friable when moist, sticky and plastic when wet	Many fine and coarse sized pores with a very high total porosity Non		Few very fine roots	Common
26-49	5YR4/6	5YR5/8	Strong, fine and	Sandy clay	slightly hard when dry,	Many fine and coarse		Few very fine roots	Common

			medium sized sub angular blocky		friable when moist, very sticky and plastic when wet	pores with very high total porosity Non			
49-80	2.5YR4/8	2.5YR5/8	Weak fine sized subangular blocky	Clay	loose when dry, friable when moist and very sticky and very plastic when wet	Common, fine pores with very high total porosity Non		Few very fine roots	Common
80-145	10R4/8	2.5YR5/8	Weak medium sized crumbs and sub angular blocky	Clay	Loose when dry, Friable when moist, very sticky and very plastic	Common, fine pores with high total porosity Non		Few very fine roots	Common

Chemical data for the soils in the profile pits

PIT #	HORIZON #	pH $CaCl_2$	Ca ppm	Mg ppm	K ppm	Na ppm	CEC me%
PROFILE PIT ONE							
1	H 1	6.19	2525	150	560	53	15.23
1	H 2	6.29	868	40	430	54	6.05
1	H 3	6.23	544	50	470	55	4.69
1	H 4	6.16	576	40	450	53	4.69
1	H 5	6.08	573	60	440	53	4.81
Average for pit 1		**6.19**	**1017.2**	**68**	**470**	**53.6**	**7.094**
2	H 1	6.27	1047	280	380	52	8.72
2	H 2	5.3	176	100	240	52	2.72
2	H 3	4.97	161	110	250	55	2.78
2	H 4	4.58	73	20	150	54	1.36
2	H 5	4.62	57	110	100	60	1.94
Average for pit 2		**5.148**	**302.8**	**124**	**224**	**54.6**	**3.504**
Classification Guide	Low	<5	<200	<50	<40	<40	<16
	Medium	5.0 – 6.0			40-100		16-24
	High	6 – 6.5	>200	>50	>100	>40	>24

Appendix 2: Purity of main chemicals used for soil extraction

Chemical	Purity
Calcium nitrate $Ca(NO_3)_2 \cdot 4H_2O$	BioXtra, ≥99.0%
DTPA $[(HOOCCH_2)_2NCH_2CH_2]_2NCH_2COOH$	≥99.0%
TEA $(HOCH_2CH_2)_3N$	≥99.0% (GC)

Appendix 3: Analysis of Variance for the field experiment (Chapter 4 and 5)

ANOVA for the effect of the treatments on soil pH

```
                     numDF denDF   F-value p-value
(Intercept)              1     8 104.53333  <.0001
Season                   1     8   0.90000  0.3706
Dam                      1     2  10.80000  0.0814
Lime                     1     6  90.13333  0.0001
Manure                   1     6   2.13333  0.1944
Season:Dam               1     8   0.90000  0.3706
Season:Lime              1     8   0.90000  0.3706
Dam:Lime                 1     6   8.53333  0.0266
Season:Manure            1     8  12.10000  0.0083
Dam:Manure               1     6   2.13333  0.1944
Lime:Manure              1     6   1.20000  0.3153
Season:Dam:Lime          1     8   0.90000  0.3706
Season:Dam:Manure        1     8   0.90000  0.3706
Season:Lime:Manure       1     8   8.10000  0.0216
Dam:Lime:Manure          1     6   0.53333  0.4927
Season:Dam:Lime:Manure   1     8   4.90000  0.0578
```

ANOVA for the effect of the treatments on soil organic matter

	numDF	denDF	F-value	p-value
(Intercept)	1	8	34.23998	0.0004
Season	1	8	3.10078	0.1163
Dam	1	2	51.60782	0.0188
Lime	1	6	0.87421	0.3859
Manure	1	6	51.16100	0.0004
Season:Dam	1	8	1.69312	0.2294
Season:Lime	1	8	0.39831	0.5456
Dam:Lime	1	6	29.83250	0.0016
Season:Manure	1	8	3.01900	0.1205
Dam:Manure	1	6	16.10157	0.0070
Lime:Manure	1	6	2.70283	0.1513
Season:Dam:Lime	1	8	6.93565	0.0300
Season:Dam:Manure	1	8	0.63162	0.4497
Season:Lime:Manure	1	8	0.05464	0.8211
Dam:Lime:Manure	1	6	0.03497	0.8578
Season:Dam:Lime:Manure	1	8	0.22590	0.6473

ANOVA for the effect of the treatments on Cd-dtpa

	numDF	denDF	F-value	p-value
(Intercept)	1	8	2.306436	0.1673
Season	1	8	1.465374	0.2606
Dam	1	2	31.470701	0.0303
Lime	1	6	2.306436	0.1796
Manure	1	6	16.992315	0.0062
Season:Dam	1	8	5.121884	0.0535
Season:Lime	1	8	0.002770	0.9593
Dam:Lime	1	6	16.992315	0.0062
Season:Manure	1	8	3.016620	0.1206
Dam:Manure	1	6	0.600384	0.4679
Lime:Manure	1	6	21.326609	0.0036
Season:Dam:Lime	1	8	0.335180	0.5786
Season:Dam:Manure	1	8	0.024931	0.8785
Season:Lime:Manure	1	8	0.002770	0.9593
Dam:Lime:Manure	1	6	5.119116	0.0643
Season:Dam:Lime:Manure	1	8	2.019391	0.1931

ANOVA for the effect of the treatments on Cu-dtpa

	numDF	denDF	F-value	p-value
(Intercept)	1	8	0.05924	0.8138
Season	1	8	0.47134	0.5118
Dam	1	2	6.67273	0.1229
Lime	1	6	52.86194	0.0003
Manure	1	6	3.83216	0.0980
Season:Dam	1	8	0.32359	0.5851
Season:Lime	1	8	2.52443	0.1508
Dam:Lime	1	6	0.95508	0.3662
Season:Manure	1	8	1.52713	0.2516
Dam:Manure	1	6	1.51644	0.2642
Lime:Manure	1	6	81.67817	0.0001
Season:Dam:Lime	1	8	0.58523	0.4662
Season:Dam:Manure	1	8	2.52443	0.1508
Season:Lime:Manure	1	8	0.52674	0.4887
Dam:Lime:Manure	1	6	25.01389	0.0024
Season:Dam:Lime:Manure	1	8	0.00962	0.9243

ANOVA for the effect of the treatments on Ni-dtpa

	numDF	denDF	F-value	p-value
(Intercept)	1	8	18.567491	0.0026
Season	1	8	0.000438	0.9838
Dam	1	2	9.844673	0.0883
Lime	1	6	1.905929	0.2166
Manure	1	6	2.800263	0.1453
Season:Dam	1	8	1.229053	0.2998
Season:Lime	1	8	0.010939	0.9193
Dam:Lime	1	6	6.727631	0.0410
Season:Manure	1	8	0.809013	0.3947
Dam:Manure	1	6	0.847079	0.3929
Lime:Manure	1	6	16.467294	0.0067
Season:Dam:Lime	1	8	0.157952	0.7014
Season:Dam:Manure	1	8	0.367972	0.5609
Season:Lime:Manure	1	8	0.886021	0.3741
Dam:Lime:Manure	1	6	0.175016	0.6903
Season:Dam:Lime:Manure	1	8	0.273463	0.6152

ANOVA for the effect of the treatments on Pb-dtpa

	numDF	denDF	F-value	p-value
(Intercept)	1	8	10.22815	0.0126
Season	1	8	0.03374	0.8588
Dam	1	2	25.90023	0.0365
Lime	1	6	0.13070	0.7301
Manure	1	6	5.58778	0.0500
Season:Dam	1	8	0.03374	0.8588
Season:Lime	1	8	0.01215	0.9150
Dam:Lime	1	6	21.56922	0.0035
Season:Manure	1	8	2.05263	0.1898
Dam:Manure	1	6	1.85692	0.2219
Lime:Manure	1	6	39.15313	0.0008
Season:Dam:Lime	1	8	0.59514	0.4626
Season:Dam:Manure	1	8	0.06613	0.8036
Season:Lime:Manure	1	8	1.65317	0.2345
Dam:Lime:Manure	1	6	5.58778	0.0560
Season:Dam:Lime:Manure	1	8	0.01215	0.9150

ANOVA for the effect of the treatments on Zn-dtpa

	numDF	denDF	F-value	p-value
(Intercept)	1	8	0.060274	0.8122
Season	1	8	0.520179	0.4913
Dam	1	2	9.762193	0.0890
Lime	1	6	1.175575	0.3199
Manure	1	6	7.542833	0.0334
Season:Dam	1	8	0.016631	0.9006
Season:Lime	1	8	0.205895	0.6621
Dam:Lime	1	6	1.554103	0.2590
Season:Manure	1	8	0.636256	0.4481
Dam:Manure	1	6	8.854721	0.0248
Lime:Manure	1	6	3.853740	0.0973
Season:Dam:Lime	1	8	0.636256	0.4481
Season:Dam:Manure	1	8	2.206580	0.1757
Season:Lime:Manure	1	8	0.188922	0.6753
Dam:Lime:Manure	1	6	8.240390	0.0284
Season:Dam:Lime:Manure	1	8	1.054571	0.334

ANOVA for the effect of the treatments on Cd-sol

	numDF	denDF	F-value	p-value
(Intercept)	1	8	22.73806	0.0014
Season	1	8	36.46959	0.0003
Dam	1	2	1.13619	0.3981
Lime	1	6	4.65443	0.0743

Manure	1	6	5.14524	0.0638
Season:Dam	1	8	2.70836	0.1384
Season:Lime	1	8	8.07317	0.0218
Dam:Lime	1	6	0.70888	0.4321
Season:Manure	1	8	0.11169	0.7468
Dam:Manure	1	6	0.58457	0.4735
Lime:Manure	1	6	5.61280	0.0500
Season:Dam:Lime	1	8	0.20387	0.6636
Season:Dam:Manure	1	8	1.57229	0.2453
Season:Lime:Manure	1	8	0.05542	0.8198
Dam:Lime:Manure	1	6	1.06207	0.3425
Season:Dam:Lime:Manure	1	8	0.03346	0.8594

ANOVA for the effect of the treatments on Cu-sol

	numDF	denDF	F-value	p-value
(Intercept)	1	8	14.840356	0.0049
Season	1	8	1.677007	0.2314
Dam	1	2	0.973930	0.4277
Lime	1	6	4.111593	0.0889
Manure	1	6	12.057314	0.0133
Season:Dam	1	8	2.923405	0.1257
Season:Lime	1	8	1.390865	0.2721
Dam:Lime	1	6	9.921965	0.0198
Season:Manure	1	8	0.003442	0.9547
Dam:Manure	1	6	6.054922	0.0491
Lime:Manure	1	6	1.985870	0.2084
Season:Dam:Lime	1	8	2.140738	0.1816
Season:Dam:Manure	1	8	0.001819	0.9670
Season:Lime:Manure	1	8	0.008900	0.9272
Dam:Lime:Manure	1	6	5.461181	0.0581
Season:Dam:Lime:Manure	1	8	0.069690	0.7985

ANOVA for the effect of the treatments on Ni-sol

	numDF	denDF	F-value	p-value
(Intercept)	1	8	1.554690	0.2477
Season	1	8	0.678588	0.4339
Dam	1	2	0.552250	0.5348
Lime	1	6	9.593127	0.0212
Manure	1	6	3.065010	0.1306
Season:Dam	1	8	0.438564	0.5264
Season:Lime	1	8	0.077761	0.7874
Dam:Lime	1	6	0.714122	0.4305
Season:Manure	1	8	1.639662	0.2362
Dam:Manure	1	6	0.019636	0.8931
Lime:Manure	1	6	0.878690	0.3847
Season:Dam:Lime	1	8	0.108281	0.7506
Season:Dam:Manure	1	8	5.310296	0.0501
Season:Lime:Manure	1	8	0.865482	0.3794
Dam:Lime:Manure	1	6	0.072497	0.7968
Season:Dam:Lime:Manure	1	8	0.022053	0.8856

ANOVA for the effect of the treatments on Pb-sol

	numDF	denDF	F-value	p-value
(Intercept)	1	8	46.20509	0.0001
Season	1	8	5.44914	0.0478
Dam	1	2	0.05471	0.8368
Lime	1	6	6.58362	0.0426
Manure	1	6	25.72894	0.0023
Season:Dam	1	8	4.29718	0.0719
Season:Lime	1	8	0.51316	0.4942

	numDF	denDF	F-value	p-value
Dam:Lime	1	6	1.38159	0.0448
Season:Manure	1	8	0.00174	0.9677
Dam:Manure	1	6	1.08056	0.0386
Lime:Manure	1	6	4.45070	0.0794
Season:Dam:Lime	1	8	0.00337	0.9551
Season:Dam:Manure	1	8	0.97295	0.3528
Season:Lime:Manure	1	8	2.15185	0.1806
Dam:Lime:Manure	1	6	1.26174	0.3042
Season:Dam:Lime:Manure	1	8	1.01479	0.3433

ANOVA for the effect of the treatments on Zn-sol

	numDF	denDF	F-value	p-value
(Intercept)	1	8	4.775114	0.0604
Season	1	8	0.780674	0.4027
Dam	1	2	0.027650	0.8832
Lime	1	6	9.793918	0.0203
Manure	1	6	1.792656	0.2291
Season:Dam	1	8	0.392336	0.5485
Season:Lime	1	8	0.008368	0.9294
Dam:Lime	1	6	0.156781	0.7058
Season:Manure	1	8	3.099794	0.1163
Dam:Manure	1	6	2.939140	0.1373
Lime:Manure	1	6	0.078151	0.7892
Season:Dam:Lime	1	8	0.181171	0.6816
Season:Dam:Manure	1	8	1.558121	0.2472
Season:Lime:Manure	1	8	0.430691	0.5301
Dam:Lime:Manure	1	6	1.068098	0.3412
Season:Dam:Lime:Manure	1	8	0.045446	0.8365

ANOVA for the effect of the fixed effects on heavy metal uptake by pumpkin leaves

Comparison	Denominator degrees of freedom	Cd		Cu		Ni		Pb		Zn	
		F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value
Season	8	2.15	0.18	1.11	0.322	1.8	0.216	4.69	0.062	265.1	< 0.000
Dam	2	32.03	0.029	5.44	0.145	21.9	0.043	20.09	0.046	12.04	0.074
Lime	6	51.097	0.000	83.4	0.000	0.000	0.994	4.51	0.048	20.7	0.004
Manure	6	7.066	0.038	5.15	0.064	1.196	0.316	1.66	0.244	21.1	0.004
Season·Dam	8	8.095	0.022	16.3	0.004	14.3	0.005	15.88	0.004	11.8	0.009
Season·Lime	8	3.557	0.096	6.88	0.031	0.11	0.753	0.004	0.949	0.014	0.909
Dam·Lime	6	7.567	0.033	1.3	0.299	1.68	0.243	0.079	0.787	0.12	0.743
Season·Manure	8	0.755	0.41	2.01	0.194	0.51	0.496	0.059	0.813	2.31	0.167
Dam·Manure	6	0.017	0.900	0.028	0.87	0.01	0.935	0.00	0.999	1.71	0.238
Lime·Manure	6	0.151	0.711	14.9	0.008	0.13	0.733	2.67	0.154	6.11	0.048

ANOVA for the effect of the fixed effects on heavy metal uptake by maize grain

Comparison	Denominator degrees of freedom	Cd		Cu		Ni		Pb		Zn	
		F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value
Season	8	4.79	0.06	0.229	0.637	1.31	0.285	14.9	0.005	1.15	0.314
Dam	2	0.53	0.542	0.0005	0.984	0.016	0.908	1.15	0.396	7.92	0.107
Lime	6	1.04	0.347	0.141	0.711	0.62	0.462	1.55	0.26	0.14	0.719
Manure	6	0.02	0.889	0.363	0.576	0.04	0.854	0.99	0.358	0.46	0.524
Season·:Dam	8	2.57	0.147	3.992	0.085	0.38	0.557	4.48	0.067	10.2	0.013
Season·Lime	8	2.57	0.147	0.016	0.894	0.064	0.806	6.2	0.038	1.26	0.293
Dam·:Lime	6	0.19	0.677	1.654	0.242	3.57	0.108	1.87	0.22	0.0006	0.981
Season·Manure	8	1.04	0.337	0.177	0.692	0.047	0.834	0.000	1.	0.323	0.585
Dam·Manure	6	0.02	0.889	1.219	0.312	0.49	0.510	0.25	0.636	0.012	0.917
Lime·Manure	6	0.53	0.493	2.326	0.179	1.43	0.277	0.062	0.812	1.09	0.337

ANOVA for the effect of the fixed effects on heavy metal uptake by maize stover

Comparison	Denominator degrees of freedom	Cd		Cu		Ni		Pb		Zn	
		F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value	F-value	P_value
Season	8	2.2	0.176	41.9	0.000	10.5	0.012	7.09	0.029	33.4	0.0004
Dam	2	0.04	0.858	2.43	0.259	0.36	0.61	0.005	0.951	11.48	0.077
Lime	6	1.29	0.299	10.9	0.016	0.11	0.749	0.120	0.741	16.25	0.007
Manure	6	0.51	0.500	32.9	0.001	2.27	0.183	1.885	0.219	15.95	0.007
Season·Dam	8	3.29	0.107	0.84	0.387	0.68	0.433	0.059	0.815	4.9	0.058
Season·Lime	8	0.47	0.513	8.46	0.0196	1.67	0.233	1.169	0.311	5.85	0.042
Dam·Lime	6	1.76	0.233	1.27	0.303	0.32	0.590	0.323	0.590	2.97	0.136
Season·Manure	8	3.1	0.116	10.7	0.011	0.79	0.399	1.240	0.298	1.73	0.224
Dam·Manure	6	0.15	0.713	2.63	0.156	1.91	0.216	3.40	0.115	0.013	0.910
Lime·Manure	6	0.48	0.514	35.2	0.001	0.099	0.764	0.323	0.590	3.03	0.132

Appendix 4: Heavy metal concentrations in crops (Mean ± standard deviation) under each treatment and season (Chapter 5)

Variable	Treatment	Concentration[a]/ mg kg^{-1}	
		Season 1	Season 2
Pumpkin leaves Cd	L1M0	0.032 ± 0.01	0.036 ± 0.00
	L0M1	0.041 ± 0.00	0.034 ± 0.01
	L1M1	0.024 ± 0.01	0.026 ± 0.02
	L0M0	0.052 ± 0.02	0.053 ± 0.01
Pumpkin leaves Cu	L1M0	35.9 ± 9	39.5 ± 5
	L0M1	57.1 ± 12	49.6 ± 8
	L1M1	45.7 ± 13	46.5 ± 1
	L0M0	56.9 ± 10	54.3 ± 6
Pumpkin leaves Ni	L1M0	2.58 ± 1.1	2.32 ± 0.8
	L0M1	2.81 ± 1.4	2.44 ± 0.8
	L1M1	2.71 ± 1.5	2.42 ± 0.4
	L0M0	2.37 ± 1.2	2.42 ± 0.3
Pumpkin leaves Pb	L1M0	0.50 ± 0.2	0.43 ± 0.0
	L0M1	0.53 ± 0.2	0.45 ± 0.1
	L1M1	0.43 ± 0.1	0.48 ± 0.1
	L0M0	0.57 ± 0.1	0.58 ± 0.1
Pumpkin leaves Zn	L1M0	25.4 ± 7.7	38.7 ± 3.8
	L0M1	39.1 ± 6.5	55.3 ± 2.9
	L1M1	25.7 ± 6.4	45.4 ± 5.6
	L0M0	27.3 ± 5.0	43.7 ± 5.1
Maize grain Cd	L1M0	0.002 ± 0.001	0.003 ± 0.001
	L0M1	0.002 ± 0.002	0.002 ± 0.001
	L1M1	0.001 ± 0.001	0.004 ± 0.001
	L0M0	0.002 ± 0.001	0.002 ± 0.001
Maize grain Cu	L1M0	1.91 ± 0.73	1.84 ± 0.23
	L0M1	1.91 ± 0.28	1.79 ± 0.28
	L1M1	1.68 ± 0.08	1.60 ± 0.14
	L0M0	1.73 ± 0.09	1.77 ± 0.19
Maize grain Ni	L1M0	0.085 ± 0.04	0.064 ± 0.03
	L0M1	0.094 ± 0.05	0.074 ± 0.01
	L1M1	0.068 ± 0.02	0.061 ± 0.02
	L0M0	0.069 ± 0.01	0.071 ± 0.02
Maize grain Pb	L1M0	0.013 ± 0.00	0.010 ± 0.00
	L0M1	0.018 ± 0.01	0.006 ± 0.00
	L1M1	0.010 ± 0.01	0.008 ± 0.00
	L0M0	0.020 ± 0.01	0.007 ± 0.00
Maize grain Zn	L1M0	13.7 ± 1.8	15.9 ± 4.4
	L0M1	13.7 ± 1.1	14.3 ± 2.0
	L1M1	13.6 ± 1.2	13.5 ± 2.2
	L0M0	14.1 ± 1.2	13.5 ± 1.9
Maize stover Cd	L1M0	0.031 ± 0.01	0.045 ± 0.01
	L0M1	0.027 ± 0.01	0.053 ± 0.01
	L1M1	0.034 ± 0.01	0.042 ± 0.01
	L0M0	0.056 ± 0.02	0.039 ± 0.03
Maize stover Cu	L1M0	10.3 ± 1.5	14.1 ± 2.7

	L0M1	7.7 ± 2.6	11.0 ± 2.7
	L1M1	12.1 ± 2.0	12.8 ± 5.1
	L0M0	18.7 ± 1.7	27.2 ± 7.8
Maize stover Ni	L1M0	3.6 ± 2.4	5.9 ± 3.4
	L0M1	2.2 ± 0.4	5.2 ± 2.3
	L1M1	3.3 ± 1.0	4.1 ± 1.3
	L0M0	3.2 ± 1.4	7.4 ± 3.9
Maize stover Pb	L1M0	0.17 ± 0.03	0.31 ± 0.09
	L0M1	0.13 ± 0.04	0.26 ± 0.14
	L1M1	0.21 ± 0.09	0.20 ± 0.09
	L0M0	0.20 ± 0.02	0.36 ± 0.27
Maize stover Zn	L1M0	4.2 ± 1.3	6.7 ± 1.2
	L0M1	3.8 ± 1.2	7.1 ± 3.0
	L1M1	4.4 ± 0.8	4.1 ± 1.8
	L0M0	7.2 ± 1.1	9.5 ± 2.0

[a]*average of four replications*

Appendix 5: Summary of the variables, parameters and settings for WHAM-VII modelling of Cu soil solution concentration, speciation and fractionation.

Variable	Settings
Suspension condition	
Suspended particulate matter (SPM)	80 g L^{-1}
Temperature (K)	293.15 K (20°C)
PCO_2 (atm)	Measured bicarbonate concentration (DIC)
Soil pH	Measured in 0.01 M $Ca(NO_3)_2$ soil suspensions.
Charge balance options	No charge balance imposed i.e. a fixed (measured) pH value was used
Adsorption phase (g L^{-1})	
Clay content	MA soils: estimated from soil texture Urban soils: measured by laser granulometry
Fe, Al and Mn oxides	DCB extraction; converted to $Fe_2O_3.H_2O$, Al_2O_3 and MnO_2
Humic and fulvic acid	Measured by alkaline extraction
Colloidal fulvic acid	Estimated from measured DOC
Major cation and anion concentration (mol L^{-1})	
Dissolved major cations (Na, Mg, Al, K and Ca)	Concentration in filtered soil suspensions (0.01 M $Ca(NO_3)_2$) *Precipitation option for Al:* One mole of precipitated $Al(OH)_3$ forms 87 g of the binding phase within the particulate soil phase (S. Lofts., pers. comm.).

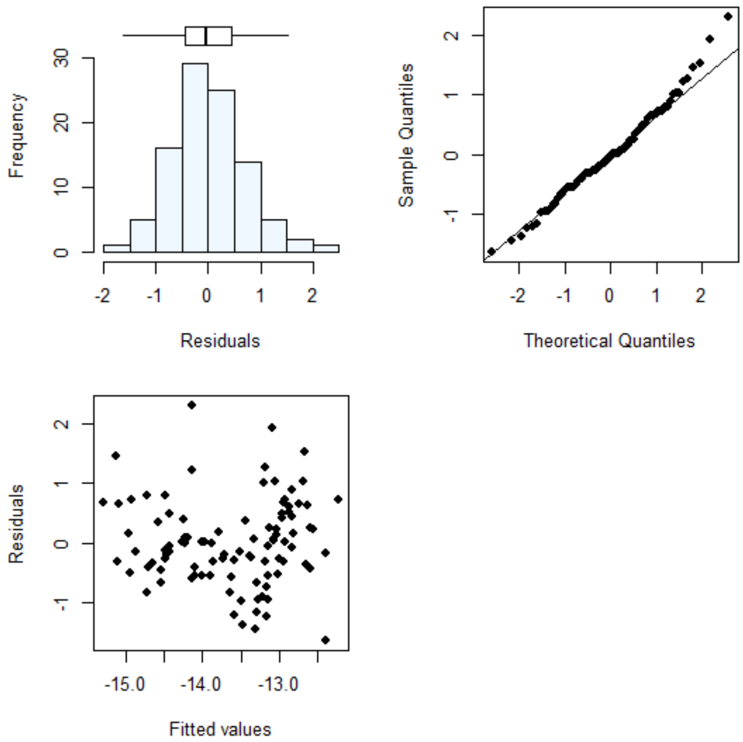
Fe^{3+} activity	*Precipitation option for Fe:* assumes that Fe^{3+} activity is controlled by $Fe(III)(OH)_3$ solubility, calculated within the model.
Dissolved nitrate (NO_3^-)	Solution concentration estimated as 0.02 M in the suspension
Reactive metal concentration in soil suspension (mol L^{-1})	
Total concentration Ni, Cu, Zn, Cd and Pb	Estimated from M-dtpa
Other settings	
Activity coefficient correction	Debye-Hückel
WHAM parameter data sets	Default master, solute and binding phase data bases.

Appendix 6: Summary statistics, scatter plots and residuals of the exploratory least-squares regression of the target variable on each of the proposed predictors

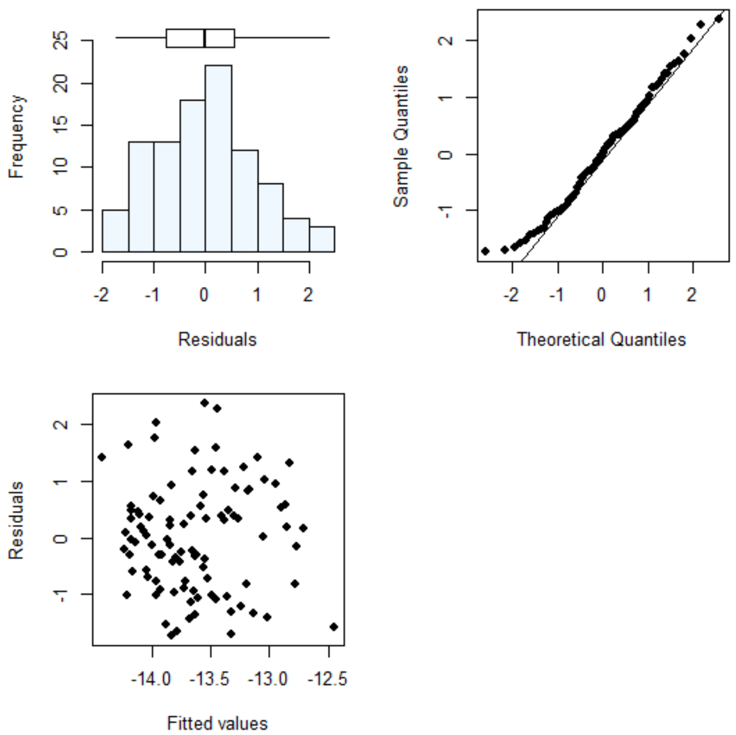
Summary statistics for residuals from exploratory fits of Cu models

Model (both variables on loge scales in all cases)		Mean	Median	SD	Skewness	Octile skewness	Number of outliers
1	Measured Aq Cu* from pred (Cu-tot)	0.00	−0.036	0.713	0.43	0.04	0
2	Measured Aq Cu* from pred (Cu-E)	0.00	−0.005	0.957	0.24	0.04	0
3	Measured Aq Cu* from pred (Cu-dtpa)	0.00	−0.023	0.449	−0.34	0.17	0
4	Measured Crop Cu from Aq Cu* (Cu-soln)	0.00	−0.007	0.351	0.76	0.06	1
5	Measured Crop Cu from pred aq (Cu-tot)	0.00	−0.019	0.353	0.64	0.13	1
6	Measured Crop Cu from pred aq (Cu-E)	0.00	−0.021	0.354	0.60	0.12	1
7	Measured Crop Cu from pred (Cu-dtpa)	0.00	−0.008	0.350	0.73	0.04	1

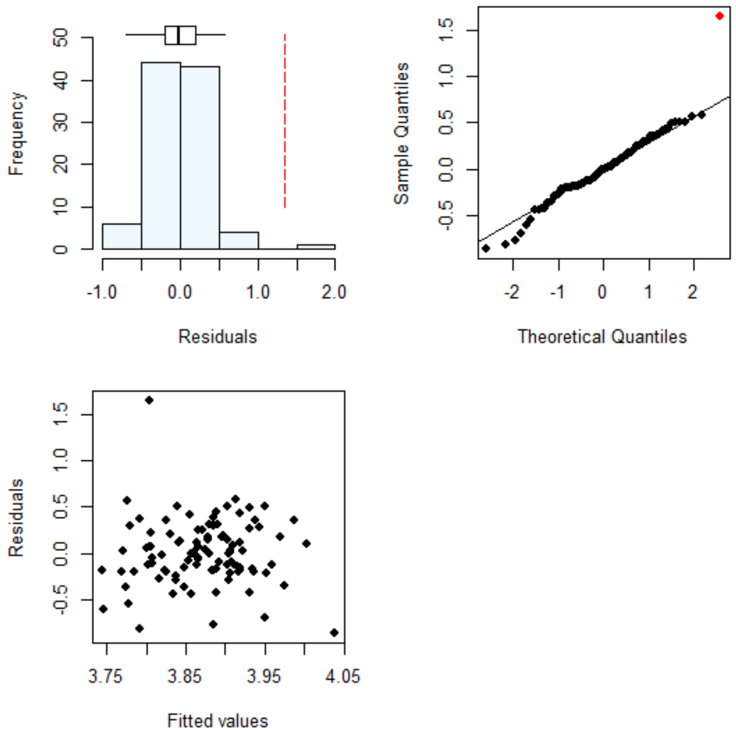
*CaNO3 extraction



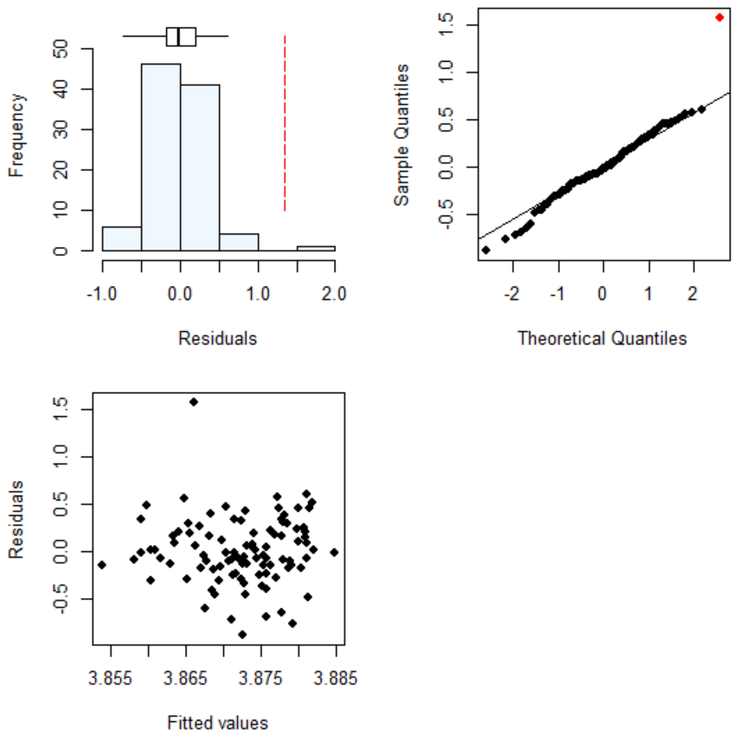
Model 1 residuals



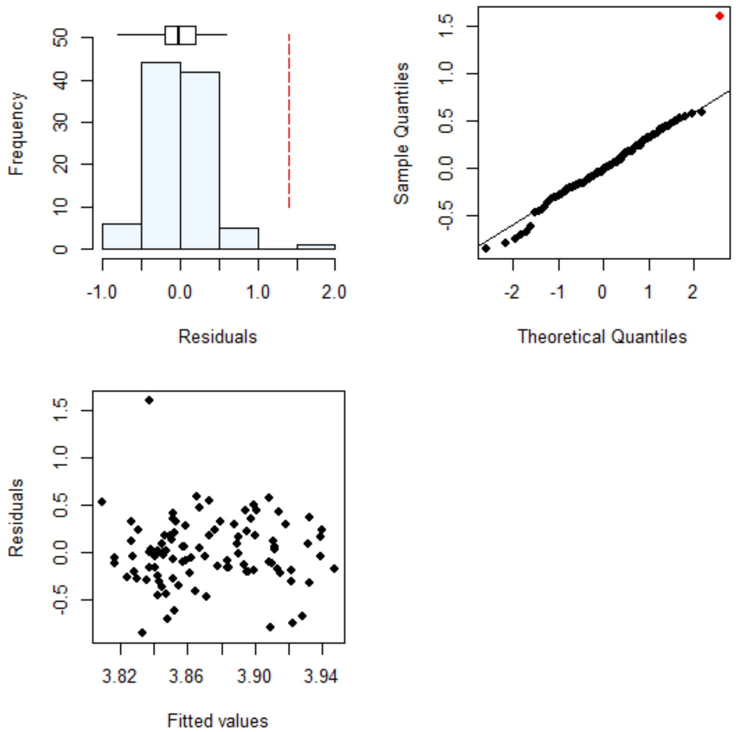
Model 2 residuals



Model 3 residuals



Model 4 residuals



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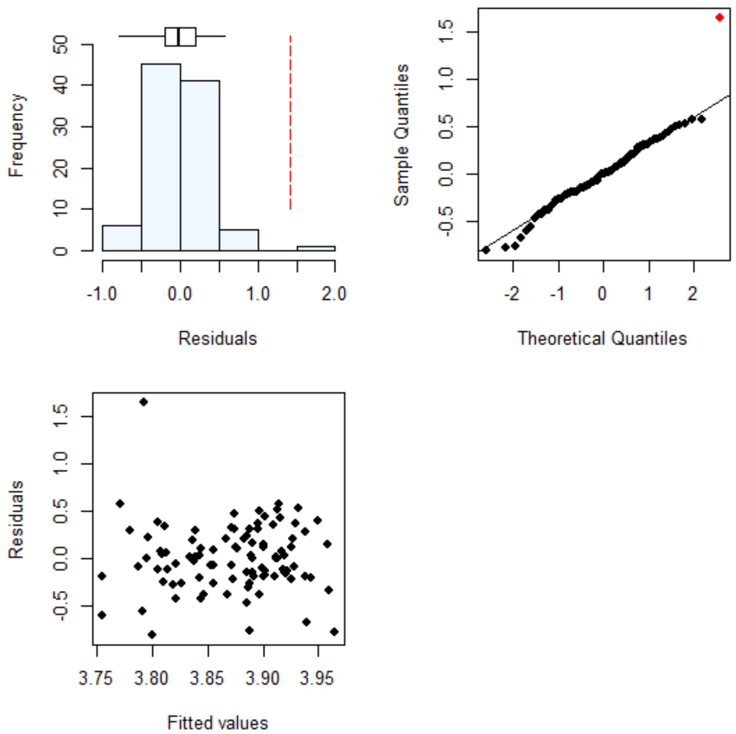
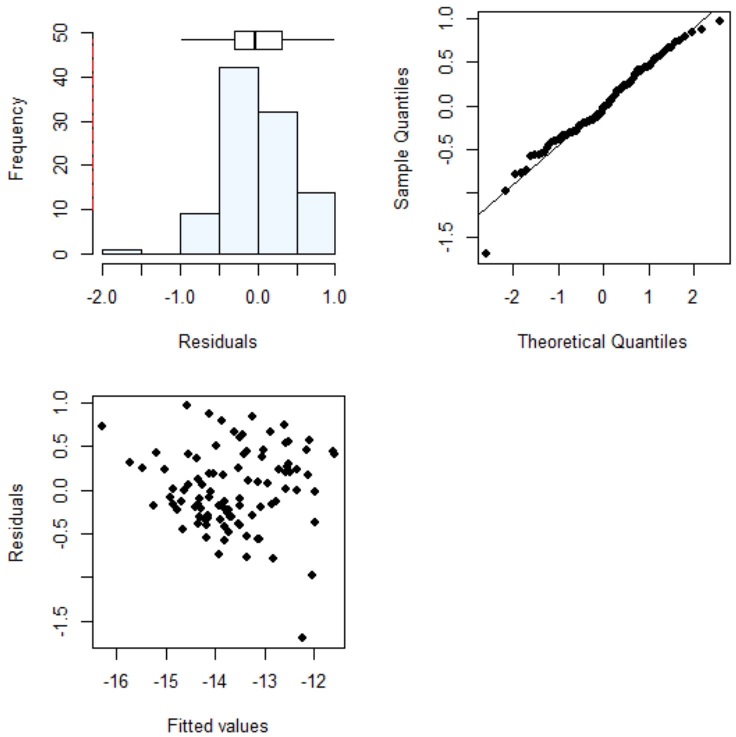
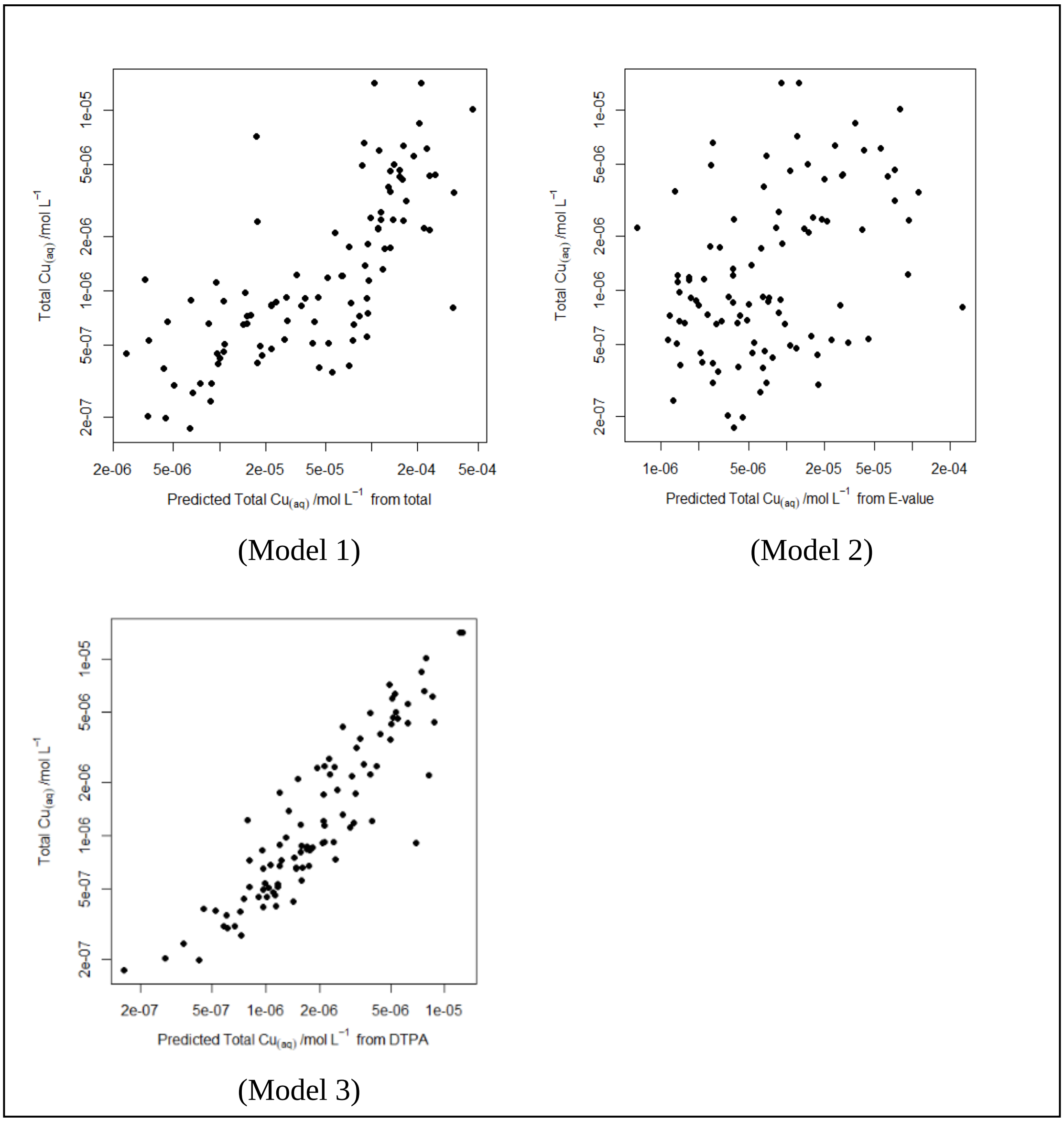


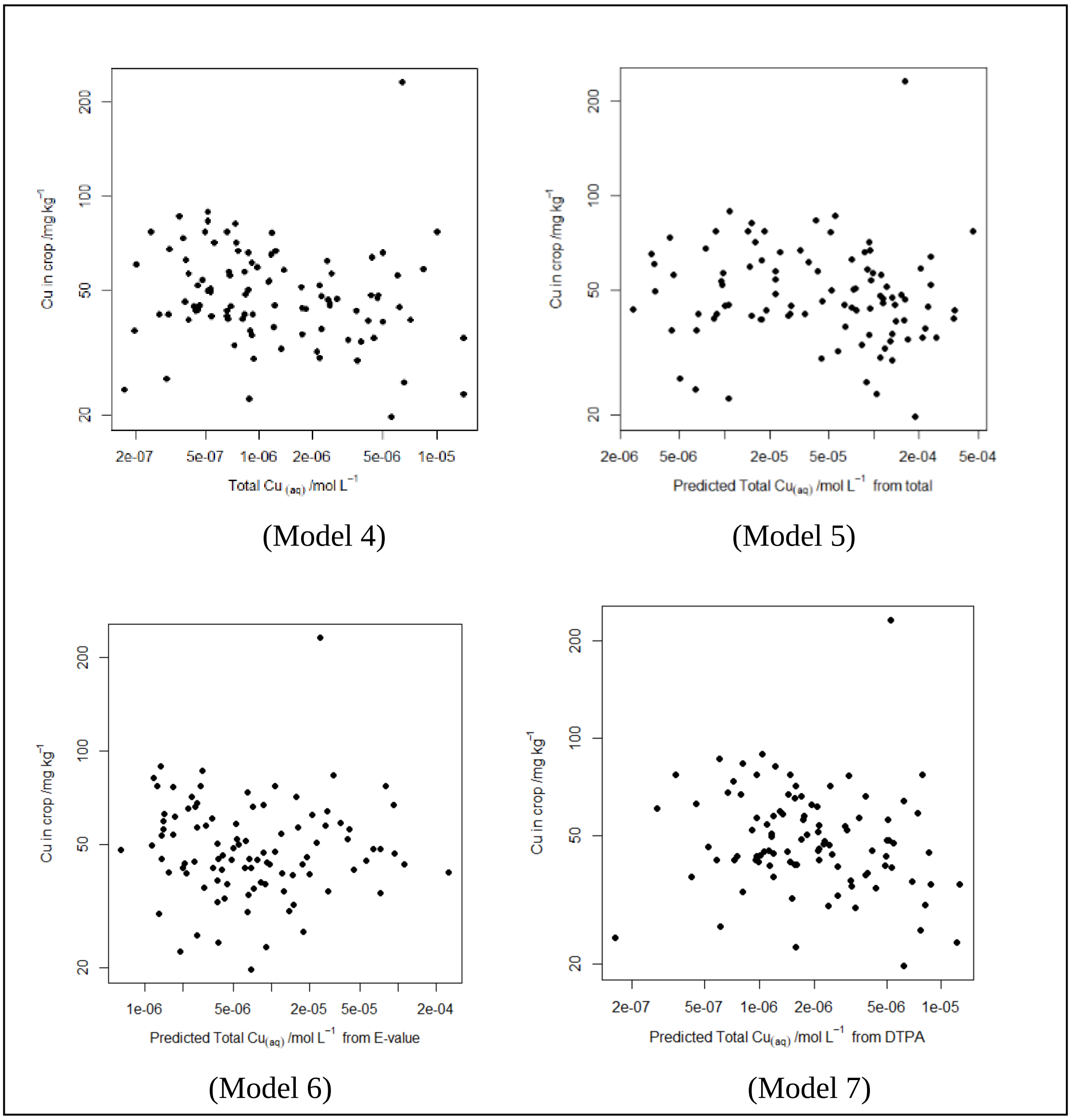
Model 6 residuals



Model 7 residuals



Scatter plots (log scales) for models describing WHAM VII prediction of soil solution Cu concentration with Cu-tot, Cu-E and Cu-dtpa as input variables



Scatter plots for models describing WHAM VII prediction of the Cu concentration in the crop with Cu-tot, Cu-E and Cu-dtpa as input variables

Appendix 7: Journal publications

Kaninga, B. K., Chishala, B. H., Maseka, K. K., Sakala, G. M., Lark, M. R., Tye, A., and Watts, M. J. (2019). Review: Mine tailings in an African tropical environment-mechanisms for the bioavailability of heavy metals in soils. *Environmental Geochemistry and Health*. https://doi.org/10.1007/s10653-019-00326-2

Abstract: Heavy metals are of environmental significance due to their effect on human health and the ecosystem. One of the major exposure pathways of Heavy metals for humans is through food crops. It is postulated in the literature that when crops are grown in soils which have excessive concentrations of heavy metals, they may absorb elevated levels of these elements thereby endangering consumers. However, due to

land scarcity, especially in urban areas of Africa, potentially contaminated land around industrial dumps such as tailings is cultivated with food crops. The lack of regulation for land-usage on or near to mine tailings has not helped this situation. Moreover, most countries in tropical Africa have not defined guideline values for heavy metals in soils for various land uses, and even where such limits exist, they are based on total soil concentrations. However, the risk of uptake of heavy metals by crops or any soil organisms is determined by the bioavailable portion and not the total soil concentration. Therefore, defining bioavailable levels of heavy metals becomes very important in HM risk assessment, but methods used must be specific for particular soil types depending on the dominant sorption phases. Geochemical speciation modelling has proved to be a valuable tool in risk assessment of heavy metal-contaminated soils. Among the notable ones is WHAM (Windermere Humic Aqueous Model). But just like most other geochemical models, it was developed and adapted on temperate soils, and because major controlling variables in soils such as SOM, temperature, redox potential and mineralogy differ between temperate and tropical soils, its predictions on tropical soils may be poor. Validation and adaptation of such models for tropical soils are thus imperative before such they can be used. The latest versions (VI and VII) of WHAM are among the few that consider binding to all major binding phases. WHAM VI and VII are assemblages of three sub-models which describe binding to organic matter, (hydr)oxides of Fe, Al and Mn and clays. They predict free ion concentration, total dissolved ion concentration and organic and inorganic metal ion complexes, in soils, which are all important components for bioavailability and leaching to groundwater ways. Both WHAM VI and VII have been applied in a good number of soils studies with reported promising results. However, all these studies have been on temperate soils and have not been tried on any typical tropical soils. Nonetheless, since WHAM VII considers binding to all major binding phases, including those which are dominant in tropical soils, it would be a valuable tool in risk assessment of heavy metals in tropical soils. A discussion of the contamination of soils with heavy metals, their subsequent bioavailability to crops that are grown in these soils and the
methods used to determine various bioavailable phases of heavy metals are presented in this review, with an emphasis on prospective modelling techniques for tropical soils.

Keywords: Heavy metals, Bioavailability, Mine tailings, Speciation, WHAM, Tropical soils

Kaninga, B., Chishala, B. H., Maseka, K. K., Sakala, G. M., Young, S. D., Lark, R. M., Tye, A., Hamilton, E. M., Gardner, A., and Watts, M. J. (2020). Do soil amendments used to improve agricultural productivity have consequences for soils contaminated with heavy metals? Heliyon, 6(11), e05502. https://doi.org/10.1016/j.heliyon.2020.e05502

Abstract: This study presents an analysis of the effects of manure and lime commonly used to improve agricultural productivity and evaluates the potential for such soil amendments to mobilise/immobilise metal fractions in soils contaminated from nearby mine tailings in the Zambian Copperbelt. Lime and manure were applied at the onset of the study, and their effects were studied over two planting seasons, i.e. 2016-17 and 2017–18. Operationally defined plant-available Cd, Cu, Ni, Pb and Zn in the soil, were determined by extraction with DTPA-TEA (diethylenetriaminepentaacetic acid-triethanolamine) and 0.01 M Ca(NO3)2, before, and after, applying the amendments.

In unamended soils, Cd was the most available and Ni the least. Lime application decreased extractable Cd, Cu, Ni, Pb and Zn. The response to lime was greater in soils with an initially acidic pH than in those with approximately neutral pH values. Manure increased DTPA extractable Zn, but decreased DTPA and Ca(NO3)2 extractable Cd, Cu and Pb. Combined lime and manure amendment exhibited a greater reduction in DTPA extractable Cd, Ni, Pb, Zn, as well as for Ca(NO3)2 extractable Cd compared to separate applications of lime and manure. The amendments had a significant residual effect on most of the soil fractions between season 1 and 2. The results obtained in this study showed that soil amendment with minimal lime and manure whilst benefiting agricultural productivity, may significantly reduce the mobility or plant availability of metals from contaminated agricultural soils. This is important in contaminated, typical tropical soils used for crop production by resource poor communities affected by mining or other industrial activities.
Keywords: Copperbelt, Lime, Manure, Mine tailings, Bioavailable metals

Kaninga, B., Lark, R. M., Chishala, B. H., Maseka, K. K., Sakala, G. M., Young, S. D., Tye, A., Hamilton, E. M., and Watts, M. J. (2021). Crop uptake of heavy metals in response to the environment and agronomic practices on land near mine tailings in the Zambian Copperbelt Province. Environmental Geochemistry and Health. https://doi.org/10.1007/s10653-021-00849-7

Abstract: A field experiment was undertaken on farmers' fields adjacent to a large mine tailings dam in the Zambian mining town of Kitwe. Experimental plots were located close to the tailings (B 200 m) or further away (300–400 m) within the demarcated land farmed by the same community. This study evaluated the uptake of Cd, Cu, Ni, Pb and Zn by pumpkin leaves and maize grown in soil amended with lime and manure applied at agronomic rates, and the subsequent risk of dietary exposure to the local community, typical of many similar situations across the Zambian Copperbelt. Treatments, combinations of lime and manure (present or absent), were applied to subplots selected independently and randomly within each main plot, which represented variable geochemistry across this study site as a result of windblown/rain-driven dust from the tailings. Total elemental concentrations in crops were determined by ICP-MS following microwave-assisted acid digestion. Concentrations of Cu and Pb in pumpkin leaves were above the prescribed FAO/WHO safe limits by 60–205% and by 33–133%, respectively, while all five metals were below the limit for maize grain. Concentration of metals in maize grain was not affected by the amendments. However, lime at typical agronomic application rates significantly reduced concentrations of Cd, Cu, Pb and Zn in the pumpkin leaves by 40%, 33%, 19% and 10%, respectively, and for manure Cd reduced by 16%, while Zn increased by 35%. The uptake of metals by crops in locations further from the tailings was greater than closer to the tailings because of greater retention of metals in the soil at higher soil pH closer to the tailings. Crops in season 2 had greater concentrations of Cu, Ni, Pb and Zn than in season 1 due to diminished lime applied only in season 1, in line with common applications on a biannual basis. Maize as the staple crop is safe to grow in this area while pumpkin leaves as a readily available commonly consumed leafy vegetable may present a hazard due to accumulation of Cu and Pb above recommended safe limits.
Keywords: Heavy metals, Pumpkin leaves, Maize, Lime, Manure, Mine tailings, Agronomic, amendments