UPTAKE OF COPPER AND COBALT BY PUMPKIN (*Cucurbita maxima*) AND CHINESE CABBAGE (*Brassica oleracea*) IRRIGATED WITH CONTAMINATED WATER ON SANDY LOAM AND CLAY LOAM SOILS

BY

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DECLARATION

I, John Volk, hereby declare that this thesis represents my own work and has not previously been submitted for a degree at the University of Zambia or any other University.

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APPROVAL

This thesis of John Volk is approved as fulfilling the requirements for the award of the degree of Doctor of Philosophy (PhD) in Soil Science of the University of Zambia.

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ABSTRACT

Pumpkins and Chinese cabbage are widely grown in peri urban areas with different soil types in Zambia using waste water which is potentially contaminated with heavy metals like copper and cobalt. The objectives of this study were to establish the effect of loading rates of heavy metals on sandy and clay loam soils and establish factors that influence transmission their uptake by pumpkin and Chinese cabbage when grown on sandy and clay loam soils when irrigated with water contaminated with Cu and Co. A green house study in which pumpkin and Chinese cabbage were grown in pots with sandy loam and clay loam soil was used. Irrigation water comprised contaminated water that had four levels of concentrations of Cu and Co in them and a control of fresh water. Comparing the chemical properties of soil before and after the experiment gave the capacities of the soils to adsorb Cu and Co in them. The fractions of Cu and Co measured in the test soils established the form in which the two elements were adsorbed in soils and provided possible explanations of transmission of heavy metals to plants from water and soil. Concentration of Cu and Co in tissues of pumpkin and Chinese cabbage established their capacity to accumulate the two metals. Yields of crops, concentration of Cu and Co in plant tissues and soil tested the relationships that explained the effect of levels of contaminants in irrigation water on crops and soils. The study found that clay loam adsorbed significantly higher ($p<0.05$) Cu and Co than sandy loam soil. The results show significantly higher ($p < 0.05$) concentrations of Cu and Co in plant tissue in sandy loam than clay loam soils. It was established clay loam retained more Cu on the organic and exchangeable fractions than sandy loam soil. Clay loam reduced significantly ($p<0.05$) the transmission of Cu and Co to crops. Lack of significant effects ($p<0.05$) of concentrations of Cu and Co in plant tissue on yield implies that crops that appear healthy may contain high levels of heavy metals and unhealthy for human consumption. The study confirms that soils with higher clay and organic matter adsorbs higher amounts of heavy metals which could potentially be used in immobilizing the same elements and prevent the contamination of crops grown on them. The lower adsorption capacity of heavy metals by sandy loam would potentially result in higher transmission of heavy metals from water to crops.

The concentrations of Cu found in Chinese cabbage (180 mg/kg) and pumpkin (116 mg/kg) were 6 times and 4 times higher respectively, than the normal range for Cu which is estimated to be around 1-30 mg/kg. Without significant negative effects on yield, or physiology from contamination, vegetables may have higher levels of heavy metals that could be unhealthy for human consumption. It is recommended that further studies seek to address understanding of how a wider range of elements each contributed to crop production, and how the various soil fractions of these elements influence availability and their uptake and yield of crops.

Key words: Contaminated water; copper; cobalt; crops; soil texture

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrophotometer
CEC	Cation Exchange Capacity
CIA	Central Intelligence Agency
CLEA	Contaminated Land Exposure Assessment Model
Co	Cobalt
CSO	Central statistical Office
Cu	Copper
DTPA	Diethylene Triamine Penta Acetic Acid
DTPA-TEA	Diethylene Triamine Penta Acetic Acid – Triethanolamine
ECZ	Environmental Council of Zambia
EDTA	Ethylene - Diamine-Tetra Acetic Acid
EPA	United States Environmental Protection Agency
EPPCA	Environmental Pollution and Protection Control Act
FAO	Food and Agricultural Organization
GDP	Gross domestic Product
GRZ	Government of the Republic of Zambia
IAEA	International Atomic Energy Agency
ICP-MS	Inductively Coupled Plasma – Multi Spectrophotometer
MOE	Ministry of Environment, Canada
NAE	National Association of Engineering
NAS	National Academy of Sciences
NEPC,	National Environment Protection Council of Australia
PRG	Preliminary Remediation Goals
RBC	Risk Based Concentrations
UK	United Kingdom
WHO	World Health Organization
ZCCM	Zambia Consolidated Copper Mines
ZEMA	Zambia Environmental Management Agency

CHAPTER 1: INTRODUCTION

1.1 Urbanization and use of waste water as source of irrigation water in Zambia

Like much of Sub-Saharan Africa, Zambia is rapidly urbanizing. In 1970, the share of urban residents in the total population was 30 percent (World Bank, 2013); currently their share is 36 percent (CIA, 2013; CSO, 2011). Zambia is one of the most urbanised countries in the third world with an urban population of 39.5 % of the nation's total population of 13,092,666 (CSO, 2013a). The proportion is even higher if you consider that 68 % of the population is concentrated on the Kafue river basin which is 20 % of the land area (CSO, 2003).

Much of this urban population growth has occurred in Lusaka, which currently contains nearly 31.5 % of the total urban population (CIA, 2013). Moreover, Lusaka is growing more quickly than the national average. Whereas, the estimated annual population growth of Zambia was 2.8 percent between 2000 and 2010, the city of Lusaka, with an estimated population of more than 1,743,000, had an estimated annual growth rate of almost 4.9 percent during the same time period (CSO, 2011). Much of this growth has been in peri urban areas where the provision of infrastructure (such as roads, electricity piped water and waste disposal) and services (such as schools, clinics, public transport and markets are uneven and has not kept pace with the rate of development (World Bank, 2002).

Peri-urban areas of Zambia are characterized by large numbers of unplanned settlements some near industries or poorly functioning sewer systems. Agriculture provides a very important source of livelihood through income generation, provision of employment and fresh food for these families (Sanyal, 1985; Rakodi, 1988; Schmidt, 2011; Kapungwe, 2013; Kapungwe, 2014; Global Economy, 2018). The land on which these farmers cultivate, generally have available year-round water supplies for irrigation in the form of waste water. This is in an environment where natural rivers and streams tend to be dependent upon rainy season for surface flow. An estimated 80 percent of wastewater in developing countries may be used for irrigation (Scott *et al.,* 2004) and cities in Zambia are no exception to this.

The sources of water for peri-urban agriculture include: effluent from industry (including mines during mineral processing), discharge from sewerage treatment plants, disused quarries, dugouts, natural streams and wetlands.

Domestic sewage waste is generated from human activities such as bathing, body elimination, food preparation and other activities such as recreation. It is estimated that a person generates about 200 litres per day (Pescod, 1992). In urban areas, 56.5 % of households in Zambia have access to piped water and only 12.7 % have access to a flush toilet that is connected to a sewer system (CSO, 2013b). The quantity of commercial sewage (from industry and mining sources) depends on the industry and management of water use and treatment before discharge in the environment. It is estimated that in 2015, the volume of sewage collected and treated in public works in Zambia was 148,700,000 m^3 which is only 40% of the water supplied for domestic, commercial and industrial purposes (CSO, 2018).

In Zambia, farming using waste water from domestic, drainage and industrial sources is very wide spread in peri-urban areas. In Lusaka, farming using waste water from sewage discharge is practiced in Chunga, a peri-urban area along the Ngwerere stream. The year round water supply comes from drainage, ground seepage, domestic and industrial discharge from the Chunga sewage treatment plant. In Kitwe, which is a town on the Copperbelt of Zambia, farmer's residing along the Uchi stream also irrigated crops using water which was mixed with effluent from the former Nkana Smelter before its closure in 2009. While the number of farmers using waste water for irrigation to grow crops for own consumption and sale is not known, the practice is wide spread in peri-urban areas of many cities in Zambia. The practice raises concern because of the health effects that comes from the consumption of food crops that are contaminated with heavy metals especially from waste water from poorly functioning sewage systems and industrial effluent discharge.

The type of irrigation systems farmer's in the peri-urban areas is commonly the flooding system. The water is usually lifted from the source by cans and buckets. In some cases small gasoline and treadle pumps are used. When irrigating using buckets, farmers flood the beds with water. If irrigation is done by gasoline powered pump, the farmers flood the furrows and allow the water to flow along the gradient while wetting the ground.

If the hazard of heavy metal contamination in food exists, then the problem is spread to a larger population. Measurement of concentration of heavy metals in water in Zambia is not regular as the use of waste water for cultivation is not practiced formerly. Besides the disposal of waste water from industries such as mineral processing and manufacturing are not well policed even though laws regarding waste water disposal exist. Heavy metals in water tend not to attract much public attention as the health effects from heavy metals are not easily seen due to their insidious nature unlike pathogen caused diseases such as cholera and dysentery.

1.2 **The problem of copper and cobalt in the environment in Zambia**

Copper (Cu) is an essential substance to all living organisms as it serves as an important catalyst in the chemistry of proteins that are used in important biological systems (Lind, 2004). However, in higher concentrations, Cu can accumulate in plant and animal tissues thereby becoming poisonous (Lind, 2004; Nordberg and Cherian, 2004; Lenntech, 2011).

Cobalt (Co) is a vital element in the synthesis of vitamin B_{12} which is important in a number of reactions in the human body including production of red blood cells (MOE, 2002). Exposure to very high levels of Co can cause health problems on the lungs, including asthma, pneumonia and wheezing. It has also been determined by the International Agency for Research on Cancer, that Co is a possible carcinogen to humans (MOE, 2002).

Copper and Cobalt are mined in many parts of Zambia but primarily on the Copperbelt and the Northwestern provinces. While these elements are commonly present in soil in trace amounts and their importance in metabolic processes is well known (MOE, 2002; Lenntech, 2011), their accumulation in water and plants to toxic levels is increasingly becoming a serious environmental and health concern (MOE, 2002; Lenntech, 2011). The increasing Cu and Co mining lately taking place in Zambia means that the problem of these elements contaminating the environment will increase proportionate to the mining and processing activities.

Evidence of high concentrations of Cu and Co have been reported in water, soils and crops on the Copperbelt in Zambia (Sawula, 1985; Kasonde, 1995; Sinkala, 1998; Sinkala *et al.,* 1998; Nkandu, 1999; ECZ., 2000; ECZ., 2001; Petersson and Ingri, 2001; Simukanga *et al.,* 2002; von der Heyden and New, 2004; SGAB *et al*., 2005; Chishala *et al.,* 2006; Lindahl; 2014: Kayika *et al*., 2017). High levels Cu in water and crops have also been reported in areas where copper mining does not exist for example around Lusaka, Kafue and Itezhi tezhi (Chishala *et al.*, 2006; Ikenaka *et al.*, 2010; Kapungwe, 2013; GRZ., 2014; Kapungwe, 2014)

The major effects arising from accumulation of Cu and Co in the environment include the toxicity to plants and humans. The effects are generally cumulative and thus take a long time to manifest themselves. The most common process in which humans get exposed to high levels Cu and Co are drinking of water and eating of food (Nordberg and Cherian, 2004). When plants are grown on land or irrigated with water contaminated with Cu or Co, these can provide a pathway in which the metals find themselves in the human food pathway (Fuge, 2004). It is thus important to prevent the accumulation of these metals in water and soil to toxic levels and then design strategies that can be used to prevent them from accumulating in food crops to levels that could be detrimental to human health.

1.3 **Legal framework and legislation**

To minimize the effect of heavy metals on the environment, most countries have enacted laws on maximum permissible limits of contaminants. The maximum permissible limits of contaminants affect different aspects of environments such as (i) waste water for disposal in the environment (ii) irrigation water, (iii) food crops for human and animal consumption, and (iv) soil. For example in Zambia, the Environmental Management Act No. 12 of 2011 (GRZ, 2011) ensures that the waste water disposed of in the environment meets the recommended disposal standards. The Food and Drugs Act of 1995 prohibits the sale of fresh foods that have metal contents above stipulated amounts. For example the legal limit for Cu allowed in fresh vegetables in Zambia is 50 mg/kg (GRZ, 1995).

Apparently, there are no national guidelines on investigating heavy metal contamination in soils, water or food crops in Zambia despite the standards that have been set for waste water disposal and food contamination. This lack of guidelines can be developed if the factors such as (i) nature of the metal in contaminated water and soil, (ii) soil factors that affect the metal in soil and (iii) factors that influence the uptake of heavy metals by plants from soils can be explained. For example, elements in water or soil occluded to organic matter will be strongly held and temporarily not available for plant uptake (Wang *et al.,* 2003; Majid, 2010). Soil's properties such as sandy textures, low organic matter content, and low Cation Exchange Capacity (CEC) would allow higher transmission of heavy metals because of low capacity for adsorption from solution (Korte *et al.,* 1976; McLean and Bledsoe, 1992). It is therefore, imperative that factors that influence these parameters are taken into consideration when one is developing models that explain the uptake of heavy metals by food crops that are irrigated with contaminated waste water.

1.4 **Statement of the problem**

The use of waste water for growing crops is a common practice in the peri urban areas of cities and towns in Zambia. At present there is a general lack of awareness amongst stakeholders of the food safety risks and there is very little supporting data on the levels and extent of the problem of heavy metal contamination in water, soils and crops in Zambia. This is probably due to the insidious nature of heavy metal contamination as compared to biological pathogens such as cholera or dysentery.

Literature shows that the bioavailability of heavy metals in sandy soils is higher compared to clay soils and this could present a serious environmental problem if crops such as pumpkin and Chinese cabbage are grown on sandy soils and irrigated with contaminated water. The absence of data on heavy metal uptake by crops like pumpkins and Chinese cabbage on different soil environments represents a serious gap in scientific information. This is bearing in mind that pumpkins and Chinese cabbage are grown on a wide scale in peri urban areas in Zambia using waste water which is potentially contaminated with heavy metals like Cu and Co. In the absence of this kind of information it is not possible to predict accurately the amount of heavy metals that could be taken up by crops such as pumpkin and Chinese cabbage for health hazard assessment.

1.5 Objectives of the study

The general objective of this study was to establish the effect of increasing concentrations of Cu and Co in contaminated irrigation water on their uptake by pumpkin and Chinese cabbage when grown on sandy loam and clay loam soils.

The specific objectives were:

1) To determine heavy metals retention on sandy loam and clay loam soils irrigated with contaminated water;
2) To determine the effect of heavy metal contaminant concentration on the fractionation of Cu and Co in the soil;
3) To determine the effect of heavy metal contamination in irrigation water on uptake by pumpkin and Chinese cabbage;
4) To evaluate the effect of Cu and Co in tissue on crop yield when irrigated with contaminated water.

1.6 Hypotheses

The study intended to test the following hypothesis

1) It was hypothesized that higher amounts of heavy metals would be retained on clay loam than sandy loam soils when irrigated with contaminated water.
2) It was hypothesized that application of contaminated water would have a significant influence on the different fractions of Cu and Co in sandy loam and clay loam soils.
3) It was hypothesized that there would be significant differences in the uptake of heavy metals by pumpkin and Chinese cabbage from contaminated water.
4) It was hypothesized that significant yield decline would be observed on crops with high Cu and Co concentrations irrigated with contaminated water.

1.7 Justification of the study

This study is designed to address some important knowledge gaps in the link between the heavy metal contaminated water used for irrigation, soil type and food crops.. The study will provide information that will be used to design guidelines for use of waste water in agriculture.

1.8 Potential benefits of the study

The benefits of this study will be local stakeholders such as Environmental Managers who can use the information to enhance environmental management and develop policies that will ensure food safety to consumers. The different city councils could use this information to develop guidelines based on the local waste water types and soil conditions on the possible use or non use of waste water. The information will also be used by scholars and environmental managers to confirm or provide an informed position on possible heavy metal hazards especially Cu and Co that could contaminate common crops grown by peri-urban farmers in Zambia. The benefits of such a study will be shared with other countries within the sub region that are faced with the problem of health issues due to the use of waste water in agriculture. The study will complement efforts in enhancing the knowledge on waste water use among peri-urban farmers worldwide. Ultimately, the beneficiary population will be those who consume food crops grown in peri-urban areas such residents of towns and cities.

1.9 Scope of the study

This study focused on sandy loam and clay loam and how they affected the accumulation of Cu and Co in vegetable crops such as pumpkin and Chinese cabbage when irrigated with contaminated water.

A literature review was carried out with the purpose of gathering information on possible Cu and Co hazards in the environment and to identify areas where information is unavailable. The selection of this study was therefore, based on the identified gaps. This study used a pot experiment that was carried out in the greenhouse at the School of Agricultural Sciences of the University of Zambia.

There were three treatments included in the experiments (1) two vegetable crops (pumpkin and Chinese cabbage), (2) two soil types (sandy loam and a clay loam) and (3) Contaminated water that was used for irrigation. The contaminated water was collected in August 2006 from Uchi stream in Kitwe. The water was a discharge from Nkana Copper Smelter that was disposed in the Uchi stream. Uchi stream is a tributary of the Kafue River. At the time the water was collected, farmers used this water to grow vegetables during the dry periods of the year. The Nkana Copper Smelter was closed in March 2009. Therefore, no effluent from the Nkana smelter was discharged into the Uchi stream from March 2009.

The contaminated water used in the experiment was diluted with rain water as the latter did not contain detectable levels of any contaminants. The dilution was done to create a gradient of decreasing contamination in the water that was used for irrigation.

In this study a relationship between three parameters, water, soil and plants, was supposed to be investigated to determine how the contaminant water would affect the soil and plants. The concentrations of Cu and Co including other parameters such as acidity, electro-conductivity and other ions present were investigated in water.

Soil samples were analysed for their physical and chemical properties before they were used in the pot study and chemical analysis was repeated after crops were harvested from the pots. Fractionation of Cu and Co species in soils was carried out on the two soils to determine the fractions the elements were likely to be distributed in soil as these influence the toxicities to plants.

1.10 **Organization of the thesis**

This thesis comprises five chapters. Chapter 2 consists of an examination of the literature that was done to help understand the state of the current knowledge and limitations in the knowledge that motivated this study. In Chapter 3, an explanation of the materials and methods that were used in the study was done. This explanation comprised the description of the chemical characteristics of the water before use in the experiments and the soil physical and chemical characteristics. The chapter also explained the study design and the methodologies that were used in the different experiments that were part of the whole study. The detailed methods for each of the experiments in the study were presented. Chapter 4 presents the results and discussion on soil adsorption and speciation of Cu and Co in the two soils used. The chapter also presents the results and discussion on the results of the plant tissue uptake of Cu and Co also the effect of concentration of these elements on plant yield. Chapter 5 presents the conclusions and recommendations.

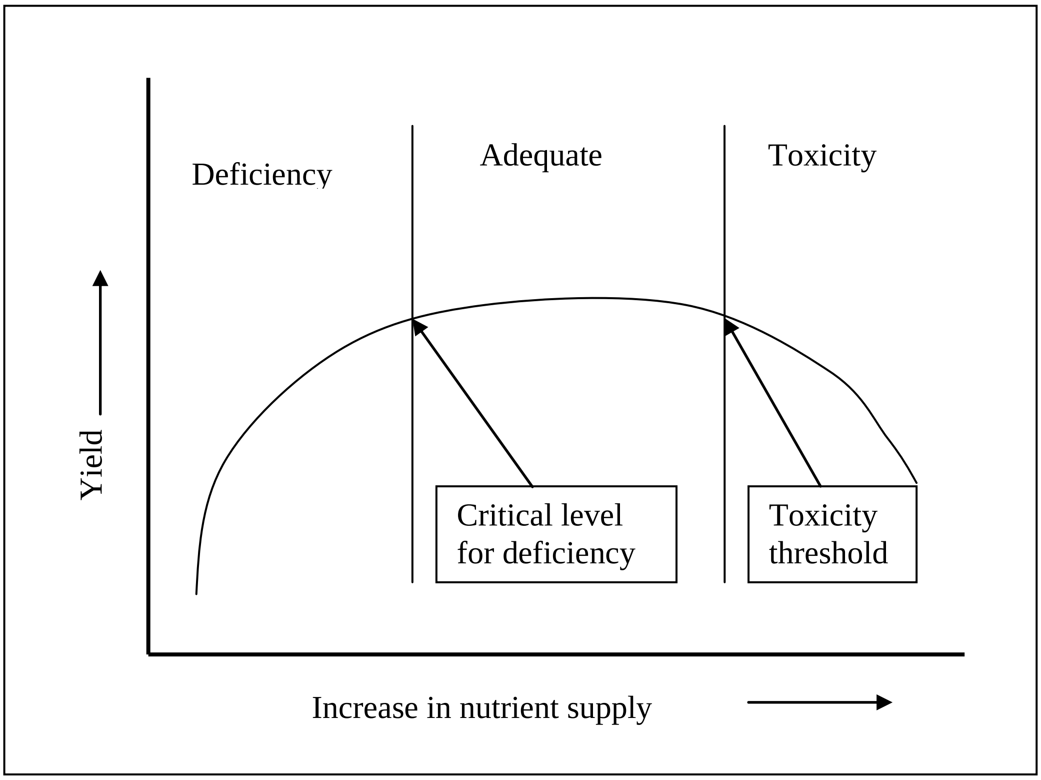
CHAPTER 2: LITERATURE REVIEW

2.1 Heavy metal contamination and toxicity

The term heavy metal is generally used for elements that have high atomic mass or metals with a density of 4500 kg/m^3 or more (Parker, 1989; Lozet and Mathieu, 1991). Heavy metals can be toxic to plants and animals at concentrations in excess of recommended amounts. These elements include: lead (Pb), zinc (Zn), chromium (Cr), mercury (Hg), arsenic (As), nickel (Ni), Cadmium (Cd), cobalt (Co) and copper (Cu). Among the elements listed, only Zn, Cu and Co are called essential and needed by plants and animals for some types of metabolic processes such as oxidation, electron transfers and enzyme reactions and important to complete their life cycles (Mertz, 1998; Lind, 2004).

In soil, when addition of an element exceeds the buffering capacity, the element becomes a pollutant (Moolenaar and Lexmond, 1998). Pollution in soil then occurs due to high concentration of available element and results in negative effects on the functions of the soil. The negative effects on the functions of soil would include reduced crop growth, and increased concentration of heavy metals in crops that will make them unhealthy for consumption by humans and animals.

A dose response curve for an essential element like Cu and Co on a plant or animal is depicted in Figure 2.1. The left part of the curve is a deficiency zone whereas the center part is adequate zone and to the right is the toxicity zone. In the deficiency zone, increasing supply of element will result in increased growth until the critical level of deficiency is reached where the yield increase ceases and deficiency symptoms disappear. Application of element beyond the critical level of deficiency will not result in any yield increase until the toxicity threshold when yield decline and toxicity symptoms appear. The zone between critical level of deficiency and toxicity threshold is ‘adequate zone’ (or tolerant zone). In the adequate zone, plants and animals can accumulate an element without signs of toxicity appearing (Mertz, 1998). Application of an element beyond the upper limit of adequate zone (the toxicity threshold) results in the adverse effects such as yield reduction and other toxicity symptoms. The toxicity threshold level is the upper limit where essential and nonessential elements exhibit adverse symptoms in plants or animals (Moolenaar and Lexmond, 1998).



(Source: Shollocks and Alloway, 1985)

Figure 2.1 A generalized dose response curve for micronutrients

One such plant that has been widely studied for its properties to accumulate toxic levels of metals that are toxic such as zinc (Zn), cadmium (Cd) and sometimes nickel (Ni) in the soil, and accumulate these metals to very high levels in the shoot without showing signs of toxic effects is *Thlaspi caerulescens*, a brassica species common in the northern regions (Europe and north America) (Milner and. Kochian, 2008). If the uptake of Cu and Co by pumpkin and Chinese cabbage is similar to what is depicted in Figure 2.1, and the crops depict differences in their threshold toxicity levels, then consuming a crop which does not show toxicity symptoms may be posing a danger to people consuming the food crop. This is because a crop that is looking healthy may be exposed to a high level contamination by heavy metal.

2.2 **Problem of heavy metal contamination in Zambia**

Commercial copper mining in Zambia started in Luanshya in 1928, although some mining activity was taking place even before that time at Kansanshi and Bwana Mkubwa (Sikamo *et al.*, 2016). Cobalt is not mined on its own but obtained as a by-product of Cu mining. Over the nine decades, Cu and Co mining has produced large quantities of waste in the environment. Some of the effects of Cu and Co mining in Zambia include generation of tailings from underground and open pits, acid deposition from SO_2 emissions, deposition of particles of Cu, Co and other metals during the ore processing. Liquid effluent from mining activities has been distributed from discharge during ore processing and overflow from tailing dams. The Auditor General report on the management of environmental degradation caused by mining activities in Zambia blamed the mining companies for discharging waste above the stipulated limits set by government (GRZ, 2014).

High levels of Cu, Co, Pb and Zn have been recorded on the soils, in water and in crops on the Copperbelt in Zambia (Sawula, 1985; Kasonde, 1995; Sinkala, 1998; Sinkala *et al.,* 1998; Nkandu, 1999; ECZ., 2000; ECZ., 2001; Petersson and Ingri, 2001; Simukanga *et al.,* 2002; von der Heyden and New, 2004; SGAB *et al*., 2005; Chishala *et al.,* 2006; GRZ., 2014; Lindahl, 2014; Kayika *et al*., 2017). The cause for the elevated levels of Cu on the Copperbelt province of Zambia is due to mining activities and processing of Cu and Co. Copper is mined in all towns of the Copperbelt province. Cobalt is mined in Luanshya, Chilibombwe and Chingola on the Copperbelt.

Copper and Co are found in very low concentrations in water, however, during the processing of Cu and Co, the end products such as slug, slimes and waste water may end up being released into the local water streams and rivers. One of the local rivers is the Kafue. The Kafue River is important as it flows through most of the towns on the Copperbelt. Most of the liquid effluents from the mines, industry and domestic on the Copperbelt end up in the Kafue River. High levels of Cu and Co have been recorded in water in the Kafue River (Kasonde, 1995; Nkandu, 1996; Berglin, 1997; Sinkala *et al.,* 1998; Petersson and Ingri, 2001; ZCCM, 2003; Ikenaka *et al.,* 2010).

Examples of high levels of Cu and Co in water have been measured by Chishala *et al.* (2006) at New farm in Mufulira, Copperbelt, where the concentration of Cu during the months between August and October was 0.53 mg/l and that of Co at the same site was 0.03 mg/l between the months of April to July. The FAO acceptable limits of Cu and Co in irrigation water are 0.2 and 0.01 mg/l respectively (Ayers and Westcot, 1985; Pescod, 1992). The concentration of Cu was more than seventeen times that of Co. When compared to FAO limits for Cu and Co in irrigation water, the concentrations were double and three times higher for the two elements respectively.

Preliminary evaluations of heavy metal contaminations in water, soils and crops by Chishala *et al.*, (2006) indicated high levels of heavy metals such as Cu, Cr, Pb and Ni in food crops. The concentrations of these heavy metals in food crops were: Cu (270 mg/kg in pumpkin leaves, 71.0 mg/kg in okra fruits, and 69.0 mg/kg in Swiss chard) and Cr (53.4 mg/kg in pumpkin leaves, 36.5 mg/kg in okra fruits, and 25.7 mg/kg in tomato fruits), Pb (8.4 mg/kg in pumpkin leaves, 5.5 mg/kg in okra fruits, and 2.4 mg/kg in Swiss chard)and Ni (6.3 mg/kg in pumpkin leaves, 10.8 mg/kg in okra fruits, and 2.7 mg/kg in Swiss chard). The concentrations of the above heavy metals apparently also varied with season (Holden and Malamud, 2006; Holden *et al.*, 2009; Holden *et al.*, 2010). The seasonal variations of heavy metals in food crops could probably be explained by soil properties like organic matter that can change seasonally due to accumulation, decomposition and mineralisation and thus affect heavy metal availability (Broekaert *et al.*, 1990, Basta *et al.*, 2005; Nolan *et al.*, 2005).

Siame *et al*. (2016) observed high concentrations of heavy metals including Cu and Co in green leafy vegetables bought from markets in Kitwe. The concentrations of Cu and Co exceeded the WHO (1989) and FAO (Ayers and Westcot, 1985) standards. They attributed the results to human activities with mining being the common contributor. Kayika *et al*. (2017) also observed high concentrations of Cu, Co and Cd in soils and mango fruits that were grown tailings in Chingola. These two studies highlight some of the environmental effects of copper and cobalt mining activities on the Copperbelt province in Zambia.

Petersson and Ingri (2001), in their study of the environmental problems of copper mining and refining in Zambia concluded that acid wastes from the mining industry would increase the dissolved loads of Ca (6 times), Mg (2.5 times), Cu (50 times) and Co (300 times) in water through acid solution.

High levels of Cu have also been recorded in water and plants in other towns of Zambia where there is no copper mining and processing factories (Chishala *et al.*, 2006). The likely sources of the high Cu content in plants and water in towns away from the Copperbelt province of Zambia could be from metallurgical and chemical industries, corrosion of copper pipes and fossil fuel combustion activities in the urban areas of Lusaka and Kafue. Presence of high levels of Cu and Co in water found in areas far away from mining areas is an indication that the problem of these trace metals contamination is more widespread. The recent increases in the production of Cu and Co by opening of new mines and reprocessing of old waste dumps to obtain more metals provides an opportunity for increased contamination of the environment. New mines are being opened in other provinces like Northwestern, Central and Luapula that could expand areas and sources of new pollution in the local streams and rivers.

The increased mining activities have generally contributed employment opportunities coupled with improvements in living standards of the population, resulting in increased demand for more resources such as cement, coal, new land for housing and roads. However, more people migrating to cities still find themselves unable to find jobs in towns and end up in informal activities such as agriculture that includes farming with any available water including water from industrial sources for irrigation. Due to lack or regular monitoring and lack of regulatory frameworks in the use of waste water that is polluted, contamination of soils and crops is likely to increase. The contribution of Cu and Co mining is increasing rapidly in Zambia and absence of scientific information from local studies that helps to explain possible environmental effects that result from waste disposal during processing is a serious scientific gap. Lack of information local studies denies environmental managers vital information for the management of waste water effluents and policies for waste water use in agriculture.

2.3. Legal guidelines on heavy metal content in water and food crops

2.3.1 Legislation on heavy metal pollution in Zambia

In the Environmental Pollution and Protection Control Act No. 12 (EPPCA) of 1990 (GRZ, 1990), provision was made for the establishment of the Environmental Council of Zambia (ECZ). The ECZ was responsible for ensuring that the waste water disposed of in the environment meets the recommended disposal standards (ECZ, 1993). The Environmental Management Act of 2011 (GRZ, 2011) repealed the EPPCA of 1990 and renamed the ECZ to Zambia Environmental Management Agency (ZEMA) however, with the same but enhanced mandate to prosecute offenders.

The Public Health Act of 1978 (GRZ, 1978) prohibits the use of waste water that is considered unhealthy for agricultural use is enforced under the Ministry of Local Government. The Food and Drugs Act of 1995 (GRZ, 1995) prohibits the sale of fresh foods that have metal contents above stipulated amounts and this is mandated to the Ministry of Health. Even though the above legislations exist, the problem has been the enforcement of these Acts by the various institutions that are mandated with the responsibility of managing the land, water use and waste management.

The Environmental Management Act relies on results of metal records provided by the sources of the waste water and thus difficult to ascertain if the information is genuine or not. With regard to waste water from municipal sewage systems, quality of waste water discharged is monitored by their laboratories and they take steps that minimize environmental problems that would result from the use of waste water that includes agriculture. The municipal councils also take measures to stop the use of waste water by farmers. However, the lack of enforcement to stop farmers in peri-urban areas to use waste water has not stopped them using the resource that is available for them to make livelihoods. This means that if waste water has high concentrations of heavy metals, crops that are grown using this water could be contaminated and it cannot be ruled out that if these contaminated food crops are consumed by humans, they could present serious health risks to consumers. It is thus on the strength of this background that this study was considered necessary.

2.3.2 **Irrigation and waste water disposal guidelines**

Discharge of waste water from industry and sewerage sources into local streams is widespread in Zambia. Although the Environmental Management Act provides regulatory measures to discharge waste water into the environment, the problem has been to implement these measures. In some instances industry has been given the mandate to monitor themselves on their compliance to disposal standards.

The World health Organisation (WHO, 1993), Zambia Bureau of Standards (ZBS, 1991) drinking water and FAO (Ayers and Westcot, 1985) irrigation water guidelines are presented in Table 2.1. This table shows that if the water just meets the minimum standards for drinking water, it would still be unsuitable for irrigation of crops because drinking water standards are lower.

Table 2. 1 Standards for waste water disposal

Institution	Element		Use of Standard
	Cu mg/l	Co mg/l	
GRZ	1.5	1.0	Waste water disposal
WHO	2.0	-	Drinking water
ZBS	1.0	0.5	Drinking water
FAO	0.2	0.05	Irrigation water
NAS/NAE		0.05[a] and 5[b]	Irrigation water

a = for continuous use; b = for short term use (<20 years); Source: NAS/NAE, 1973; Ayers and Westcot, 1985; ZBS, 1991; GRZ, 1993; WHO, 1993.

There is very little evidence from literature on Co toxicity being used to design guideline for the metal. However, the 0.05 mg/l of cobalt in irrigation water was derived from National Academy of Sciences / National Association of Engineering Report (NAS/NAE, 1973) that recommended 0.050 mg/l for continuous use on all soils and 5.0 mg/l for short term use (< 20 years) on neutral and alkaline fine textured soils.

The disposal of waste water in Zambia is guided by the Water Pollution Control (Effluent and Waste Water) Regulations Act (GRZ, 1993). The waste disposal guidelines for waste water recommend that the amounts of Cu and Co that can be disposed off in the aquatic environment are 1.5 and 1.0 mg/l respectively for the two metals. The Waste Water Disposal Act (GRZ, 1993) sets out the processes and limits that industries must adhere to obtain a license to discharge effluent in aquatic environments. The Zambia waste water disposal guidelines however, do not provide for situations that requires specific loading rates on the environment that would be determined by the environmental conditions or land uses in the affected areas. Possibly the rationale for such guidelines were precautionary because of the absence of basic information of contamination of the disposed effluents, edaphic conditions and land uses in the affected areas.

Ayers and Westcot (1985) in their recommendation on the use of irrigation water in agriculture provided generalized guidelines for heavy metal pollutants in water (Table 2.2 for Co and Cu guidelines). Their recommendation included the effect of different elements to crop growth and in some cases effects on use by humans. However, these guidelines do not indicate properties that influence bioavailability of heavy metals in soils. They however, recommended examination of potential toxicity under local conditions such as nature of the soil, climatic conditions, nature of effluent and crops grown. It is with this background that guidelines specifying metal content in waste water are different from country to country and they depend on varying risk management approaches they have adopted.

Table 2. 2 Recommended maximum concentrations of Co and Cu in irrigation water

Element	Recommended maximum concentration (mg/l)	Remarks
Co	0.05	Toxic to tomato plants at 0.1 mg/l in nutrient solution. Tends to be inactivated by neutral and alkaline soils.
Cu	0.20	Toxic to a number of plants at 0.1 to 1.0 mg/l in nutrient solutions.

Source: Ayers and Westcot, 1985.

To minimize the effect of heavy metals on the environment, most countries have enacted laws on maximum permissible limits of contaminants, countries in North America, Australia, Japan and Europe have legal frameworks to identify and deal with soil contamination problem. For example, in the United Kingdom, the commonly used guidance for screening acceptable soil contamination values are the Soil Guideline Values published by Environment Agency (UK Environmental Agency, 2009). These are screening values that demonstrate the minimal acceptable level of a substance in soil. These have been derived using the Contaminated Land Exposure Assessment Model (CLEA). The input parameters in the CLEA model include health criteria values; age and land use (UK Environmental Agency, 2009). The output of a CLEA model is a probabilistic output. Other national standards include the United States Environmental Protection Agency (EPA) Preliminary Remediation Goals (PRGs) (EPA, 1996), the EPA Risk Based Concentrations (EPA RBCs) (EPA, 2012) and National Environment Protection Council of Australia Guideline on Investigation Levels in Soil and Groundwater (NEPC, 2011).

These legislated limits usually differ from one country to another and the differences arise from the context in which the guidelines were made. In addition, these guidelines may differ from one country to another due to the models in which they are based and other local variables that exist in a country or region like climate, soils, level of development, legislation and policy. Therefore, these legislated limits of heavy metals may not be applicable in all situations.

Generalised recommendations present their own problems because they do not specify the type of waste water, the species of the elements that are in the waste water, nature of the soil conditions, uses (legal or illegal) that the waste water could be used for. The problem is that even if generalised guidelines may result in reduced metal disposal, safe levels for contamination of soils and crops would not be guaranteed.

It is thus imperative to establish the safe heavy metal content of the different types of waste water since the effect may vary if the source of the waste water is industrial where the amount of organic matter may be low or sewage where you may have large amounts of organic matter that could adsorb the metals.

2.3.3 **Food guidelines**

Guidelines for heavy metal pollution in crops are produced in different countries in order to prevent the consumption of the crops or fodder in humans and farm animals. Different countries and organisations have also produced guidelines for standards of safety of heavy metals in food. The most commonly used food guideline is the Codex Alimentarius (FAO/WHO, 1995). The Codex Alimentarius is a collection of internationally recognised standards, codes of practice and guidelines for food production and safety. The Codex Alimentarius Commission was established by the Food and Agriculture Organisation (FAO) and the World health Organisation (WHO) in 1963.

In Zambia, allowed legal limits for heavy metals in food stuffs are stipulated in the Food and Drugs Act (GRZ, 1995). The act stipulates acceptable limits for the presence of As, Cu, Fe, Pb, Hg, Sn, Zn and F, in fresh fruits and vegetables. The legislated acceptable level of Cu in fresh fruits and vegetables is 50 mg/kg. There are no guidelines for cobalt in food stuffs in the GRZ Food and Drugs Act. The Canadian Ministry of Environment (MOE, 2002) suggests concentrations between 25-100 mg/kg to be a threshold for toxicity of cobalt in plants.

If a crop is a hyper accumulator and it can accumulate above 50 and 100 mg/kg of Cu and Co in their tissue, its consumption would cause toxicity to animals or humans even when the plants look healthy. Based on these findings, regular measurements of heavy metals in food crops is important as even health looking food crops could have high concentration of these metals.

2.4. **Human health and environmental effects of Cu and Co**

Copper and cobalt are found in many kinds of foods and in drinking water. The absorption of Cu and Co is necessary, because the elements are essential for human health. Although Cu and Co are important for biological systems, high concentrations of these elements can cause health problems.

2.4.1 **Copper**

Copper (Cu) is a reddish brown metal that is malleable, ductile, and an extremely good conductor of both heat and electricity. Copper is classified as a transition metal because it is located in group 11of the periodic table. It has an atomic mass of 63.546 g/mol and a density of 8.9 g/cm^3 at 20°C. Copper is an essential substance to all living organisms. Copper has unique properties that allow it to be found in either oxidized (Cu (II)) or in reduced Cu (I) states. For this reason, Cu ions serve as important catalytic co-factors in redox chemistry of proteins that are used in important biological systems (Lind, 2004).

Copper can accumulate in plant and animal tissues thereby becoming poisonous (Lind, 2004; Nordberg and Cherian, 2004; Lenntech, 2011). Crops that have been irrigated with Cu contaminated water or soils that are contaminated with metal may end up poisoning the consumers of the crops. Although cases of Cu poisoning in healthy humans are rare, it is however, toxic when one is over exposed to the element. Excess amount of Cu in drinking water has been found to cause diarrhoea and stomach problems (Lind, 2004; Lenntech, 2011). The maximum amount of Cu in drinking water is 2.0 mg/l (WHO, 1993). These standards for drinking water are set taking into consideration the long term accumulation of an element in the body.

Copper has also been found to cause headaches and kidney failure in some instances (Lenntech, 2011). In some cases chronic Cu toxicity has been recorded to be hereditary or acquired (Centano *et al.,* 2005). In humans this is exemplified by Wilsons or Menkes diseases and Indian childhood cirrhosis (Centano *et al.,* 2005). It has been found to cause anaemia, liver and kidney damage, stomach and intestinal irritation and diarrhoea (Nordberg and Cherian, 2004). At levels of 2 mg/l in drinking water, Cu has been found to cause diarrhoea in children at 10 mg/l it has been found to increase the risk of odour and taste in water (Nordberg and Cherian, 2004).

In plants, high levels of Cu above 20 mg/kg are considered toxic and the effects of this toxicity are seen in stunted growth and chlorosis (Shorrocks and Alloway, 1985). In soil, Cu toxicity is common where concentration of plant available Cu is above 30 mg/kg (Shorrocks and Alloway, 1985). Copper toxicity in plants primarily affects the roots by stunting their growth (Tiller and Merry, 1981, Sheldon and Menzies, 2005).

In fresh vegetables and fruits, the legal limits allowed in Zambia for Cu is 50 mg/kg (GRZ, 1995) or 40 mg/kg (FAO/WHO, 1995). Copper is toxic to man at concentrations above 250 mg/day (Bowen 1979). These limits of standards can be exceeded in food crops when the amounts of metal in either soil or water are too high. The problem is that examination of food crops for contamination from microbiological or chemical sources is not regular. Foods are only usually examined only when problem arises. The problem for heavy metals is usually insidious and may take a long time before the symptoms are seen (Goyer, 1991; Demirozu and Saldamli, 2002). It is therefore, important that measures are taken to prevent contamination of heavy metals by agronomic and land use measures.

2.4.2 **Cobalt**

Cobalt (Co) is a silver-white, hard, lustrous, brittle element. Cobalt is classified as a transition metal because it is located in group 9 of the periodic table. It has an atomic mass of 58.9332 g/mol, and a density of 8.9 g/cm^3 at 20°C and also considered to be a heavy metal

Cobalt is commonly present in small amounts in soil, water and plants. Cobalt is important in trace amounts to human and plant life. Cobalt is a vital element in the synthesis of vitamin B_{12} which is important in a number of reactions in the human body including production of red blood cells (MOE, 2002). One of these important reactions is the enzyme ribonucleotide reductase that requires Vitamin B_{12} for the reduction of ribonucleotides to corresponding deoxyribonucleotides (Lind, 2004). Isotopes of Co-60 are used in nuclear medicine, research and preservation of foods (Lenntech, 2011)

Concentration of Co in freshwater is generally low, below 0.001mg/l (MOE, 2002) and higher concentrations are associated with pollution from industry or mining areas (Lenntech, 2011). Toxicity of Co has been found to be very low compared to other metals. Exposure to very high levels of Co can cause health problems on the lungs, including asthma, pneumonia and wheezing. It has also been determined by the International Agency for Research on Cancer, that Co is a possible carcinogen to humans (MOE, 2002). When Co is added to beer, it has been found to cause endemic problems of cardiomyopathy among beer drinkers and resulted in fatalities (Nordberg and Cherian, 2004). This was related to cobalt sulphate being added to beer as stabilizer of beer foam (Centano *et al.,* 2004).

In plants, threshold tissue concentration of Co is 25-100 mg/kg (MOE, 2002). Toxicity of cobalt in plants is not common but like all compounds when taken in excess amounts can possibly result in toxicity and possibly death of plants (Breckle, 1991; Lind, 2004). Symptoms of Co toxicity in plants are seen by reduced root elongation and yield. Misra *et al.,* (1994) observed that high levels of Co in soil reduced root elongation in broad beans. Wallace *et al.,* (1977) also observed a reduction in leaf dry weight in bush beans when the soils had high levels of Co.

There is no report on the toxicity experiences of Co in human beings or plants in Zambia. Even when elevated levels beyond recommended limits of Co have been recorded in water and soils, the absence of specific Co toxicity incidences does not necessarily mean there is no problem with Co contamination especially when monitoring of heavy metals in the environment is almost absent.

2.5 **Behaviour of Cu and Co in soils**

Copper and cobalt are found in one or more of several types of pools in the soil, as described by Shuman (1991), these include (1) dissolved in the soil solution; (2) occupying exchange sites on inorganic soil constituents; (3) specifically adsorbed on inorganic soil constituents; (4) associated with insoluble soil organic matter; (5) precipitated as pure or mixed solids; (6) present in the structure of secondary minerals; and/or (7) present in the structure of primary minerals. In polluted environments as through human activities, Cu and Co are usually found associated with the first five pools. Native Cu and Co may be associated with any of the pools depending on the geological history of the area.

2.5.1 **Copper**

Copper is found in all natural soils but content can sometimes be so low that it affects the healthy growth of plants (Shorrocks and Alloway, 1985). Copper content in the soil is largely determined by parent materials and anthropogenic sources such as mining and industrial waste, pesticides, manures and sewerage sludge can also be responsible for the high amounts (Shorrocks and Alloway, 1985; Nordberg and Cherian, 2004).

Parent materials are important sources of most nutrients including Cu. From parent materials, elements are usually released slowly during the processes of physical and chemical weathering. In unpolluted soils, the mean amount of total concentration of Cu is usually around 25 mg/kg (Shorrocks and Alloway, 1985; Adriano, 2001; Garret, 2004). Sandy soils usually have low Cu contents in the range of 2-10 mg/kg, clays between 10-27 mg/kg and organic rich loams usually have highest between 1-100 mg/kg with a mean of 30 mg/kg (Thornton, 1979; Baker, 1990)

The most dominant chemical species in soil and water, without accounting for ion pairs or complex-ion species, for Cu is Cu^{2+} (Logan and Traina, 1993). The most toxic species is Cu^{2+} if we consider the degree of bioavailability (Logan and Traina, 1993).

2.5.2 **Cobalt**

Cobalt is only needed in small amounts for good health of plants and animals. Cobalt is found naturally in most rocks, soils, plants and water (MOE 2002; Smith and Carson 1981). In unpolluted soils, the mean amount of total concentration of Co is usually about 1-20 mg/kg and can be higher in geologically rich areas where total concentration can be within 1-50 mg/kg range (Smith and Carson, 1981; Hamilton, 1994; Nagpal, 2004; Lison, 2005). Cobalt is also found in many different chemical forms and usually associated with other metals such as Cu, Ni, Mn and As (Smith and Carson, 1981; MOE, 2002).

Cobalt content in the soil is largely determined by weathering of parent materials, volcanic emissions and breakdown of organic matter where there is vitamin B12. (Moore, 1991; Thecdi, 2006). Anthropogenic sources such as mining and industrial waste, burning of coal and oil, use of fuels in motor vehicles and airplanes, application of manures and sewerage sludge can also be responsible for the high amounts of Co that can be found in soils (Nagpal, 2004; Thecdi, 2006;). High amounts of Co in soil have been reported in areas near ore deposits and where industries impact the environments (Smith and Carson, 1981; Hamilton, 1994).

In the environment, Co is found in the form of Co^{2+} or Co^{3+} oxidation states and largely in inorganic and organic salts. The most dominant chemical species in soil and water, without accounting for ion pairs or complex-ion species, for Co is Co^{2+} (Logan and Traina, 1993). The most toxic species is Co^{2+} if we consider the degree of bioavailability (Logan and Traina, 1993).

Most studies on toxicity of heavy metals such as Cu and Co have been carried out in the developed world where the pathway to environmental contamination may not necessarily be the same such as the use of contaminated industrial waste water for irrigation. It is thus imperative that effects using contaminated industrial waste water are studied to fill the gap for this information.

2.6 Bioavailability of Cu and Co to plants

Heavy metals are found in different forms (fractions) in soil. Some of the forms cannot be taken up by plants for its metabolic functions. The term bioavailability refers to that fraction of the total element content in either the soil or solution that can be taken up by plants as the bulk of the metal fractions are not in soluble form for transport to plant roots (Lasat, 2002) Bioavailable fraction depends on the soil metal content, soil texture, soil organic matter, content and pH (Chaney, 1998).

The adsorption of Cu to organic compounds is known (McGrath, 1996; Mehra *et al*., 1999; Kabata-Pendias and Pendias, 2001). Therefore, the mobility and availability of Cu is largely governed by the organic fractions in soils. Cobalt is usually not freely available in the environment but in cases where the element is disposed to the environment due to pollution and the element not adsorbed to soil or sediments, the element can be toxic to plants (Gopal *et al*., 2003; Li *et al*., 2009) and humans when ingested (MOE, 2002; Nordberg and Cherian, 2004). Studies have shown that Co in soils is commonly adsorbed in the exchangeable and organic fractions (Maclaren *et al*., 1986; Borggaard, 1988; Bashir *et al*., 2014)

To understand the behaviour of heavy metals in the environment such as their mobility, pathways and bioavailability, it is important to know the information about the physicochemical forms. Measuring the bioavailable concentrations of heavy metals in soils provides more information on the environmental impact of the elements of interest. Therefore, determining total content of heavy metals is insufficient to assess the environmental impact of contaminated soils and sediments (McLaren and Crawford, 1973a; McLaren and Crawford, 1973b; Tessier *et al*., 1979), because it is the chemical form that determines metal behaviour in the environment and its remobilization ability.

2.7 Methods of measuring total and bio available metal concentrations in soil

Continuous application of waste water loaded with heavy metals results in increase of these toxic elements in soil. What becomes an important issue are the bioavailable forms of these heavy metals. One can use either the total or bioavailable concentrations of these heavy metals to interpret their effect on the environment. The determination of the total content of heavy metals alone is insufficient in assessing their environmental impact, since it is the chemical forms that determine metal behaviour in the environment and their mobilization ability (McLaren and Crawford, 1973a; McLaren and Crawford, 1973b; Tessier *et al.*, 1979). It is thus necessary to assess both total contents of the hazardous substances, as well as the chemical forms in which they may be present. Several studies have shown that total metal concentrations are generally a poor indicator of metal toxicity because elements exist in various solution and solid phase forms. The toxicity of elements is dependent upon its chemical form, mobility, degree of transformation and bioavailability (Tessier *et al.*, 1979; Kabata-Pendias and Pendias, 2001; Basta, *et al.,* 2005). Elements in soil may be found in one or more of the following forms: (a). dissolved in solution, (b) exchangeable: in organic and inorganic form), (c) as structural components of soil minerals, and (d) insoluble precipitates. The first two forms are available for uptake by plants whereas the rest may be potentially available in the long term (Cottenie *et al.*, 1980; Gibson and Farmer 1986; Maskall and Thornton 1998). The trend has been moving towards the use of bioavailable forms of heavy metals when looking at their environmental impacts as they tend to have a stronger correlation with plant uptake (Amacher, 1996; Basta, *et al.,* 2005; Nolan *et al.,* 2005).

Extraction procedures are the most common methods for assessing bioavailability of trace metals in soils. The methods commonly use simple aqueous washes, acids, bases, or organic solvents. These and related methods provide what may be termed ‘bioaccessibility’ data, but the final assay is always chemical rather than biological. The underlying assumption of this approach is that the less accessible the metal, the harsher would be the conditions required to remove it from the soil (Tessier *et al.,* 1979).

However, there are different methods which have been developed to determine bioavailable trace metals in soils and there is no consensus as to which of the numerous methods is the acceptable one as to reflect plant uptake. Bioavailable trace elements are measured by first extracting the trace elements form soil into solution. The dissolved elements in solution are measured using an Atomic Absorption

Spectrophotometer (AAS) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS).The AAS is most frequently used tool in analytical chemistry. The ICP_MS is expensive and can measure elements in very low concentrations. Many different compounds have been used in the different methods to extract bioavailable forms of heavy metals in soils. Some of these compounds include chelating compounds such as: Diethylene Triamine Penta Acetic Acid (DTPA); Diethylene Triamine Penta Acetic Acid – Triethanolamine (DTPA-TEA); Ethylene - Diamine-Tetra Acetic Acid (EDTA); Ammonium acetate: Calcium chloride and Water (Table 2.3). The value of each of the methods is dependent upon the strength of the compounds used.

Table 2. 3 Methods of extracting bioavailable trace metals in soil

Chemical Extractant	Authors
Diethylene Triamine Penta Acetic Acid (DTPA)	Lindsay and Novell, 1978
Diethylene Triamine Penta Acetic Acid – Triethanolamine (DTPA-TEA)	Lindsay and Novell, 1978
Ethylene-Diamine-Tetraacetic Acid (EDTA)	Soane and Saunder, 1959: Brun *et al.,* 2001
Ammonium acetate	Brun *et al.,* 2001
Calcium chloride	Murray and Evans, 2002; Murray *et al.,* 2003

Chelating agents are used in the assessment of bioavailable trace metals because they combine with free ions in solution and those that are adsorbed on the exchangeable complexes of soils. However, some authors disagree on the use of chelating agents as they over estimate amounts of metals than plants extract form the soil (Macgrath and Cegara, 1992; Murray *et al.,* 2003).

Lindsay and Novel (1978) observed that Zn and Cu extracted using DTPA, a mild chelating agent, correlated well with crop response to these metals. Soane and Saunder (1959) and Brun *et al.,* (2001) using EDTA observed a strong correlation between soil bioavailable Ni and Cd content and concentration of the same metals in plants. Murray and Evans (2002) recommended the use of 0.01 $CaCl_2$ when determining plant available trace metals in soils after they found strong correlation between Brome grass metal content and that of soils. Brun *et al.,* (2001) found strong correlation between plant metal content and Ammonium acetate extracted soil metal content. Murray *et al.,* (2001) recommended dilute $CaCl_2$ as a universal soil extractant after obtaining strong linear regressions between soil and crop heavy metal contents.

It is thus important to find out the impact that these fractions have on the uptake of Cu and Co in soils where contaminated waste water is used to irrigate crops. This will provide answers to the differences that may be observed over different edaphic areas on the concentration of heavy metals in plants. In this study, the intention is to relate the metal content in soil and that found in plants. Therefore, the Lindsay and Novel (1978) would be more appropriate method in this study for extracting bioavailable elements because of the strong correlation between the metal content in soil and plant response.

2.8 Influence of soil properties on heavy metal adsorption

A number of factors influence the adsorption of heavy metals like Cu and Co when applied to the soil through irrigation water. These factors according to Dube *et al.,* (2001) may immobilize heavy metal ions. Immobilisation of heavy metals is due to the sorption properties of the components of soils such as clay types, organic matter, pH, water content and properties of the metal ions (White, 1987; Alloway, 1995; Birley and Lock, 1999; Dube *et al.,* 2001).

A soils' cation exchange capacity is influenced by the type of the clay mineral present, including the element(s) that form the bonding with clay minerals. Illites, for example, have a higher cation exchange capacity than, for example, kaolinite minerals (Carroll, 1959; McBride, 1987; White, 1987). Adsorption of heavy metals on soils may have some temporary benefits for soils where crops are grown using contaminated water. The adsorption of heavy metals by soil clays may make some portion of the metals not available to plants and thus the concentration of these metals reduced in plant tissue.

A soil's organic matter content is another factor affecting the uptake of heavy metal cations (McGrath and Loveland, 1992; Holmgren *et al.,* 1993). Higher levels of organic matter content increases the CEC in soils, and thus greater uptake of heavy metals can result. The availability of trace elements to plants is governed by a variety of reactions that include complexation with organic and inorganic ligands, ion exchange and adsorption, precipitation and dissolution of solids, and acid-base equilibria. A key role is often played by organic matter (OM), mostly humic compounds, in enhancing availability to plants and in reducing toxicity effects of the free cation. The ability of humic compounds to form stable complexes with polyvalent cations essential to plants, as well as toxic ones, has been well established (Frimmel *et al.,* 1983; Chen and Aviad, 1989; Chaney and Ryan, 1993; Chaney *et al*., 1997: Stevenson, 1994). The formation of these complexes facilitates the mobilization, transport, segregation and deposition of trace metals found in soils and aquatic systems.

Several soluble metals including Cu are primarily associated with organic matter. Trace elements that would ordinarily convert into insoluble precipitates (such as carbonates, sulphides or hydroxides) at the pH levels found in many soils are maintained in solution through chelation. Knowledge of the nature of the organic ligands that form complexes with metal ions, and of properties of the complexes thus formed will lead to a better understanding of the factors that affect trace element availability to plants.

The soil pH is also an important factor controlling chemical behaviour and therefore heavy metal uptake by soils. (Alloway, 1995; Naidu *et al.,* 2003). Heavy metal cations are more mobile in acidic conditions. Therefore, if the pH is increased in soil, the bioavailability of the heavy metal ions is reduced. This ultimately implies that toxicity of metals will vary in different soils even if they have the same total metal concentration and this can be attributed to their sorptive capacities (Naidu *et al.,* 2003)

The strength with which metal ions are held to soil particles is dependent on the size of charge. Ions with higher charges such as Al^{3+}, is strongly held to soil than one with smaller charge like K^+. However, for metals of the same charge such as Cu^{2+} and Co^{2+}, the ionic radius and rank of hydration is important (Dube *et al.,* 2001). The bigger the ionic radius, the smaller the electric field it emits around it and consequently less hydrated than ions with smaller radius (Ibid).

Soils generally have different chemical and physical properties as a result of the various factors that influence their formation and processes taking place in them. These properties affect heavy metal adsorption and desorption, consequently the availability of these elements to crops that grow on them. In view of the above explanations on the factors that influence metal adsorption and desorption, uniform guidelines on the use of waste water on soils with different characteristics may not provide adequate protection against contamination by heavy metals.

2.9 Speciation of heavy metals in soils

The term fractionation is frequently used interchangeably with speciation. The term speciation has been defined as the identification and quantification of the different, defined species, forms or phases in which an element occurs (Tack and Verloo, 1995). The term fractionation has been used to describe the concept of subdividing a total content (Tack and Verloo, 1995). That is why laboratory analytical procedures for separating metal species are referred to as fractionation. The behaviour of different fractions of heavy metals in soil is determined by different physical and chemical processes. These physico-chemical processes influence the way in which the heavy metals can be available or not for plant uptake in soil. The principle behind fractionation is that each subsequent leachant should be either stronger in chemical action than the previous one or be of a different nature (Hlavay *et al.,* 2004)

A number of environmental, chemical and biological processes influence the accessibility of heavy metals to plants. In general availability of heavy metals depends upon the properties of soil such as, CEC, pH, electro-conductivity, salinity and the concentration of organic and inorganic complexation agents. Thus the determination of the total content of heavy metals alone is insufficient in assessing their environmental impact, since it is the chemical forms that determine metal behaviour in the environment and their mobilization ability (McLaren and Crawford, 1973a; McLaren and Crawford, 1973b; Tessier *et al.,* 1979, Domanska, 2008). It is thus necessary to assess both total contents of the hazardous substances, as well as the chemical forms in which they may be present.

The information about the physicochemical forms of heavy metals is important to understanding the mobility, pathways, bioavailability in the environment. Therefore, when assessing hazards and risks that metals and metal compounds pose, such properties such as speciation, bioavailability, bioaccumulation, persistence, and toxicity need to be considered.

The differences in Cu concentrations in soils with different physical and chemical characteristics are a result of the physical and chemical processes that adsorb the element to the soil (Baker 1990). Total content of Cu in soil is usually made up of five major fractions (McLaren and Crawford, 1973a; McLaren and Crawford, 1973b; Tessier *et al.*, 1979). These fractions (or pools) are classified depending on the physico-chemical behaviour of the metals (Sposito *et al.*, 1982; Shorrocks and Alloway, 1985; Fedotov and Spivakov, 2008). The fractions are: (i) exchangeable, (ii) water soluble, (iii) organic, (iv) carbonate and (v) residual (Table 2.4).

Table 2.4 Types of fractions, bonding forms, mobility, bioavailability of commonly used leachants.

Fraction	Possible bonding forms	Mobility	Bioavailability	Commonly used leachant
Water soluble	Free ions	Mobile	Easily available	Deionised water
Exchangeable	Exchangeable on weak electrostatic interactions	Mobile	Easily available	$CaCl_2$, $MgCl_2$, KNO_3, $Ca(NO_3)_2$, $CH_3CO_2NH_4$
Carbonate (Acid soluble)	Adsorbed on carbonates	Easily mobile	Easily available	KF, EDTA, NaOAc ($pH \leq 6$)
Reducible (Fe, Al, Mn)	Adsorbed on Fe, Al, Mn oxides	Range from moderate to poorly mobile	Range from moderate to poorly available	NaOH $NH_2OH \cdot HCl$ (pH 3.2),
Organic bound (Oxidizable)	Adsorbed on organic complexes	Range from moderate to poorly mobile	Range from moderate to poorly available	NaOH, HNO_3, H_2O_2, NH_4OAc, $Na_4P_2O_7$
Residual (Sulphide)	Retained within crystal structures of minerals	immobile	unavailable	$HF\text{-}HClO_4$, HNO_3, $HClO_4$

Sources: McLaren and Crawford, 1973a; McLaren and Crawford, 1973b; Stover *et al.*, 1976; Tessier *et al.*, 1979; Sposito *et al.*, 1982; Miller and McFee, 1983; Vojtekova *et al.*, 2003; Fedotov *et al.*, 2008

The exchangeable, water soluble, carbonate forms are the most important in supplying nutrients for plant growth (Xian, 1987; Aydinalp and Marinova, 2003; Violante *et. al.,* 2010). Heavy metals present in these categories have different remobilization behaviour under changing environmental conditions (Forstner, 1985). It is from this knowledge of chemical and binding forms that has given the basis for measuring of bioavailability and mobility of contaminants in the environment (Sposito *et al.,* 1982; Broekaert *et. al.,* 1990; Dube *et. al.,* 2001). The availability, non availability and toxicity of heavy metals in soils are largely influenced by the forms in which they are adsorbed (Sposito *et al.,* 1982; Xian, 1989; Nolan *et. al.,* 2005). The different forms of heavy metals in the soils are important to explain temporal and immediate availability of elements in soil that can be taken up by plants (Tessier *et al*., 1979; Sposito *et al.,* 1982; Song and Breslin, 1998; Ryan *et al*., 2002).

The exchangeable fractions are those that are weakly adsorbed on the soil. They are retained on the soil due to weak electrostatic interactions between the negative charges on the soil and the positive charges on the cation. Their behaviour is dictated by ion exchange processes. For example, Cu that is adsorbed on the exchange complex can be released (or immobilized) in solution when the pH drops. This immobilized Cu can then be taken up by plants through root interception (Jarvis, 1981; Shorrocks and Alloway, 1985).

The water soluble fraction contains the elements in the form of free ions, mineral salts and metal complexes. The water soluble fraction is the most biologically active fraction with the highest potential to contaminate crops and animals in the food chain as well as surface and ground water (Leita and De Nobili, 1991; Iwegbue *et al*., 2007). Khurana and Bansal (2008) observed that concentration of Ni in *Brassica juncea* and *Brassisca campestris* grown on soils irrigated with sewage water was highest where the concentration of exchangeable and water soluble fractions were highest. In the same study by Khurana and Bansal (2008), low concentrations of Ni were observed where the highest fractions of the metal were in the oxide and residual fractions.

Reducible fractions of metals are those that are bound in the soil by Fe and Mn hydroxides. These fractions of metals are unstable under reducing conditions

(Fedotov and Spivakov, 2008). Elements in the residual forms are completely immobile and considered to be not bioavailable (Iwegbue *et al*., 2007). Iron and manganese oxides (reducible fractions), existing in nodules, concretions, cement between particles or as acting on particles and are excellent trace element scavengers (Nachtegaal and Sparks, 2004) have been implicated in the precipitation of heavy metals in the environment (Scheidegger *et al.,* 1998). Heavy metals that are adsorbed in residual fraction may be a long-term source of contamination since they could be released if there are changes in the redox status of the soil (Wassay *et al.,* 2001; Kim and Kim, 2010).

The organic fraction contains an element that is bound to the organic materials. The element is released in solution as a result of oxidation and decomposition of organic matter and sulphides when there is a change in the physico-chemical conditions in soil (Tessier *et al*., 1979: Fedotov and Spivakov, 2008). Copper becomes unavailable for plant uptake when it is found occluded in stable organic complexes (Shorrocks and Alloway, 1985). In soils, copper is largely found in the soil minerals or occluded to organic matter (McLaren and Crawford, 1973a; McLaren and Crawford, 1973b; McGrath, 1996; Mehra *et al.* 1999; Kabata-Pendias and Pendias, 2001). Copper has been found to be highly adsorbed on organic matter and consequently soils with high organic matter such as peats have been found to exhibit Cu deficiencies (Shorrocks and Alloway, 1985). Osu *et al. (*2013) found that different elements were associated with different fractions but Cu was largely found in the organic fraction on automobile workshop dump sites.

Increasing the amount of organic matter in soil has been used as a low cost method for remediation of soil contaminated with heavy metals. Uwumarongie-Ilori *et al.* (2012) showed that addition of cow dung to soil increased the Organic matter in soil, which resulted in immobilization of Pb and Cr in soil contaminated by these metals. They observed that this immobilization largely affected the water extractable fractions of Pb and Cr in soil resulting in low uptake of these metals by maize plants that were grown for 2 months in the green house. Sposito *et al*. (1982) and Iwegbue *et al*. (2007) in different studies also observed that addition of sewage sludge to soil increased the organic fraction of trace metals in soils.

The carbonate fraction comprises elements that are co-precipitated with carbonates. Ramos *et al.* (1994) observed that sequestration of heavy metals in the carbonate fraction is an important mechanism in the mobility and availability of heavy metals in the environment. The elements that are precipitated in the carbonate fraction are released into water phase when there is a change from neutral (pH~7) to acid phase (pH~5) (Fedotov and Spivakov, 2008). The pH has also very strong influence on the availability of Cu to plants. Under alkaline conditions Cu deficiencies are more pronounced (Shorrocks and Alloway, 1985). Some studies (Doner and Lynn, 1989; Lambert *et al.*, 1997) have reported that carbonates are only stable in soils with high pH values.

The Residual (or sulphide) fraction is one which is fixed to the primary or secondary minerals in mineral lattices, which is not released into water phase under normal conditions in the environment (Xian, 1989; Broekaert *et al.,* 1990). The destruction of primary and secondary mineral lattices releases heavy metals which have been held within the soil fractions as a result of isormophous substitution. This will release the ‘residual’ forms.

Given the chemical basis for metal absorption, it is clear that soil type is a fundamental determinant of heavy metal transport and fate. The information about the physico-chemical forms of heavy metals is important to understanding the mobility, pathways, bioavailability Cu and Co in soils. This kind of information provides more information on the susceptibility of crops to contamination from the heavy metals in that soil. In addition, adsorption of heavy metals on soil fractions provides a temporally removal of the elements away from the crops if the source of pollutants are in irrigation water. Higher amounts of clays with high adsorption capacity and organic matter would be beneficial in the short run to adsorb heavy metals in solution that would contaminate plants.

2.10 Methods of fractionation of heavy metals in soils

The common method of quantifying contamination of soils is by determining the total content of element in soil. The total content of an element in soil is not the best method as it does not give the best reflection of biological availability for plant uptake (Broekaert *et al.,* 1990; Fedotov and Spivakov, 2008). The toxicity of heavy metal elements in the soil and their uptake by plants is dependent upon their mobility, form, degree of transformation and bioavailability (Broekaert *et al.,* 1990; Basta *et al.,* 2005; Nolan *et al.,* 2005).

During fractionation, operationally defined forms can be extracted by simulation of natural conditions, whereby metals associated with soils can be released. For example 'exchangeable forms' or 'acid soluble forms' can be released by changing the ionic composition where some ions will replace others on the exchangeable complex (adsorption- desorption) or change the pH where a decrease in pH results in some elements being more mobile (released) in solution

It is with this background that analytical methods have been developed to quantify chemical and binding form of elements in soils that can be used to correlate to their availability for plant uptake and mobility in soils. Fractionation does not separate pure chemical compounds but their chemical combinations (Fedotov and Spivakov, 2008) such that each chemical leachant used will most likely influence a group of compounds with similar properties.

Sequential extraction of elements from soil is a form of fractionation of elements (Templeton *et al.,* 2000; Fedotov and Spivakov, 2008). Sequential extraction methods have been commonly used for studying metal mobility and availability in contaminated soils.

Different sequential extraction methods can isolate or separate different fractions from soils (Table 2.2). Numerous extraction methods for soils have been described in literature (McLaren and Crawford 1973a; Stover *et al.,* 1976; Tessier *et al.,* 1979; Sposito *et al.,* 1982; Miller and McFee, 1983; Fedotov *et al.,* 2002; Vojtekova *et al.,* 2003). The procedure by McLaren and Crawford (1973a) was developed to isolate four fractions; exchangeable, specifically sorbed, organically bound and oxide bound. The Tessier *et al.* (1979) is the most commonly used procedure which is similar to the McLaren and Crawford (1973a) but uses different reagents and sequence of procedure. The Kersten and Forster (1995) method is based on the Tessier *et al.,* (1979) method but allows one to separate metals bound to the Mn and Fe oxides separately. The Stover *et al.* (1976) was developed to separate metals into exchangeable, sorbed, organic bound, carbonate, and sulphide fractions using KNO_3, KF, $Na_2P_2O_7$, EDTA and HNO_3 respectively as extractants. Sposito *et al.* (1982) method was based on the Stover *et al.* (1976) to separate fractions of trace metals in exchangeable, sorbed, organic bound, carbonate, and sulphide fractions. These trace metal forms are extracted using KNO_3, deionised water, NaOH, Na_2EDTA and HNO_3 for the following forms: exchangeable, sorbed, organic bound, carbonate, and sulphide. The significance and relevance of the different methods is largely dependent upon the use and type of samples.

Fedotov and Spivakov (2000) however, tabulated some limitations in the use of sequential extraction methods. They observed that results are usually dependent on the type of leachant used, the contact time, intensity of shaking of the mixture, ratio of sample weight to reagent volume and specific features of the soils under investigation. The other problems the authors identified included lack of versatility, poor reproducibility of results, the long time required to perform the procedure, metal redistribution among phases during extraction and inconsistency of results under natural and simulated conditions.

Despite the problems related to the selectivity of the various leachants, fractionation methods provide qualitative evidence of the forms of association of trace metals and, indirectly, of their mobility and bioavailability (Harrison, 1981).

2.11 Contamination of food crops by heavy metals in the environment

During their growth, plants require macro and micronutrients. Macro nutrients comprise N, P, K, S, Ca, and Mg and micronutrients include Fe, Zn, Mn, Ni, Cu, Co and Mo. Nutrients are commonly obtained through roots and translocated to various organs of plants using different membrane transporters (Rascio and Navari-Izzob, 2011; Manara, 2012). Uptake and translocation of nutrients are complicated mechanisms that are sensitive to the type and concentration of metal ions (Rascio and, Navari-Izzob, 2011). It has been observed that some membrane transporters have specific roles to transport of divalent cations, but do not recognize mono or trivalent ions (Lasat, 2002; Rascio and, Navari-Izzob, 2011).

Some elements such as Cu, Co, Zn, Mn, and Ni are essential micronutrients. In most plants, concentration of these elements does not exceed 10ppm or just what the plants require. On the other hand, some plants can accumulate very high amounts of metals than what their metabolic needs require (Lasat, 2002). Plants that accumulate high concentrations of contaminants are called hyper-accumulators. Hyper accumulators have been defined by Lasat (2002) as those species that can accumulate more than 100 times more contaminants than typical shoots of accumulator plants. The accumulation of heavy metals by plants can vary according to species of plants (Davis and Carlton-Smith, 1980; Ross and Kaye, 1994; Lasat, 2002; Chove *et al.*, 2006; Singh *et al.*, 2010).

Plants can be contrasted by the nature of their metal uptake: Ross and Kaye (1994) defined three groups of plants by their metal uptake nature: (i) Excluders: restricted uptake of toxic metals, (ii) Index plants: reflect soil metal content and (iii) Accumulators: plants which actively concentrate heavy metals in their tissues. Lasat (2002) added a fourth group called hyper-accumulators. Hyper-accumulators are those plants which have the ability to accumulate large amounts of metals into their tissues without exhibiting signs of toxicity. Lasat (2002) noted that some hyper-accumulators can concentrate up to 1000mg/kg Co in them.

Davis and Carlton-Smith (1980) reported the importance of plant genotype in accumulation of heavy metals from a pot trial in which 39 cultivars of crop plants were grown on two contaminated soils. They found that spinach and cabbage were high accumulators of heavy metals for cadmium and zinc. Research examining the levels of pesticides and heavy metals in Egyptian leafy vegetables and some aromatic medicinal plants found 97% of leafy vegetables contaminated with heavy metals with 37% exceeding maximum limits (Dogheim *et al.*, 2004).

Sharma and Kansal (1986) found leafy vegetables irrigated with contaminated sewerage water accumulated more heavy metals due to their leafy growth. Alam *et al.* (2003) in Bangladesh, observed that vegetables irrigated with contaminated water, accumulated very high amounts of As, Cd, Pb, Cu, and Zn, with Cu and Zn accumulation being greater for leafy plants. Finster *et al.* 2004) found that there was greater accumulation of heavy metals by leafy vegetables than fruit crops from polluted soil of urban gardens in Chicago.

Reasons for leafy vegetables being the greatest accumulators of heavy metals are related to the surface area of the plant’s root, root cation exchange capacity, and the rate of evapotranspiration. A plant with larger root surface will be able to absorb a greater amount of heavy metal ions, and a plant with a large cation exchange capacity will have the potential for greater uptake than a plant with a lower cation exchange capacity.

The evapotranspiration rate influences the movement of ions to the point of absorption (i.e. the root) and evapotranspiration affects soil solution mass flow (Alloway, 1995). Leafy plants have the greatest leaf area and thus higher evapotranspiration rates and potential for higher uptake rates. Therefore, if leafy crops are grown using contaminated irrigation water or on contaminated soil, they may have higher uptake rates and thus higher contamination levels.

2.12 Predicting the uptake of heavy metals in water-soil-plant systems

Predicting uptake of heavy metals in polluted environments presents a number of challenges. The challenges come about as a result of the multitude of influences that affect heavy metal uptake by plants which can include the following: differences among plant species, differences among soil types, difficulties in quantifying pollutant deposition and inadequacies of measuring methods such as total concentration of metals.

However, despite the above listed problems, attempts have been made to predict heavy metal concentrations in polluted environments by using mass balance methods and water- soil-plant-systems to predict contamination.

2.12.1 **Mass balance approaches for heavy metal prediction in plants**

The mass balance method is used to predict pollution by heavy metals in the environment (Moolenaar and Lexmond, 1998). The approach is based on measurement of inputs and outputs from systems for example a field or region. Metal dispersion is predicted for the different compartments within the field or region. Using mass balance methods present challenges as the dynamic nature of the environment changes with seasons (dry and rainy seasons); methods of measuring heavy metals whether total or bioavailable and variable nature of inputs. Different mass balance models are employed for example the dynamic mass-balance models that are used to predict how heavy metal pollutants concentrations change over time. These dynamic models require more detailed information such as current and historic depositions of heavy metals, environmental conditions such as geology and weathering rates. While it is possible to obtain for example inputs from industrial sources for elements like Cu, it is the lack of information on the accepted input data such as crop contamination, soil contamination (on different soils), different qualities of contaminants and interactions between the soil, water and plants that is required.

2.12.2 **Water-Soil-Plant models for heavy metal prediction in plants**

The use of water-soil-plant models for heavy metal prediction in plants is the most common method that has been used to model soil-plant tissue uptake factors in health and ecological risk assessments (Brooks *et al.,* 1977; Nirmal-Kummar, 2009). The uptake factors that Brooks *et al.,* (1977) used are the ratio between the mean concentrations of the element in the plant against the mean concentration of the bioavailable element in the soil. This approach helps to provide a visual trend of mobility of heavy metals from soil to crop plants. The method has largely been used by researchers to model heavy metal transfer in soils where sewage sludge is used as amendments in soil. The uptake of trace elements from soil is influenced by several factors such as the type of crop, pH, soil organic matter, clay type and content, water content and properties of the metal ions (Birley and Lock, 1999; Dube *et al.,* 2001). This implies that the uptake of heavy metals by plants should be explained with respect to soil conditions and plant species for the results to be more meaningful.

2.13 Exposure pathways from irrigation water derived heavy metals

Exposure to heavy metals rarely happens by direct ingestion or drinking contaminated water or eating contaminated soil. Most times heavy metals are ingested from food sources, where human beings or animals consume contaminated crops.

Exposure to heavy metals in the pathways shown in Figure 2.2 is usually limited by various mechanisms that can exclude the metals from reaching both plants and animals. These mechanisms include adsorption on clay, organic matter or competition from other ions (Alloway, 1995; Dube *et al.,* 2001; Naidu *et al.,* 2003; Fedotov *et al.,* 2008). Heavy metals in water that are applied to soil may end up being excluded from reaching the plants by adsorption on clay, organic matter or other metals such as Fe and Mn (Alloway, 1995; Fedotov *et al.,* 2008).

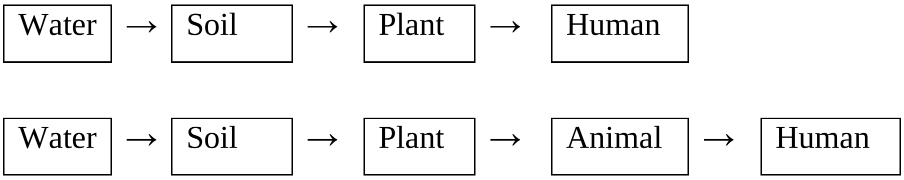


Figure 2.2 Pathways for human exposure to heavy metals from contaminated water.

The uptake of the heavy metals by plants may be excluded as a result of competition from other elements in solutions (Kabata-Pendias and Pendias, 1992; Dube *et al.,* 2001). For example Copper uptake is thought to be inhibited by Zinc, Calcium, Potassium and Ammonium (Graham, 1981), while Zinc uptake is thought to be inhibited by Copper and Hydrogen cations with Iron and Manganese having no effect (Barber, 1995). Plant uptake of heavy metals in solutions can also be influenced by different preferences for heavy metals in soil solution (Lasat, 2002; Nirmal-Kumar *et al.,* 2009) and transfer coefficients. The transfer coefficient of a heavy metal can give an indication of which heavy metals are the most readily taken up by plants and then moved through the plant system. Kloke *et al.* (1994) listed transfer coefficients that included Cu among those with highest coefficients.

Plant uptake can also be influenced by differences in species and the nature of their metal uptake (Brooks *et. al*., 1977; Ross and Kaye, 1994; Lasat, 2002). Plants that are hyper accumulators may accumulate large amounts of metals into their tissues without exhibiting signs of toxicity because they have developed a number of strategies that help them to resist the toxicity of heavy metals. Such strategies include efflux pumps, sequestration in cells and intracellular compartments where metals do least harm, and binding of heavy metals inside the cells by strong ligands like phytochelatins or free amino acids (Cobbett and Goldsbrough, 2002; Küpper and Kroneck, 2005).

Hyper accumulation of heavy metals in metal-rich environments in many parts of the world, serves as a defence mechanism against pathogens and herbivores (Boyd *et al.,* 2002; Hansen *et al.,* 2003; Jhee *et al.,* 2005). The heavy metals that could end up accumulating in plants may reach humans by direct consumption of contaminated crops or through consumption of animals which have eaten crop products that have been contaminated with heavy metals.

The above pathways for exposure of heavy metals provide an opportunity to explain the problems about the spread of heavy metal contamination and information that can be used to find solutions. An example of a problem is if a crop is a hyper accumulator of a heavy metal and does not show any signs of metal toxicity consuming the crop by humans or animals may pose a danger of exposure to high levels of contamination by the metal present.

The information could also give an opportunity to make recommendations on heavy metal loadings that specify the environmental conditions such as type of soil on which contaminated water would be used.

2.14 Use of pot methods to determine metal uptake in soils

The uptake of heavy metals by plants has been studied in either field or in controlled conditions. These two conditions present different challenges and benefits and results on the uptake of heavy metals by crops could vary depending on these conditions. Results from pot studies cannot be extrapolated to field conditions due to different environmental conditions that can influence the results under the two situations (de Vries, 1980). The environmental conditions include (a) size of pots could restrict the volume for root development (b) microclimate conditions in greenhouse are different from field conditions, (c) watering and fertilization regimes may differ between the two method due to small soil volumes in pots and (d) the careful management of pot experiments and their environmental controls will not necessarily replicate conditions in the field where uncertainties are difficult to control like temperatures or interferences from weeds and pests.

In experiments used to measure uptake of elements by plants from soils, it has been found that pot studies tended to give higher levels of contaminants in plants than from field experiments (Kayser *et al*., 2000; Othman, 2001; Schmidt, 2003; Wang, 2008). Kayser *et al*. (2000) in a study to investigate enhancement of phytoextraction of Zn, Cd and Cu from calcareous soil, observed threefold increase in Cd in tobacco (*Nicotiana tabacum* L.) and sunflower and seven fold increase in Indian mustard from pot study than in the field. Schmidt (2003) postulated that higher heavy metal contents in plants grown in pots as compared to field conditions was a result of the plant roots being always in contact with amendments. In addition, the smaller soil volume increases efficiency by the roots as the roots are in contact with amendments. Other studies have however, shown no significant differences between greenhouse and field experiments. McBride *et al.*, (2000) while investigating Mo uptake by forage crops, observed similar elevated uptake for both greenhouse and field conditions where the average for green house was 2.5 mg/kg and for field was 3.0 mg/kg in clover.

The benefits of using pot experiments are (a) they can allow one to test different concentration levels on a smaller area (b) they can simplify measurements of relevant parameters and (c) can be useful in the designing of field experiments (Schmidt, 2003).

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

3.1.1 Irrigation water used in the study

The contaminated water (CW) used in the study was obtained from the Uchi stream in Kitwe at the road bridge (S 12º 50.266, E 028º12.856, Elevation 1220 m). The water is believed to be contaminated with heavy metals because it has effluent from the Nkana Copper Smelter. The Uchi stream is a tributary of the Kafue River. At the time of collecting this contaminated water, farmers used the water from the Uchi stream and Kafue River to irrigate crops during the dry season of the year from April to October. The Nkana Copper Smelter was closed in 2009 and since the effluent is no longer discharged in the Uchi stream. The contaminated water was collected from this station because water samples had been collected from the Uchi stream including crop and soil samples every for 24 months during the time the Smelter was operational.

The contaminated water (CW) was collected in August 2006 and stored in two 210 litre plastic barrels. The 210 litre plastic barrels were washed first washed in acidified water at the University of Zambia, Soil Science Laboratory. In the field, the containers were rinsed three times using the same water from the Uchi stream before filling it. A 10 litre plastic bucket was used to lift the water from the stream and decanted in the water barrels. The water was transported to Lusaka and kept in a store room under room temperature. The temperature, pH and electro-conductivity were recorded at the time of collecting the water. These parameters were read in the field using an Eijkelkamp 18.28 Multi-Parameter Analyser. Sub samples of this water were collected and drops of acid added. The sub samples were collected to be analysed for metal elements.

The rain water (FW) used in the study was collected in February 2007 at University of Zambia. The rain water was stored in two 210 litres plastic barrels. The barrels had been washed using rain water three times before filling.

For the purpose of the study, the contaminated water (CW) was diluted with rain water (FW) so that a gradation of contamination concentrations was created. The water with only rain water (FW) was used as control and the other had increasing levels of contamination. The highest level of contamination was CW 100% where no dilution was done. The following levels of contamination were created:

(a) FW. This treatment comprised only rain water only;

(b) CW 25%. The treatment comprised adding one part contaminated water to three parts of rain water;

(c) CW 50%. The treatment comprised adding one part contaminated water to one part of rain water;

(d) CW 75%. The treatment comprised adding three parts contaminated water to one part of rain water

(e) CW 100. The treatment comprised only contaminated water only.

A number of stations including Uchi stream in Kitwe, Chilumba farm in Mufulira, Kafue, Kaunda Square and Chingwere sewage treatment plant in Lusaka were observed every month for 24 months to observe the crops, soil and waste water used by farmers. The concentrations of Cu and Co in water at the station it was collected was consistently around 26 mg/l and 10 mg/l respectively for these elements especially in the dry periods of the year. Several other stations on the Copperbelt especially near tailing dams had Cu and Co concentrations same even higher than these and farmers also used waste water from these sources. Similar values for these elements are found in various literature like those from the report of Auditor General on the management of environmental degradation caused by mining activities in Zambia (GRZ, 2014) the Copperbelt Environment Management Program (ZCCM, 2003). The concentrations of elements in streams and other water channels has also been observed to decrease away from the source and this has been attributed to dilution from other water sources and adsorption to sediments (Mohiuddin *et al.*, 2010; Sekabira, 2010). Thus the dilution of the contaminated water (CW) to 25, 50 and 75 percent shows the use of this water away from the sources.

The rain water (FW) was control and contained undetected concentrations of Cu, Co and other metal element found in contaminated water. The standard allowed concentrations for discharge for Cu and Co in the environment in Zambia are 1.5 and 1.0 mg/l (GRZ, 1993). The highest concentrations used was the same as reported from mine discharges and the lowest was just about 3 times the allowed limits for Cu and Co in waste water. The Control treatment (FW) had undetected amounts of Cu and Co thus well below the allowed waste water discharge allowed for Cu and Co, including the other contaminants.

These five irrigation water types were stored separately for use in the green house experiment. The water containers were kept at room temperature for the duration of the study. Subsamples of each of the above solutions was analysed for the following parameters: pH, total acidity, electro conductivity, sulphates, chlorides and elements including Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn.

3.1.2 Soils used in the study

Two soil types were selected for this study, a sandy loam and clay loam soil. These two soil types were selected because they had different textural characteristics where one has higher clay and organic matter (clay loam) content than the other (sandy loam). The soils had similar physical characteristics to the ones farmers in peri urban areas especially around Lusaka grew crops and irrigated with waste water. The soils were selected away from the areas where waste water was used as they did not have contamination from this type of water.

The sandy loam soil was collected from Liempe Farm which is located about 18 km along the Great East road, to the east of Lusaka. The latitude and longitude position for the site the soil was collected was: S15º 15’ 15.4” and E 023º 08’ 25.8” respectively. The soil was collected 400 m from the Great East road, on the part of the farm that was fallow. The soil on this part of Liempe farm is classified as Choma soil series (Fine loamy kandiustalf) (Veldkamp, 1984). The soil was collected from the surface between 0 and 15 cm.

The clay loam soil was collected from the Great East Road campus. The latitude and longitude position for the site the soil was collected was: S15º 22’ 47.9”, E 028º 27’ 51.8”. The soil was collected from grassland that floods seasonally. The soil is classified as Kafue soil series (Ustic paleustert, clayey, montimorillonitic isohyperthermic) (Veldkamp, 1984). The soil was collected from the surface between 0 and 15 cm.

The soils were collected from the surface down to a depth of 10 cm. The vegetation was first removed from the surface using a spade. Thereafter, a hoe was used to dig the soil and then bulked in a polythene sack.

3.1.3 Crops used in the study

The crops grown in this study were Pumpkin (*Cucurbita maxima*) and Chinese cabbage (*Brassica oleracea var chinensis*). These two crops are widely grown by farmers in peri urban areas and reported commonly in studies where waste water use and soil pollution studies have been done (Chisanga and Silembo, 2004; Chishala *et al.*, 2006; Holden *et al.*, 2009; Holden *et al.*, 2010; Kapungwe 2013; Kapungwe, 2014; Siame *et al*., 2016; Chapoto *et al*., 2017; Kayika *et al*., 2017). A local variety of pumpkin which is grown for its edible leaves and fruits were selected as these are the ones farmers grow in their gardens in the dry season. The pumpkin seeds were extracted, washed and dried in the sun for 5 days. For Chinese cabbage, the variety used was Sjinese Kool (Chihilli). The Chinese cabbage seed was produced by Mayford Seed Company of South Africa.

3.2 **Methods**

3.2.1 **Chemical characteristics of water**

This involved the analysis of water for the following parameters: pH, Electrical Conductivity, cations, sulphates, and chlorides.

3.2.1.1 **pH of water**

A sub sample of the water was collected in a 100 ml beaker. This water was then filtered using Whatman® No. 125 filter paper. The pH was measured using a Radiometer PHM82 standard pH meter. Standard buffer solutions of pH 7 and 4 were used to calibrate the pH meter.

3.2.1.2 **Total acidity of water**

The total acidity of water was determined by a method that was described by Gupta and Varshaney (1989).

A 100 ml aliquot of sample water was transferred into a conical flask. Then 5 drops phenolphthalein solution was added to the water in the conical flask. Standard 0.1 M NaOH was then used to titrate to a permanent pink end point. The total acidity in water was calculated using equation 1.

$$\text{Total acidity in meq/l} = \text{ml NaOH} \times 0.1 \qquad (1)$$

3.2.1.3 Electrical Conductivity (EC) of water

The Electrical Conductivity (EC) of water was determined by a method that was described by van Ranst *et al.* (1999).

A sub sample of water was collected in a 100 ml beaker. The water was then filtered using Whatman® No. 125 filter paper. The EC was measured using a WTW D8120 LBR 40 (Wissenschaftlich Technische Werkstatten D8120 Wellheim) EC analyzer. Temperature corrections were made after every five samples read.

3.2.1.4 Cations in water

A sub sample of the water was collected in a 100 ml beaker. The water was then filtered using Whatman® No. 125 filter paper. After filtration, the cation concentrations were read on the Atomic Absorption Spectrophotometer (Perkin Elmer AA Analyst 400). The AAS was calibrated using standards for each element that are made in distilled water. Each element was then read using a specific lamp.

3.2.1.5 Sulphates in water

The procedure to measure sulphates in water was described in detail by Gupta and Varshaney (1989). The determination of sulphates in water was preceded by first testing whether there were sulphates in the water. This was done by first collecting 10 ml of water sample. To the water sample, was added drop by drop concentrated HCl and a few drops of $BaCl_2$. Appearance of white precipitate indicated presence of sulphate.

The procedure used to determine sulphates involved pipetting 3 ml of sample into a test tube. This was followed by adding 3 ml of Barium Gum solution. The suspension was then shaken for 1 minute. The absorbance of the suspension was read on a Genesys20 thermoSpectronic® spectrophotometer. The spectrophotometer was set at a wavelength of 430nm to read the sulphate absorbance.

3.2.1.6 **Chlorides in water**

The Mohr method was used to determine chlorides in water. The procedure used was described by Gupta and Varshaney (1989).

The determination of chlorides in water was preceded by first testing whether there were chlorides in the water. This was done by first collecting 5 ml of water sample. To the water sample were added 2-3 drops of dilute HNO_3 and 5-6 drops of $AgNO_3$. Appearance of white precipitate indicated presence of chlorides.

The procedure involved the following steps: Pipetting 25 ml of sample into a conical flask. This was followed by adding 8 to 10 drops of potassium chromate indicator solution. The next step involved adding standard 0.05N $AgNO_3$ (silver nitrate) from a burette slowly while shaking the flask constantly until the red colour formed by addition of each drop by drop begins to disappear more slowly. The titration with silver nitrate continued drop by drop until a faint reddish brown colour was obtained. The titration was repeated until 2 concordant readings differing by 0.1 ml were obtained. The quantity of chlorides in the water was calculated using equation 2

$$meq/l\ Cl = (Titre - Blank)\frac{0.05X1000}{Volume\ taken} \qquad (2)$$

3.2.2 **Soil physical and chemical parameters**

The soil was characterized in terms of its physical and chemical parameters. The parameters analyzed included: particle size composition (texture), pH, cation exchange capacity, concentration of exchangeable trace elements, and organic matter content.

3.2.2.1 Texture of the soils

The soil texture was analysed using the hydrometer method. The method has been described by Anderson and Ingram (1993). The hydrometer method was selected because it is rapid and easier to carry out. The procedure involved the following steps:

First, the moisture content of the soil was measured by using the gravimetric method. This was done by first weighing a soil sample before drying to obtain w_1. This was followed by putting the soil sample in an oven at 60°C until a constant weight, w_2, was obtained. The moisture percent was calculated using equation 3.

$$Moisture\,content\,\% = \frac{w1 - w2}{w1}100 \qquad (3)$$

After moisture determination, the soil was sieved on a 2 mm mesh and then 50 g was collected and placed in a 1000 ml of sedimentation cylinder. Calgon was used dispensing agent. A plunger was used to mix the contents thoroughly. The amount of silt and clay was measured by taking the hydrometer reading and temperature of the suspension 40 seconds after mixing. The clay content of the sample was obtained by the hydrometer reading carried out after 2 hours. The correction factor of the dispensing agent was determined by adding 50 cm^3 of dispensing agent to a clean sedimentation cylinder, making it up to 1 dm^3 with distilled water and taking a hydrometer reading. The temperature corrections for the 40 second and 2 hours readings were calculated by adding or subtracting 0.4 units for every degree above or below 20°C. The particle size distribution was calculated using equations 4 to 7.

% (Silt + Clay) = (40 second reading – Dispensing agent correction factor ± 40 second temperature correction factor) X 100/50 (4)

% Clay = (2 hours reading – Dispensing agent correction factor ± 2 hours temperature correction factor) X 100/50 (5)

% Sand = 100% - %(Silt + clay) 6)

% Silt = 100 - %Sand - % Clay (7)

The textural name of the soil is determined by using the textural triangle.

3.2.2.2 **pH of the soils**

The procedure for analysing soil pH was described by van Ranst *et al*. (1999). The method involved air drying the soil and then taking a 10g sample and placing it in 25 ml of 0.01M $CaCl_2$ solution. The mixture was stirred using a glass rod. The pH was measured after 15 minutes using a Radiometer PHM82 standard pH meter that has a single glass and calomel electrode

3.2.2.3 **Exchangeable acidity of the soils**

The procedure for analysing exchangeable acidity involved the extraction of the H^+ and Al^{3+} that is adsorbed to the soil using 1M KCl and then titrating with a base (0.01 M NaOH) until a permanent pink end point. This procedure is described in detail by Ryan *et al.,* (2001) and van Ranst *et al.,* (1999).

The amount of base used was equivalent to the exchangeable acidity in the aliquot taken.

To measure the amount of the H^+ ions, sodium fluoride (NaF) solution was added to the filtrate collected in the procedure described above. The solution was titrated 0.01 M HCl until pink colour disappeared. The miliequivalent of acid used in the titration was equivalent to the exchangeable H^+.

The amount of Al^{3+} was calculated by the difference between total exchangeable acidity and the exchangeable H^+.

3.2.2.4 **Organic matter content in the soils**

The Walkely and Black method was used to determine the C content in soil. The method used was described by Anderson and Ingram (1993). The method uses the reduction of Cr^{+6} ions by organic matter in sulphuric acid medium. The procedure involved adding 1N $K_2Cr_2O_7$ and concentrated H_2SO_4 to a sample of soil. After shaking and settling, phosphoric acid (H_3PO_4) was added and then excess dichromate was titrated with iron (II) sulphate (Fe (II) $SO_4.7H_2O$) solution. A blank titration (without soil) was carried out throughout the procedure to standardize the Cr_2O_7 solution.

The amount of organic matter was calculated by multiplying the organic carbon portion by two because it is assumed that organic matter consists of 50% organic carbon.

3.2.2.5 **Total cations in the soils**

The method described here was used to analyse the total content of heavy metals in soil. The method has been described by van Ranst *et al.,* (1999). The total content of HM metals therefore gives a higher value as it includes forms that are not soluble and available for plant uptake and assimilation. Air dry soil was sieved on a 2 mm mesh and roots, gravel, stones and other materials that remain on the sieve were discarded. A 1 g of sub sample was collected and placed in a 250 ml flask. A solution of *Aqua regia* (1:3 HNO_3: HCl) was used as extracting solution. The mixture was digested on a hot plate. The digest was filtered and filtrate was then taken for reading on a Perkin Elmer AA Analyst 400 Atomic Absorption Spectrophotometer (AAS).

The AAS is calibrated using standards for each element that are made in *Aqua regia*. A blank sample and standard reference sample (IAEA Soil 7) were also read. The value of the blank was used in correcting the readings of the samples.

3.2.2.6 **Plant available (exchangeable) cations in soil**

The method described here was used to analyse the plant available content of heavy metals in soil. The method was described by van Ranst *et al.,* (1999). The plant available content of HM metals therefore gives a lower value as it includes only forms that are soluble and available for plant uptake and assimilation.

Air dry soil was sieved on a 2 mm mesh and roots, gravel, stones and other materials that remain on the sieve were discarded. A 10 g sub sample of the soil was placed in a 250 ml Erlenmeyer flask. Twenty millilitres of Diethylene triamine Penta Acetic Acid (DTPA) was added to the soil as extracting solution. The mixture was shaken for 2 hours on a mechanical shaker. After shaking, the mixture was filtered using

Whatman® 1001 125 filter paper. The filtrate was then taken for reading on a Perkin Elmer AA Analyst 400 Atomic Absorption Spectrophotometer (AAS).

The AAS is calibrated using standards for each element that are made in DTPA. A blank sample and standard reference sample (IAEA Soil 7) were also read. The value of the blank was used in correcting the readings of the samples.

The Cation Exchange Capacity (CEC) of the soils was calculated by summation of the measured bases in soil (Warncke, and Brown, 1998; Sumner and Miller, 1996). The bases used in the summation to calculate CEC included Ca, Mg, K, Na, H and Al (CEC = $Ca^{2+} + Mg^{2+} + K^{+} + Na^{+} + H^{+} + Al^{3+}$).

3.2.2.7 **The soil fractionation experimental procedure**

The separation of Cu and Co and fractions in the soils was carried out using the sequential extraction technique. The sequential extraction technique that was used was described by Sposito *et al.,* (1982). They used this procedure to estimate heavy metals on soil amended with anaerobically digested sludge. The estimated metals in the following fractions: exchangeable, sorbed, organic, carbonate and sulphide forms. The modification only involved shaking time and used to measure five different fractions of Cu and Co in soils from the green house study. The five different fractions of Cu and Co evaluated comprised the following: exchangeable, water soluble, organic, carbonate and the residual forms.

There is generally no agreed procedure in soil fractionation. The number of steps can vary from three (Silviera and Somers, 1977) to nine (Krishnamurti and Naidu, 2002). The Sposito *et al.* (1982) procedure was selected because it was better suited to the analytical methods and equipment that are found at the University of Zambia Soils laboratory where the study was conducted. In the Sposito *et al.* (1982) procedure, the soluble component was combined with exchangeable component. It has been recommended (Hass and Fine, 2010) that the water soluble should be separated from the exchangeable component. For the soluble component, water is a more appropriate extractant than salt solutions. Use of salt solutions can lead to coagulation and precipitation of organo-metal complexes, leading to underestimation of soluble component. The study used contaminated water without sludge in it. It was thus decided to look at the soluble component separately than combined with the exchangeable component. In sequential extraction procedures, the subsequent stages use extractants that are more aggressive than the previous. Therefore, using water for the soluble fraction may not have influenced the next phase which is organic, that requires NaOH as extractant.

Shaking time is important in extraction experiments as it allows complete dissociation of the phase of interest. Twelve hours shaking time was selected because earlier test runs on shaking time for the samples did not show differences in the results between 16 hours and 12 hours shaking.

In recent years there appears to be more widespread use of the BCR (Community Bureau of Reference) procedure because it has been applied in different kinds of samples including mine spills (Pueyo *et al.*, 2008). However, the procedure could not be used due to non availability of the required reagents for its application. The Silveira *et al.* (2006) and Tessier *et al.* (1979) procedures have been found to give better results of the fractions with sludge amended samples. The samples in this study were not amended with sludge but waste water from mineral processing.

The experiment involved determining total metal fractions followed by sequential extraction procedure.

(a). Determination of total metal fractions

Two grams of air dry soil samples were placed in a 50 ml flask. To the soil 12.5 ml of 4 M HNO_3 solution was added and shaken briefly. The suspension was then placed on a hot plate for digestion at 80 ºC for 12 hours. After digesting, the solution was cooled down and then filtered with Double ring filter paper No. 102. The extract was collected in a 50 ml flask. The Cu and Co in the extract was read on the Atomic Absorption Spectrophotometer. Standards for calibrating the Atomic Absorption Spectrophotometer were made in a background of HNO_3. Triplicate soil samples were used for this part of the experiment.

(b). The sequential extraction procedure

The sequential extraction involved the following steps. A 2 g sample of air dry soil was collected and then placed in a 50 ml flask. This was followed by weighing 25 g of the following reagents: 0.5 M KNO_3, Deionised water, 0.5 M NaOH, 0.05 Na_2 EDTA, and 4 M HNO_3. The deionization the water was done on an Aquarius GX-50 distillation and deionising machine at the Chemistry Department at University of Zambia.

The extraction was carried out in the sequence as illustrated in Table 3.1 and explained in the sections below. Standard solutions for Cu and Co were prepared for each of the extraction steps in a background solution of each extraction reagent. Triplicate soil samples were used for this part of the experiment.

Table 3. 1 Sequential extraction procedure.

Step	Extractant	Time (hours)	Temperature	Fraction
1	0.5 M KNO_3	12	Room	Exchangeable
2	Deionised water	6	Room	Water soluble
3	0.5 M NaOH,	12	Room	Organic
4	0.05 M Na_2 EDTA	6	Room	Carbonate
5	4 M HNO_3	12	80 ºC	Residual

Step 1. Determination of exchangeable Cu and Co fractions in the soils

Two grams of air dry soil was placed in a 50 ml flask. Then 25 g of 0.5 M KNO_3 was added to the flask. The suspension was then placed on the shaker and shaken for 12 hours. The suspension was then left for overnight to settle down and the supernatant solution was decanted. The decanted supernatant solution was filtered using Double ring No. 102 medium filter paper and collected in a 50 ml flask. The remaining suspension was washed with deionised and the excess water decanted. The weight of remaining solution was measured (in grams) and recorded. The extracted solution was read on the AAS for Cu and Co. Standard solutions for Cu and Co were prepared in a solution of KNO_3.

Step 2. Determination of water soluble Cu and Co fractions in the soils

To the sample that had remained from the above (Step 1), 25 g deionised water was added. The suspension was placed on the shaker and shaken for 6 hours. The suspension was then left for overnight to settle down and the supernatant solution was decanted. The decanted supernatant solution was filtered using Double ring No. 102 medium filter paper and collected in a 50 ml flask. The weight of remaining solution was measured (in grams) and recorded. The procedure was repeated two more times making a total of 3 extractions. The extracted solutions were read on the AAS for Cu and Co. Standard solutions for Cu and Co were prepared in a solution of water.

Step 3. Determination of organic Cu and Co fractions in the soils

To the sample that had remained from step 2, twenty five grams of 0.5 M NaOH solution was added. The suspension was placed on the shaker and shaken for 12 hours. The suspension was then left for overnight to settle down and the supernatant solution was decanted. The decanted supernatant solution was filtered using Double ring No. 102 medium filter paper and collected in a 50 ml flask. The remaining suspension was washed with deionised and the excess water decanted. The weight of remaining solution was measured (in grams) and recorded. The extracted solution was read on the AAS for Cu and Co. Standard solutions for Cu and Co were prepared in a solution of NaOH.

Step 4. Determination of carbonate Cu and Co fractions in the soils

To the sample that had remained from step 3, twenty five grams of 0.05 M Na_2 EDTA solution was added, and the suspension was placed on the shaker and shaken for 12 hours. The suspension was then left for overnight to settle down and the supernatant solution was decanted. The decanted supernatant solution was filtered using Double ring No. 102 medium filter paper and collected in a 50 ml flask. The remaining suspension was washed with deionised and the excess water decanted. The weight of remaining solution was measured (in grams) and recorded. The extracted solution was read on the AAS for Cu and Co. Standard solutions for Cu and Co were prepared in a solution of Na_2 EDTA.

Step 5. Determination of residual Cu and Co fractions in the soils

To the sample that has remained from step 4, twenty five grams of 4 M HNO_3 solution was added. The suspension was placed on the shaker and shaken for 12 hours. The suspension was then left for overnight to settle down and the supernatant solution was decanted. The decanted supernatant solution was filtered using Double ring No. 102 medium filter paper and collected in a 50 ml flask. The weight of remaining solution was measured (in grams) and recorded. The extracted solution was read on the AAS for Cu and Co. Standard solutions for Cu and Co were prepared in a solution of HNO_3.

The amount of metal extracted by a given extracting reagent was calculated using equation 8 (below) that was recommended by Sposito *et al.,* (1982).

$$\mu g\, extracted = C\ x\ 25g - C'\ x\ M \qquad (8)$$

In the equation 1, C is concentration in (µg/g) of metal in solution

C´ is concentration in (µg/g) of metal in the preceding step of sequence.

M is mass of solution (g) carried over to the present extraction from the preceding one.

Equation 8 contains a correction for the amount of metal in the solution entrained in the soil sample after shaking which follows each extraction. This correction is made in lieu of washing the sample with deionised water after shaking and decanting, as suggested by Stover *et al.,* (1976) because washing may lead to metal loses and therefore low recovery of metal from the soil in a given step.

3.2.3 **Determination of Cu and Co in plant samples**

To determine the Cu and Co in plants, a wet destruction method was used. This method mineralizes the elements into solution and then the elements are determined by Atomic Absorption Spectrophotometer (AAS). The method used was described by Cottenie *et al.,* (1982).

A1.0 g of plant material was placed in a 250 ml conical flask. Then 25 ml of concentrated Nitric acid (HNO_3) was added to the flask with plant material. The flask was then placed on a hot plate to digest the plant material. The digestion was allowed to go on until all the organic matter had been destroyed but not allowing the solution to dry up.

When the digestion was finished, the solution was cooled. Then, 10 ml of distilled water was added, followed by 10 ml of Percloric acid ($HClO_3$). The digestion was repeated on a hot plate until the solution was clear or when white fumes started coming from the digest.

The solution was again cooled. After cooling, 25 ml of distilled water was added to the solution and then the boiling was repeated on a hot plate for 30 minutes. The flask was removed from the hot plate and allowed to cool. The cooled solution was filtered using a Double ring No. 102 medium filter paper into a 50 ml volumetric flask. The filtrate was then made up to 50 ml with distilled water.

The filtrate was again transferred to a 100 ml plastic container and then taken to the AAS machine for reading. The AAS used in the soil sciences laboratory was a Perkin Elmer AA Analyst 400. The AAS was calibrated using standards for each element that were made in 5% nitric acid. Each element was then read using a specific lamp e.g. Cu, and Co have specific lamps for their analysis. For samples reading higher than the highest standard, a dilution was done and 5% Nitric acid was used to bring to volume. A blank sample was also read and the value of the blank was used in correcting the readings of the samples. The machine was recalibrated after reading 20 samples. After the concentrations of the samples had been read on AAS, calculations were then made for the elements in the original sample.

3.2.4 Experimental designs

3.2.4.1 Design of the pot experiment

The experiment used a Randomized Block Design with three replicates. The treatments comprised two crops (namely Pumpkin and Chinese cabbage), two soil types (sandy loam and clay loam) and five types of contaminated water differentiated by concentration of contamination of the water. The experiment had 20 treatment combinations in a factorial arrangement. A coding system was used for the treatments as shown in Table 3.2. The treatment combination with code 124 would mean that it is Pumpkin, planted on clay loam soil and irrigated with CW 75% water.

Table 3. 2 The numbering system of treatments used in the greenhouse experiment

Number	Crop	Soil	Water
1	Pumpkin	Sandy loam	FW
2	Chinese cabbage	Clay loam	CW 25%
3	X	X	CW 50%
4	X	X	CW 75%
5	X	X	CW 100%

In the greenhouse, the treatments were arranged as shown in Figure 3.1 The treatments were randomly arranged and the randomization was generated using Genstat3® statistical program.

Replicate1		Replicate 2		Replicate 3	
125	211	123	121	214	112
112	122	212	115	124	125
214	213	111	221	213	114
114	225	211	124	122	225
115	111	223	213	211	111
212	121	215	125	212	123
221	224	222	224	223	121
124	223	214	112	113	222
113	123	225	114	115	224
222	215	122	113	221	215

→ N

Figure 3. 1 A random arrangement of experimental treatments in the green house

3.2.4.2 Design of the fractionation experiment

Composite soil samples from the treatments which comprised soil types with same water for irrigation were collected and bulked. The bulked soil sampled weighed about 1000 g. The bulked soil samples were then divided into three sub samples which represented the replications. The soils then comprised the following soil types and treatments: (a) sandy loam soil with five water types (FW, CW25%, CW50%, CW75% and CW100%) (b) Clay loam soil with five water types (FW, CW25%, CW50%, CW75% and CW100%). (c) Initial sandy loam soil that was not irrigated with any of the contaminated or fresh water. (d) Initial clay loam soil that was not irrigated with any of the contaminated or fresh water.

3.2.5 Experimental management

The experimental management involved soil preparation, planting of the seeds and thinning them. It also included the regular observations for pests, diseases, deformities, and discolorations. Other activities involved regular irrigation, and harvesting plants and soil sample collection at the end of the experiment.

3.2.5.1 The pots used in the experiment

The pots used for this study were made of plastic and had a volume of 3 litres. The pots were perforated at the bottom with two 4 mm holes. The holes at the bottom were made to drain any excess water that could have accumulated at the bottom and also to aid in aeration of the soil. A plastic plate was placed at the bottom to collect any possible leachate from the pot.

The pots were filled with soil up to the top. The pot contained 3.0 kg of dry soil when filled to the brim. The pot had a mouth with a diameter of 17 cm and thus exposing 230 cm^2 of soil surface.

3.2.5.2 Soil treatment for pot experiment

Before placing the soil each pot, it was sieved on a 0.5 mm mesh and then. 2 grams of D’ compound fertilizer (N:P:K = 10:20:10) was added and mixed. The soil was then packed into each pot that had been labelled with the treatment which was to be used. All pots were be filled with 3 kg of either sandy loam or clay loam soil.

3.2.5.3 **Planting of seeds and thinning of seedlings**

The seeds were planted in the pots directly. For pumpkin, 8 seeds were planted per pot and later thinned to four. Fifteen to twenty seeds of Chinese cabbage were initially planted in each pot and latter thinned to 4 plants per pot. The idea of leaving 4 plants in each pot was to reduce competition among the plants in pot and also to ensure that mortality did not affect the final number of plants at harvest. In order to reduce competition of plants in the pots, the seedlings were thinned on two occasions. The first thinning reduced the plants that had germinated to about 50%. At the second thinning, four plants were left in each pot.

3.2.5.4 **Irrigation of plants in the pots**

The crops in the experiment were irrigated every day. Each pot was irrigated with the type of water according to the experimental design. To prevent the mix up of water types during irrigation, all water holding containers were labelled and a colour coded sticker was glued to it (Figure 3.2). Each water type had its own specific cup for applying water and had a coloured sticker similar to that on the holding container.

FW CW 25% CW 50%

CW 75% CW 100%

Figure 3. 2 Water holding containers and cups for irrigating the pot experiment

Each pot also had a same colour sticker to that on the holding container and the watering cup. One water type was applied to the experiment at a time until all necessary pots were irrigated before moving to the other water type. In addition, the water was applied to the soil surface and not the plant in order to prevent the leaves from being contaminated by the irrigation water. A plate was placed at the bottom of every pot to collect water that would have drained in case excess was added.

All water applications were recorded so that the total amount of water applied up to the time of harvesting was known. The watering of the pots was started from the day the seeds were sown. On the first two days after planting, the amount of water applied was 350 ml and thereafter reduced to 175 ml. The amount of water being applied was 80 % of the soils field capacity. The watering was done every day for the duration of the experiment. Appendix 8.1shows the watering days and amounts applied during the course of the experiment. The last watering was done a day before the plants were harvested from the experiment. At the end of the experiment, each pot had received 8575 ml of water. The amounts of water and days applied are presented in Appendix 8.1.

3.2.5.5 **Environmental conditions in the green house during the study**

The greenhouse is located at latitude 15º23039.2” S, longitude 28º20003.9” E, and altitude of 1266 m above sea level. The glasshouse covers an area of 47.3 m^2 and ventilated using window openings on the roof. No external temperature or humidity measures were used in the greenhouse to change the climate effects. Daily dry and wet bulb thermometer readings inside the greenhouse were recorded each morning for the duration of the study. The mean dry bulb readings were 29.6 °C (±2.1 °C) and the wet bulb readings were 19.6 °C (±2.3 °C). The mean relative humidity during the period of study was 55.1% (± 2.1%). The mean maximum and minimum temperatures obtained from the meteorological station at the University of Zambia during the study period were 23.1 °C (±1.7 ºC) and 11.2 ºC (±1.0 ºC) respectively.

3.2.5.6 **Pest and disease management**

Plants were checked regularly for signs of pest presence. There were no pest or disease attacks on the crops during the duration of the experiment.

3.2.5.7 **Harvesting and treatment of plant samples**

The plants were harvested from the experiment after growing for 57 days from planting. Chinese cabbage and pumpkin do not necessarily have similar growth patterns. The decision to harvest at this date was based on the leaf sizes and that harvesting on the same date means that both crops received the same amount of irrigation water. If the crops were harvested on different dates, they would have been irrigated with different amounts of water.

The pumpkin leaf sizes in control treatments (FW) were about 15 to 22 cm in length and 6 to 8 cm in width. Chinese cabbage leaf sizes in control treatments (FW) were about 20 to 26 cm in length and 9 to 11 cm in width. The crops were harvested at this stage because pumpkin leaves and Chinese cabbage of this size have been found to be sold in local markets. The plants were harvested in two parts; above ground and below ground parts. The above ground plant parts were harvested by cutting the plant with a surgical blade at the ground level. All the four plants from each pot were bulked and placed in a paper bag to constitute a sample.

Both above and below ground plant parts were washed before drying. Before the plants parts were taken for drying, they were cleaned on by removing soil particles and other foreign matter that was attached to them. The cleaning was done using rain (FW) water. Running water was poured over the plant samples while shaking gently to remove soil particles and any particles that had to be cleaned. No water was reused during cleaning process. After cleaning, they were then placed in the brown paper bag. Figure 3.3 shows below ground plant sample from the greenhouse experiment.

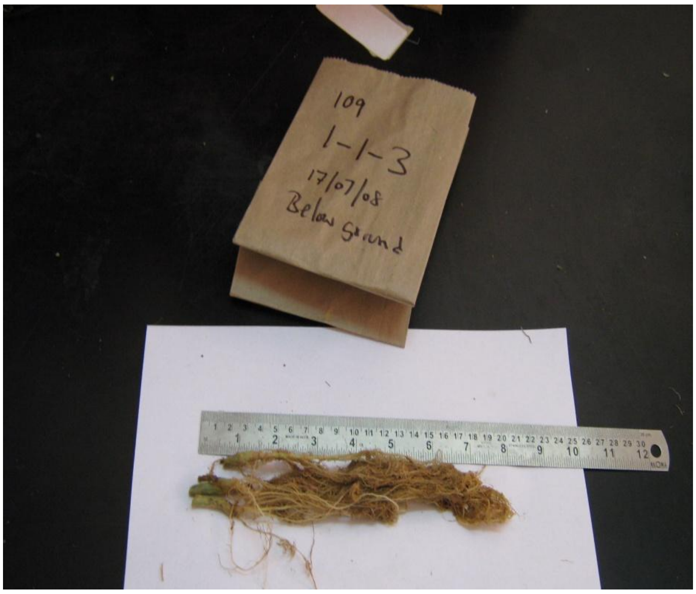


Figure 3. 3 Below ground plant sample before placing in a brown bag for drying.

The plant samples were placed in the oven where the temperature was set at 60 ºC and were left to dry for 24 hours until a constant weight was obtained. Weight of the plant samples were recorded for each treatment.

3.2.5.8 **Collection and treatment of soil samples at the end of the pot experiment**

The soil samples were selected from each treatment. The collection of soil samples from the pots was done after the removal of the roots and below ground stems. The soil that remained was placed in a 20 litre plastic bucket and mixed thoroughly by hand. A subsample of about 500 grams was collected from the main sample that had been earlier mixed and taken for drying in an oven at about 60 ºC for 24 hours. Dried soil samples were stored in a labelled plastic bag until they were analysed for plant available Cu and Co and species of these elements.

3.2.6 **Data analyses**

All the data for the variables for this experiment was obtained in form of quantitative continuous data. All the variables collected comprised random continuous variables. Random continuous variables are observations that can have any of the infinitely many non negative real numbers (such as 0.1, 0.001, 0.25, 2.06 etc) and these tend to vary due to natural variation.

Data analyses were conducted using GenStat Release 7.2 DE, 3rd edition. Statistical analyses were carried out to determine normality of data, means, range and standard errors to the various data sets in the study. Analysis of Variance (ANOVA) and Duncan’s multiple-range test test was used to compare the significance of the differences at 95% confidence level (LSD $p<0.05$). The assumption was that errors were normally and independently distributed with the mean of zero and constant variance.

Correlation Analysis was used to test the strength of association of leaf Cu and Co concentration against yield. Correlation is a method used to measure the degree of linearity of an association between random variables, which have a joint distribution. The statistic that is used for measuring correlation is the product moment correlation coefficient or simply correlation. It is also called Pearson's product moment correlation and is denoted R^2. A simple correlation is used to measure the degree of strength of relationships between any two variables.

The specific areas in which different types of analyses were employed and the use of the outputs are specified in the subsections below.

3.2.6.1 Irrigation water

To analyse the measured data, arithmetic means and standard errors were calculated for pH, total acidity, electrical conductivity, chlorides, sulphates and concentration of cations.

Data analysis of irrigation water comprised analysis of variance (ANOVA) of the pH, total acidity, electrical conductivity, chlorides, sulphates and concentration of cations in the different water treatments (FW, CW25%, CW50%, CW75% and CW100%) to test the significance of differences among these water treatments. Mean concentrations of cations in the irrigation water treatments were compared with maximum allowed concentrations.

3.2.6.2 Initial Soil

The results for particle size analysis were used in the textural triangle for determination of the texture of the two soils. Means for pH, total acidity, electrical conductivity, chlorides, sulphates, organic matter content, cation concentrations were compared between the two soils.

3.2.6.3 Final soil samples

In each group, concentrations of the following key variables were obtained: total, exchangeable, water soluble, organic carbonate and residual forms of Cu and Co.

Measured data on total, exchangeable, water soluble, organic carbonate and Residual forms of Cu and Co were subjected to the analysis of variance (ANOVA). The ANOVA was used to test the significance of differences in Cu and Co extracted amongst the treatments.

3.2.6.4 Plant samples

The variations measured on sampled plants such as yield and metal content in above and below ground plant tissues were subjected to the analysis of variance (ANOVA) to test the significance of differences among the treatments.

Simple regression was used in the study to establish relationships between variables and to estimate some parameters in the regression model. Specifically, in the study, simple regression was used in estimating/predicting the responses of yield to the level of metal content in plants. Regression was also done to estimate metal uptake by crops against different fractions in soils.

CHAPTER 4:RESULTS AND DISCUSSION

4.1 Chemical properties of the irrigation water

The results presented in this section are the chemical properties of the water that was used for irrigating the soils. The parameters analysed included: pH, total acidity, Electrical conductivity (EC), chlorides, sulphates and metal elements. The concentrations in contaminated (CW) and rain water (FW) were compared to recommended standards for irrigation water. It was assumed that these parameters would affect the adsorption of heavy metals in soil and ultimately uptake by plants.

4.1.1 pH of the water

The pH for irrigation water used in the experiment is presented in Table 4.1. The pH value for CW 100% was 2.35. Rain water (FW) had a pH of 6.06. The pH for other water treatments was: 2.47, 2.54 and 2.68 for CW75%, CW50% and CW25% respectively. The results of pH readings indicate a significant difference ($p<0.05$; LSD = 0.20) only with fresh water. Dilution of contaminated water with rainwater did not result in significant differences in pH values among diluted contaminated water treatments.

Table 4.1 Chemical properties of irrigation water.

Water type	Parameter				
	pH	Total Acidity (meq/l)	Electrical conductivity (10^3 µS/cm)	Chlorides (meq/l)	Sulphates (meq/l)
FW	6.06 (0.15)	0 (0.00)	0.00 (0.00)	0.06 (0.03)	0.01 (0.01)
CW (25%)	2.68 (0.05)	0.35 (0.02)	1.00 (0.00)	0.165 (0.03)	1.96 (0.98)
CW (50%)	2.54 (0.03)	0.73 (0.03)	2.30 (0.00)	0.235 (0.07)	5.67 (2.84)
CW (75%)	2.47 (0.01)	1.03 (0.08)	3.30 (0.00)	0.25 (0.10)	8.62 (4.33)
CW (100%)	2.35 (0.03)	1.34 (0.02)	3.70 (0.15)	0.375 (0.09)	16.02 (8.01)
$LSD_{0.05}$	0.20	0.10	2.13	0.21	3.96
CV %	4.0	9.8	6.7	65.1	39.8

The numbers in parenthesis are standard error of means.

The low pH of contaminated water was also caused by the high amounts of dissolved metals. The lack of significant differences among contaminated water treatments could have been due to concentrations of the elements being already in the saturation ranges. For the pH to change significantly, large amounts of rain water than was used were necessary.

4.1.2 **Total acidity of water**

The total acidity in irrigation water is presented in Table 4.1. The total acidity for CW100% was 1.34 meq/l whereas rainwater (FW) was neutral in terms of acidity. Dilution of contaminated water resulted in a decrease in the total acidity. The total acidity values for other water treatments were: 1.03 meq/l for CW75%, 0.73 meq/l for CW50% and 0.35 meq/l for CW25%. The total acidity values were significantly different ($p < 0.05$, $LSD_{0.05} = 0.10$) meq/l among all the treatments. Total acidity of water comprises all titrable acid present in the water sample. Metals dissolved in water contribute to total acidity of the water. The significant differences in total acidity among contaminated water treatments were a result of the dilution that reduced the amounts of dissolved metals per unit volume.

4.1.3 **Electrical Conductivity (EC) of the water**

The electrical conductivity (EC) values for irrigation water are presented in Table 4.1. The EC value for CW100% was 3.7×10^3 µS cm^{-1}while FW had negligible EC. Dilution of contaminated water resulted in a decrease in the EC. The EC values for other water treatments were: 3.3×10^3 µS cm^{-1} for CW75%, 2.3×10^3 µS cm^{-1} CW50% and 1.0×10^3 µS cm^{-1} CW25%. Statistically significant differences ($p<0.05$; $LSD_{0.05} =2.13$) were only observed between FW and water treatments CW50%, CW75% and CW100%.

Salts and other chemicals that dissolve in water breakdown into positively and negatively charged ions. The purity of water can be observed by its EC. In rain water the EC was 0 because there were no dissolved salts and electrically charged ions. As the contaminated water was diluted, the proportion of dissolved elements per unit volume decreased, resulting in decreased EC. The EC values were within the range for irrigation water which is between 0 – 300 x 10^3 µS cm^{-1} as recommended by the FAO (Ayers and Westcot, 1985).

4.1.4 **Chlorides in water**

The concentrations of chlorides in the irrigation water are presented in Table 4.1. The amount of chlorides in CW100% was 0.375 meq/l while FW had 0.09 meq/l. The amounts of chlorides in other water treatments were 0.25 meq/l for CW75%, 0.235 meq/l CW50% and 0.165 meq/l CW25%. The amounts of chlorides in water increased with contamination levels however, significant differences ($p<0.05$; $LSD_{0.05} = 0.21$) were only between FW and CW100%.

High amount of chlorides in water causes foliar burn if the water is deposited on leaves. The contaminated water did not contain high amounts of chlorides because ions come from salts which could not have been a significant component of the pollutants. The amounts of chlorides were below the minimum levels for irrigation water which is 3.0 meq/l and within the usual range of 0 – 30 meq/l as recommended by the FAO (Ayers and Westcot, 1985).

4.1.5 **Sulphates in water**

The amounts of sulphates in irrigation water are presented in Figure 4.1. The amount of sulphates in CW100% was 13.58 meq/l whereas FW had 0.03 meq/l. The amounts of sulphates in other water treatments were 8.72 meq/l for CW75%, 5.96 meq/l for CW50% and 2.0 meq/l for CW25%.

Sulphates in contaminated water in the study were likely to have come from sulphuric acid which is used in the processing of copper ores. Copper ores also may contain sulphur (e.g. chalcopyrite, $CuFeS_2$) which could be released during processing of the metal. Waste effluent from mineral process of Cu is usually neutralized with lime ($CaCO_3$) before being discharged in into the environment. This process results in the concentration of Sulphates in contaminated water. The concentration of sulphates in the CW100%, which was highest, was within the usual range in irrigation water of 0 – 20 meq/l as recommended by the FAO (Ayers and Westcot, 1985).

4.1.6 **Concentration of Cu and Co in water**

The concentrations of copper, cobalt calcium, chromium, iron, potassium, magnesium, manganese, sodium, nickel, lead, and zinc in the irrigation water are presented in Table 4.2.

Table 4.2 also shows maximum permitted limits for selected elements in irrigation water as recommended by the FAO (Ayers and Westcot, 1985). All elements except for Pb indicated highly elevated concentrations in the contaminated water that exceeded maximum permitted levels in irrigation water. The high concentrations of the elements in contaminated water were due to the nature of the materials being processed, for example Cu ores. Other materials besides Cu ores contain the other elements in them. The low pH results in dissolution of most elements hence making their concentration high in this type of contaminated water.

The concentrations of Cu in CW100% were 26.25 mg/l. The concentrations of Cu in other contaminated water treatments were 22.43 mg/l for CW75%, 14.83 mg/l for CW50% and 7.18 mg/l for CW25%. Copper was not detectable in rain water (FW). The amount of cobalt in contaminated water was 10.52 mg/l. The amounts of Cobalt in contaminated water that was diluted were 10.33 mg/l (diluted to 75%), 7.44 mg/l (diluted to 50%) and 4.33 mg/l (diluted to 25%). Cobalt was not detectable in rain water (FW). As the contaminated water was diluted, concentration of the elements was reducing. Similarly, it was observed that as water flows through streams and seepage water is mixed with contaminated water, the latter gets diluted (Idriss and Ahmad, 2012). Some of the heavy metal contaminants also get adsorbed to stream sediments as the water moves along channels.

Table 4.2 Concentration of elements in Rain water (FW) and contaminated water (CW)

Element	Irrigation water type					LSD$_{0.05}$	CV	Limits in irrigation water
	FW	CW 25%	CW 50%	CW 75%	CW 100%			
			mg/l			mg/l	%	mg/l
Ca	nd	11.90 (0.29)	16.46 (5.19)	22.88 (5.02)	26.93 (0.20)	9.43	44.9	1.0
Co	nd	4.33 (0.03)	7.44 (0.02)	10.33 (0.04)	10.52 (0.15)	0.19	1.9	0.05*
Cr	nd	nd	0.05 (0.04)	0.19 (0.04)	0.37 (0.04)	0.06	37.0	0.1*
Cu	nd	7.18 (0.03)	14.83 (0.07)	22.43 (0.17)	26.25 (0.17)	0.35	1.5	0.2*
Fe	nd	2.54 (0.03)	4.97 (0.03)	7.11 (0.03)	7.37 (0.78)	1.08	15.0	5.0*
K	nd	47.91 (0.14)	69.07 (0.25)	84.33 (0.40)	92.08 (0.53)	1.14	1.3	2.0
Mg	nd	1.15 (0.03)	2.55 (0.17)	3.42 (0.05)	3.33 (0.02)	0.23	7.2	0.4
Mn	nd	0.26 (0.01)	0.51 (0.01)	0.76 (0.02)	0.89 (0.01)	0.02	3.3	0.2*
Na	nd	41.73 (0.24)	66.81 (0.47)	88.43 (0.47)	98.90 (0.82)	1.58	1.8	1.7
Ni	nd	0.44 (0.04)	1.04 (0.02)	1.55 (0.04)	1.83 (0.02)	0.05	30.5	0.2*
Pb	nd	nd	nd	0.06 (0.03)	0.29 (0.04)	0.06	54.2	5.0*
Zn	nd	1.55 (0.02)	2.99 (0.23)	5.29 (0.14)	8.46 (2.51)	3.44	51.0	2.0

Numbers in parenthesis are standard errors of means. nd = not detected in samples; *(Ayers and Westcot, 1985).

4.2 Soil properties

The results presented in this section are the physical and chemical properties of the two soils that were used in this study. The properties analysed included: texture, acidity, organic matter, cation exchange and metal elements. The elements were measured in terms of the total and plant available forms. The elements of interest to the study, Cu and Co were also determined in terms of the fractions (species) they were held in soils. It was assumed that these properties would affect the adsorption of Cu and Co in soil and ultimately influence their uptake by plants.

4.2.1 Soil texture

The textural analysis of the soils that were used in the pot experiment is presented in Table 4.3. The clay fraction content of clay loam was apparently more than three times that in sandy loam soil. The silt fraction content was similar in sandy loam and in clay loam soils.

Table 4.3 Textural characteristics of soils

Textural class	Textural fraction			Total
	Sand	Clay	silt	
		%		
Sandy loam	65.65	7.6	26.75	100
Clay loam	46.4	27.6	26.0	100

Clay minerals are usually very fine in size (usually less than 0.002 mm). The importance of clay in soil is that it has large specific surface and predominantly negatively charged which is important to adsorb nutrients and prevent their leaching and reacts with hydrogen and aluminium ions, while buffering the soil against extreme pH changes (Aboudi Mana *et al.*, 2017). The sand fraction is usually composed of minerals which are very resistant to weathering such as quartz and have low specific surface area. Silt is generally similar in mineralogy to the sand fraction except that its size range (0.05 mm to 0.002 mm) is less than that of sand (2.0 mm to 0.05 mm). Thus the clay loam soil in this study has more than 3.6 times more clay than sandy loam soil. If clay retains more ions on its large surface area then the clay loam used in the study should adsorb large amounts of ions from the contaminated water than sandy loam soil.

4.2.2 **Soil acidity**

The soil pH and exchangeable acidity values are presented in Table 4.4. The mean pH of the soils were 5.91 (±0.18) for sandy loam soil and 5.85 (±0.08) for clay loam. The exchangeable acidity for both soils was 2.5 meq/100g. When the source of the acidity was segregated between Al^{3+} and H^+, results show that large part of the exchangeable acidity on both soils was from the latter.

Table 4.4 The pH and exchangeable acidity of the soils

Soil Type	pH ($CaCl_2$)	Exchangeable acidity	Exchangeable Al^{3+}	Exchangeable H^+
			meq/100 g soil	
Sandy loam	5.91 (0.18)	2.5 (0.5)	0.44 (0.08)	2.06 (0.47)
Clay loam	5.85 (0.08)	2.5 (0.5)	0.72 (0.14)	1.78 (0.44)

Numbers in parenthesis are standard errors of means

4.2.3 **Soil Organic Matter Content**

The soils organic matter contents that were determined are given in Table 4.5. The organic matter content in the soils showed that clay loam had more than double the amount than sandy loam. The amount of organic matter in clay loam soil was 4.32 % and in sandy loam it was 2.13%. These values are largely typical for the soils in the subtropical areas like Zambia (Sanchez, 1976; Donovan and Casey, 1998).

Table 4.5 Organic matter and organic carbon content in soils

Soil Type	Total Organic Carbon	Organic matter
	%	%
Sandy loam	1.06 (0.28)	2.13 (0.56)
Clay loam	2.16 (0.12)	4.32 (0.25)

Numbers in parenthesis are standard errors of means.

4.2.4 Concentrations of Cu, Co and other Trace Elements in the Soils

Concentrations of total and plant available trace elements in sandy loam and clay loam soils used in the pot experiment are depicted in Table 4.6. The initial total concentrations of Cu in soils were 0.7 (±0.5) mg/kg in sandy loam and 13.5 (±4.0) mg/kg in clay loam while for Co it was 17.7 (±0.5) mg/kg in sandy loam and 16.1 (±0.5) mg/kg in clay loam. The initial soil plant available concentrations of Cu in soils were 0.2 (±0.0) mg/kg in sandy loam and 0.7 (±0.0) mg/kg in clay loam. The concentrations for Co were 0.6 (±0.0) mg/kg in sandy loam and 0.3 (±0.0) mg/kg in clay loam.

Table 4.6 Total and plant available trace element content of soils

Element	Clay loam soil		Sandy loam soil	
	Total mg/kg	Plant Available mg/kg	Total mg/kg	Plant Available mg/kg
Ca	14203 (686.1)	611.3 (1.8)	16950.0 (651.8)	122.0 (1.1)
Co	16.1 (0.5)	0.3 (0.0)	17.7 (0.5)	0.6 (0.0)
Cr	27.3 (1.0)	nd	13.65 (4.0)	Nd
Cu	13.5 (4.0)	0.7 (0.0)	0.7 (0.5)	0.2 (0.0)
Fe	15922 (306.7)	13.7 (0.2)	10121.6 (390.2)	10.9 (0.1)
K	2908.4 (164.7)	152.95 (2.9)	21525 (1487.7)	132.8 (4.0)
Mg	1722.8 (85.4)	303.6 (10.0)	359.4 (3.2)	63.4 (3.2)
Mn	131.2 (3.4)	38.5 (0.3)	84.0 (4.0)	31.9 (1.9)
Na	832.6 (238.6)	106.0 (0.2)	1008.6 (85.1)	87.4 (0.2)
Pb	nd	0.5 (0.0)	nd	0.5 (0.1)
Zn	526.2 (5.2)	1.9 (0.1)	517.5 (16.1)	5.8 (0.1)

Numbers in parenthesis are standard errors of means.

These concentrations of plant available Cu and Co in clay loam and sandy loam soils determined were still well below the levels of these two elements that would be considered toxic for plant uptake in soil. Copper and cobalt are generally present in most soils with average content of the former at around 30 mg/kg while that of the latter is about 8 mg/kg (Sumner, 1999).

4.2.5 Cation exchange capacity (CEC) of the soils

The CEC of the soils were calculated by summation of the measured bases in soil (Warncke, and Brown, 1998; Sumner and Miller, 1996). The CEC for sandy loam soil was 3.54 meq/100g and clay loam had 8.0 meq/100g (Table 4.7). CEC on soil arises from clay minerals and organic matter. Clay loam soil used in this study apparently holds more cations than sandy loam due high clay and organic matter content. If these elements were to be replaced by those in contaminated water, clay loam soil would thus adsorb more heavy metals than sandy loam thereby reducing the metals that could be taken up by plants.

Table 4.7 Cations and cation exchange capacity (CEC) of the soils

Element	Sandy loam	Clay loam
	me/100g	
Al^{3+}	0.4	0.48
H^+	1.28	1.08
Na	0.38	0.46
Ca	0.61	3.06
K	0.34	0.39
Mg	0.53	2.53
CEC = (Al+H+Na+Ca+K+Mg)	3.54	8.0

4.3 Retention of Cu and Co on sandy loam and clay loam soils irrigated with contaminated water

The results and discussion presented in this section relate to objective 1 of the study. It was assumed that the differences in the physical and chemical properties of sandy loam and clay loam would influence adsorption of Cu and Co in the two soils. These differences in adsorption of Cu and Co by the two soils would ultimately affect crowing growing on them. It was also assumed that increasing the loads of contaminants in the irrigation water would result in an increase in the concentrations of the elements on the different fractions.

4.3.1 **Plant available Cu and Co in soils at end of the experiment**

The mean concentrations of plant available Cu and Co in sandy loam and clay loam soils are presented in Figure 4.1. It is observed that the mean concentration of available Cu was 16.5 mg/kg in sandy loam and 27.7 mg/kg in clay loam. The concentration of plant available Co was 8.1 mg/kg soil in sandy loam and 13.6 mg/kg soil in clay loam soil. There was a significantly higher ($p<0.05$; $LSD_{0.05} = 4.4$ and 0.8 mg/kg for Cu and Co respectively) concentration of available Cu and Co in clay loam than sandy loam soil.

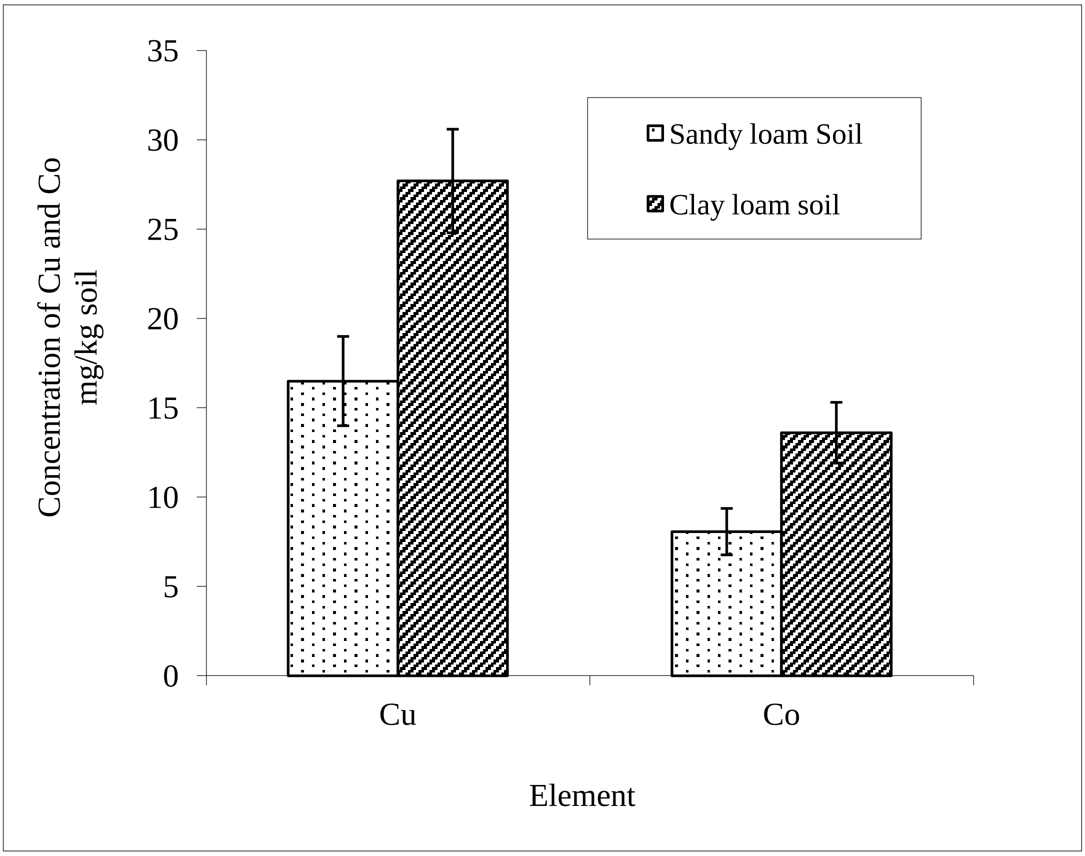


Figure 4. 1 Concentration of Cu and Co in the soil at harvest. The error bars shown are the standard error of means.

The findings conform to factors that contribute to the adsorption of cations in soils and the effect of some of them such as clay and organic matter. The higher concentration of Cu and Co on clay loam than on sandy loam soil can be attributed to the higher cation exchange capacity, surface complexation and electrostatic interaction on the surfaces (Adriano, 2001; Basta *et al.*, 2005; Wahba and Zaghloul, 2007). Wahba and Zaghloul (2007) observed that montimorillonite removed high amounts of dissolved heavy metals from aqueous solutions than kaolinite. They alluded these findings to outer sphere ion exchange with the permanent surface sites of montimorillonite. Basta *et al.,* (2005) and Adriano (2001) observed that trace elements can adsorb to soil organic matter and other forms of humified natural organic matter and that formation of metal chelates reduces solubility of several metals in soil. The clay loam soil used in this study had 27.8 % clay while sandy loam had 7.6 % clay.

Another factor that can contribute to higher adsorption of Cu and Co on clay soil is organic matter (Baker 1990; Dube *et al*., 2001). The clay loam soil used in the study had 4.11% organic matter while sandy loam had 1.56%. The higher quantities of clay and organic matter on clay loam soil that was used in the experiment could have contributed to the higher adsorption of Cu and Co than what was observed on sandy loam soil. Dube *et al.* (2001) explained that higher attraction of Cu on clay soil can be attributed to the smaller atomic radius of Cu (0.73Å) compared to that of Co (0.745Å) that makes the former to have a higher affinity by organic fraction in the soil than the latter element. In this study the contaminated water used for irrigation had twice the amount of Cu than Co and the initial content of the two elements could also contribute to the observed high content of Cu than Co on soils.

If clay loam adsorbs more contaminants from irrigation water than sandy loam, the result is that crops that would be grown on these two soil types will possibly not have the same concentration of the contaminants in tissues. This is due to removal by adsorption some of the possible contaminants. It is this important that in field studies, soil characteristics are included to get better explanations of the consequences of using waste water on different soil types.

4.3.2 **The effects of increasing concentration of contaminants in irrigation water on plant available Cu and Co in sandy loam and clay loam soils**

Figure 4.2 shows the concentration of Cu in soils irrigated with contaminated water. Significant differences ($p<0.005$; $LSD_{0.05} = 9.93$) in Cu concentration were largely between CW treatments and FW in sandy loam soil. The concentration of Cu in FW was 1.7 mg/kg and 12.9 mg/kg in FW 25%. For clay loam, significant differences ($p<0.05$; $LSD_{0.05} = 9.93$ mg/kg) in Cu concentrations were observed between CW treatments and FW. There were however, no significant differences in Cu concentrations in soils among CW 50%, CW 75% and CW 100% water treatments.

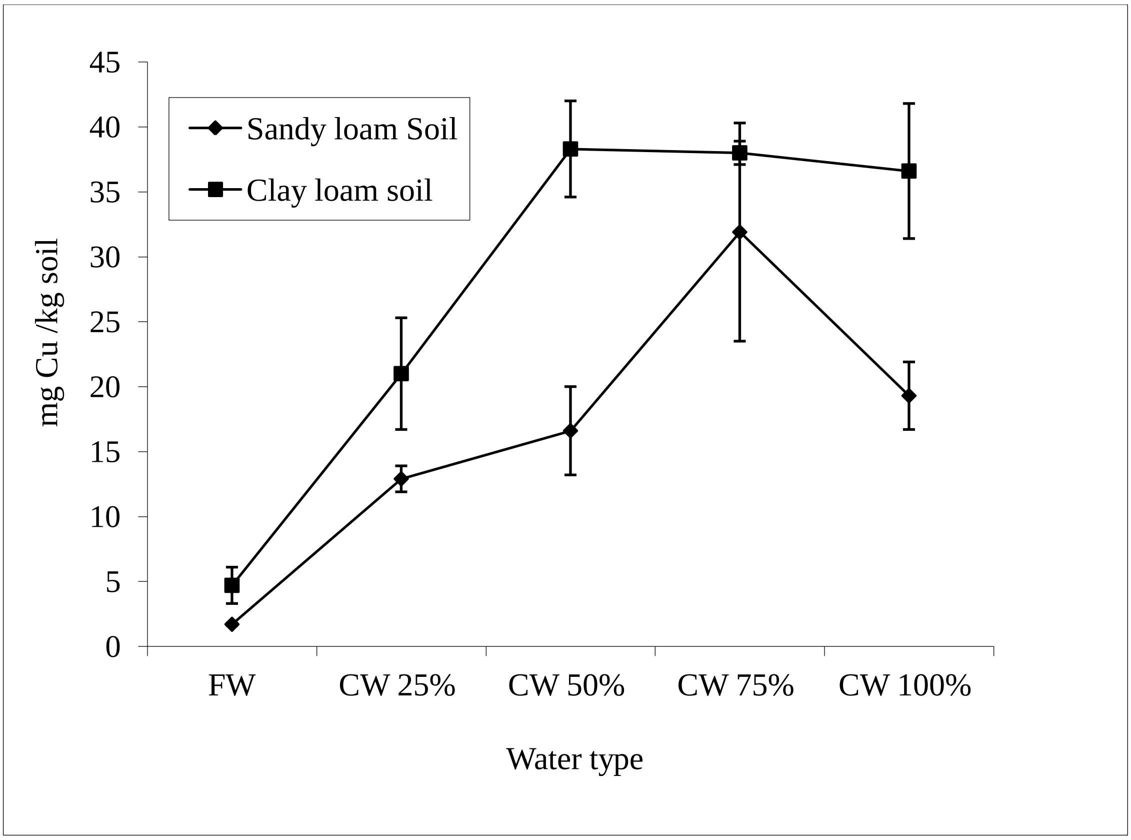


Figure 4.2 Concentration of Cu in soils irrigated with contaminated water.

The error bars shown are the standard error of means

Figure 4.3 shows the concentration of cobalt in soils irrigated with contaminated water. The concentration of cobalt in soils significantly ($p<0.05$; $LSD_{0.05} = 5.88$ mg/kg) increased with increasing levels of contamination in irrigation water.

In sandy loam, the concentration of Co increased from 0.09 mg/kg soil with FW to 15.31 mg/kg when irrigated CW100%. Significant differences ($p<0.05$; LSD = 7.98 mg/kg) in Co in sandy loam was observed between FW, CW75% and CW100%. In clay loam, the concentration of Co increased from 0.46 mg/kg soil with FW to 20.75 mg/kg when irrigated CW50%. There were significant differences ($p<0.05$; LSD = 7.98 mg/kg) in Co concentrations in clay loam soil among irrigation water treatments.

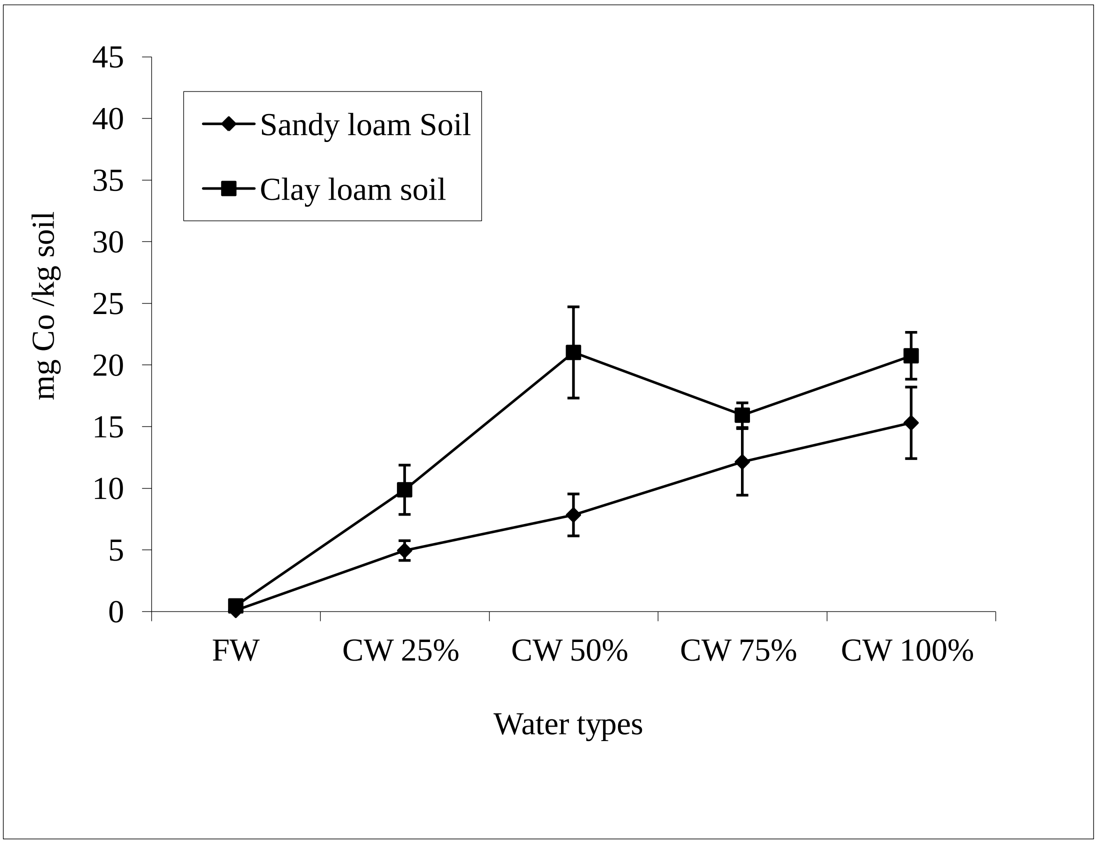


Figure 4.3 Concentration of cobalt in soils irrigated with contaminated water. The error bars shown are the standard error of means

Apparently from figures 4.2 and 4.3, Cu and Co in clay soil increased and reached a peak when the concentrations of the two elements were about 38 and 21 mg/kg soil respectively. On the other hand, concentration of available Cu in sandy soil reached the peak when the concentration of Cu is about 16 mg/kg soil.

It is most likely that concentration of Cu and Co in soils could be determined by the soils capacity to hold cations. Since clay loams have a higher capacity to hold cations than sandy loam, the peak of the levels tends to be higher for the former than later soil. This could be the possible explanation of the observed results where the peak Cu on clay loam was 38 mg/kg and on sandy loam was 16 mg/kg. The highest concentration of Cu and Co in irrigation water used in this study could be adsorbed up to this concentration. There was no excess irrigation water applied to the pots in this study as the irrigation only reached 80% of the field capacity.

4.3.3 Amounts of Cu and Co in soils before and at the end of the greenhouse study

The amounts of total Cu and Co extracted from the soils before and after the greenhouse experiments are shown in Table 4.8. Each pot in the experiment received 8575 ml of water. The concentrations of Cu and Co in the contaminated water used in this study are presented in Table 4.6. Therefore, the amount of Cu applied to each pot ranged from 0 mg in FW, 61.57 mg in CW 25%, 127.17 mg in CW 50%, 192 mg in CW 75% and 225.09 mg in CW 100%. For Co the amount ranged from 0 mg in FW, 37.13 mg in CW 25%, 63.80 mg in CW 50%, 88.58 mg in CW 75% and 90.21 mg in CW100%.

The general trend indicated that there was an increase in the amounts of Cu and Co extracted from the two soils at the end of the experiment. The trend showed higher Cu and Co extracted from clay loam than sandy loam soil.

Table 4.8 Total Cu and Co extracted from soils before and after use in the greenhouse experiment.

Soil type	Cu		Co	
	Before	After	Before	After
	mg	mg	mg	mg
Sandy loam	21.58 (0.47)	137.0 (21.6)	9.60 (1.20)	41.6 (4.22)
Clay loam	39.32 (1.39)	304.0 (21.6)	9.17 (0.39)	81.7 (4.22)
Lsd ($p<0.05$)	ns	45.4	ns	12.5

The numbers in parenthesis are standard errors of means. Detection limits for Cu = 0.001 mg/l and Co = 0.006 mg/l.

Results presented in Table 4.8 show that before the experiment, the amounts of total Cu extracted from sandy loam was 21.58 mg and 39.32 mg from clay loam. At the end of the greenhouse experiment the mean amounts of Cu extracted from sandy loam increased to 137 mg. and in clay loam to 304.0 mg. The difference in the amount of extracted Cu from the soil before and after the experiment was 115 mg for sandy loam and 264 mg for clay loam.

For Co the amount extracted from sandy loam before the experiment was 9.60 mg and 9.17 µg from clay loam. The amounts of Co extracted from the soil after the green house experiment increased to 41.6 mg from sandy loam and 81.7 mg on clay loam soil. The difference in the amount of extracted Co from the soil before and after the experiment was 32 mg for sandy loam and 72 mg for clay loam. The effect observed also depends also on texture as it determines how much remains in the root zone. Clay loam has higher capacity to hold elements on exchange sites and surface than sandy loam thus the subsequent availability in the root zone for uptake.

The proportion increase in amounts of recovered Cu in sandy loam soil was more than 6 times, and 7 times in clay loam soil. For Co, the increase was more than 4 times in sandy loam and 8 times in clay loam soil.

Heavy metals are adsorbed on the soil by cation exchange and chelating processes. The latter process is most common with organic matter. The clay loam used in this experiment had 27.6% clay and 4% organic matter while sandy loam had 7.6% clay and 1.5% organic matter.

Soils with high clay content, especially the smectites (or montimorillonites) tend to have high specific surface and thus provide a large surface area for adsorption of metals on them (Zubillaga *et al*., 2008; Du *et al.,* 1997; Miller and Donahue, 1995). Clay minerals have a larger amount of negative charges on them in the range of 15 - 30 $cmol_c$/kg soil compared to sandy loam with about 5 - 10 $cmol_c$/kg soil (Miller and Donahue, 1995). The difference between the two soils indicates that clay loam has about 3 times the surface area to hold metals than sandy loam soil. In addition clay loam soils have slower drainage compared to sandy loam soils increasing the possibility of holding more cations on the soil.

The other factor that governs adsorption of cations in soil is the organic matter (Logan and Traina 1993). Organic matter adsorbs cations on negative charges that exist on their surfaces through a process called chelation. The clay loam soil used in this study had 4% organic matter (or 1.56% TOC) compared to 1.5% (0.79% TOC) on sandy loam. The higher organic matter on clay loam contributed to the higher adsorption of Cu and Co, than sandy loam.

4.4 Effect of contaminated water on the fractions of Cu and Co in soils

The results and discussion presented in this section relate to objective 2 of the study. It was assumed that Cu and Co in contaminated water when used for irrigation would be adsorbed in soils on different fractions thereby influencing their availability for plant uptake.

4.4.1 Fractions of Cu and Co in soils before and at end of the greenhouse experiment

The results of fractions of Cu and Co extracted from soil samples before (initial soil) and after use in greenhouse are presented in Table 4.9.

Initial soil indicates that for sandy loam soil, Cu fractions were found in the following decreasing order: organic > residual > exchangeable > water soluble > carbonate. The Cu carbonate fractions were below detection in the initial sandy loam soil. The levels extracted of the Cu fractions in sandy loam soil were 7.7, 6.0, 3.9, and 1.8 µg in organic, residual, exchangeable and water soluble fractions respectively. In clay loam soil, Cu was only found in organic and Residual fractions at 8.16 and 4.5 µg respectively.

Table 4.9 Cu and Co fractions before and after the soils were used in the experiment

Fraction		Soil before use in green house				Soil after use in green house			
	Soil type	Sandy loam	Clay loam	Sandy loam	Clay loam	Sandy loam	Clay loam	Sandy loam	Clay loam
	Element	Cu	Cu	Co	Co	Cu	Cu	Co	Co
			µg					µg	
Exchangeable		3.93	0.00	0.22	0.00	4.22	10.21	4.56	6.5
Water soluble		1.82	0.00	6.37	0.92	0.12	0.00	1.64	0.41
Organic		7.73	8.16	0.00	0.00	23.12	43.5	0.00	0.00
Carbonate		0.00	0.00	0.00	0.00	0.05	0.4	0.48	0.00
Residual		5.96	4.50	0.00	0.00	4.81	5.31	0.00	0.00
$LSD_{0.05}$						1.63	1.63	0.423	0.423
CV %						24.5	24.5	43.1	43.1

Detection limits for Cu = 0.001 mg/l or 1 µg/l and Co = 0.006 mg/l or 6 µg/l

For Co on sandy loam soil, the element was only extracted on exchangeable and water soluble fractions with the highest recovery in the later fraction. The extracted quantities of Co from sandy loam were 6.37 and 0.22 µg as water soluble and exchangeable fractions respectively. On the other hand on clay loam, Co was extracted in water soluble fraction only. The amount of water soluble Co extracted from clay loam was 0.92 µg.

Changes in the composition of fractions of Cu and Co in the soils can be observed by comparing the amounts of Cu and Co extractions in different fractions between the initial and final soils after cultivation. Upon irrigation with contaminated water, the amounts of Cu and Co increased in both soil types on different fractions as observed in recovered amounts

In the final sandy loam soil statistically significant differences ($p<0.05$, $LSD_{0.05}$ = 1.63) in Cu fractions were observed. Recovered amounts of Cu fractions on sandy loam soils after use in the green house experiment were found in the following decreasing order: organic > residual > exchangeable > water soluble > carbonate. The amount of Cu recovered from the organic fraction in sandy loam soil after use in the greenhouse study was 23.1 µg and the least in carbonate form was 0.05 µg.

The pattern of trend of fractions of Cu in clay loam soil changed between the soil before being irrigated (initial) and after use in green house study (final). The highest Cu fraction in final clay loam had organic (43.5 µg) as the highest followed by exchangeable (10.2 µg). In the final clay loam soil statistically significant differences ($p<0.05$, $LSD_{0.05}$ = 1.63) in Cu fractions were observed. The pattern of the different Cu fractions extracted was decreasing in the following manner: organic > exchangeable > residual > carbonate > water soluble.

In the final clay loam soil statistically significant differences ($p<0.05$, $LSD_{0.05}$ = 0.423) in Co fractions were observed. Cobalt was extracted in the exchangeable and water soluble fractions in clay loam soil at the end of the experiment. The exchangeable Co fractions were observed in clay loam at the end of the experiment but were not detectable in the initial soil. For sandy loam at the end of the experiment, Co was extracted from the soil in the following order: exchangeable >

water > carbonate while the initial soil had the fractions in the following order: water soluble > exchangeable.

The final sandy loam soils showed statistically significant ($p<0.05$, $LSD_{0.05} = 0.423$) differences in Co fractions in soil with (elevated levels of Co in the exchangeable (4.6 µg) followed by water soluble (1.6 µg) and least carbonate (0.5 µg) fractions. For clay loam, Co fractions in the final soils were highest in the exchangeable fractions (6.5 µg) followed by water soluble (0.41 µg). Fractions of Cu comprising the exchangeable and organic were significantly higher ($p<0.05$) in clay loam than sandy loam soil. The other fractions were somewhat higher in clay loam but not statistically significant. For Co, the organic and residual fractions were below detection in both clay and sandy loam soils.

4.4.2 Effects of increasing concentration of contaminants in Irrigation Water on fractions of Cu and Co in Soils

Table 4.10 shows the various fractions of Cu and Co in sandy loam and clay loam soils that had been irrigated with different concentrations of contaminated water. On the sandy loam soil, Cu was largely extracted in the soil on the organic followed by the exchangeable and residual fractions in decreasing order. On the clay loam soil, Cu was largely extracted in the soil on the organic followed by the exchangeable, residual and carbon fractions in decreasing order.

Increasing concentrations of contaminants in water used for irrigation resulted in increased amounts of Cu and Co that were extracted from the soils. There also appeared to be differences in the amounts of Cu and Co from the different fractions between the soils and among the different levels of concentrations in water for irrigation.

Table 4.10 Fractions of Cu and Co in Sandy loam and clay loam soils irrigated contaminated water.

Soil Type	Water Type	Fraction									
		Exchangeable		Water soluble		Organic		Carbonate		Residual	
		Cu	Co	Cu	Co	Cu	Co	Cu	Co	Cu	Co
						µg					
Sandy loam	FW	2.36	0.0	0.62	2.795	6.06	0.0	0.0	0.0	6.92	0.0
Sandy loam	CW25%	4.31	3.775	0.0	1.650	27.72	0.0	0.0	0.0	3.76	0.0
Sandy loam	CW50%	3.16	0.917	0.0	1.691	22.81	0.0	0.0	0.0	4.13	0.0
Sandy loam	CW75%	6.20	0.0	0.0	1.169	25.45	0.0	0.0	0.0	3.72	0.0
Sandy loam	CW100%	5.09	18.100	0.0	0.902	33.55	0.0	0.26	2.417	5.54	0.0
Clay loam	FW	0.0	0.0	0.0	0.937	11.19	0.0	0.0	0.0	5.68	0.0
Clay loam	CW25%,	0.0	0.0	0.0	0.678	31.60	0.0	0.0	0.0	7.30	0.0
Clay loam	CW50%	5.36	12.817	0.0	0.0	46.97	0.0	0.0	0.0	3.93	0.0
Clay loam	CW75%	26.36	7.433	0.0	0.437	63.50	0.0	0.69	0.0	4.35	0.0
Clay loam	CW100%	19.32	12.250	0.0	0.010	64.25	0.0	1.33	0.0	5.30	0.0
LSD $p<_{0.05}$		1.51	0.95	ns	0.95	3.65	ns	1.111	0.586	3.574	ns
CV %		12.2	20.9	91.5	53.3	13.3		24.9	44.7	25.3	

4.4.2.1 **Organic fractions**

On sandy loam soil, all the treatments with contaminated water, had significantly higher ($p<0.05$; $LSD_{0.05} = 3.65$) Cu extracted from the organic fraction than that of control treatment (FW). The amount of organic Cu extracted was higher than 22 µg from treatments irrigated with contaminated water whereas in control treatment (FW) was around 6 µg. There were apparently significant differences ($p<0.05$; $LSD_{0.05} = 3.65$) of Cu extracted in the organic fraction among the treatments CW 25% and CW 50%, CW 25% and CW 100%, CW 50% and CW 100%, CW 75% and CW 100% treatments. The highest extracted Cu on the organic fraction on sandy loam was 33.5 µg on CW100%.

On clay loam soil, the organic Cu fractions were also significantly higher ($p<0.05$; $LSD_{0.05} = 3.65$) on all CW treatments than FW. There was however, no significant differences observed in the organic Cu fraction between CW75% and CW100%. The organic Cu fraction on clay loam soil increased from 11 µg in FW to 64 µg in CW100%. The highest extracted Cu on the organic fraction on clay loam was 64 µg on CW100%. Apparently no Co fractions were extracted on both sandy loam and clay loam soils.

The organic fraction is that where the metal element is bound on the organic and sulphide fractions (Salomons and Forstner, 1980; Fedotov and Spivakov, 2008). The elements bound on these forms are released into the water phase when the oxidation and decomposition of the organic matter takes place in soil.

Results from the study shows higher Cu on the organic fractions in both sandy loam and clay loam soils than Co. The higher preference for Cu to organic colloids in soils was reported by Baker (1990). The attraction of metals to colloids is usually affected by their ionic radii (Dube *et al.*, 2001; Alloway 2005). The attraction of the organic colloids with the metal ions is electrostatic. The atomic radius of Cu is 0.73Å and Co is 0.745Å (Dube *et al.,* 2001). The smaller atomic radius of Cu thus affects its higher affinity by organic fraction in soil than Co.

The other observation is that organic Cu fraction extracted from clay loam was more than double that obtained in sandy loam. Initial soil analysis showed that the clay loam used in the experiment had 1.56% Total Organic Carbon (TOC) compared to sandy loam with 0.79% TOC. Clay loam soil has double the TOC than sandy loam and this ratio conforms closely to the amounts of Cu recovered from the two soils on the organic fractions.

4.4.2.2 **Exchangeable fractions**

The exchangeable fractions of Cu and Co increased between the control treatments and those where contaminated water was applied in both sandy loam and clay loam soils. The exchangeable fraction of Cu was generally higher on clay loam by more than two times on the sandy loam soil. The mean Cu extracted from sandy loam was 4.22 µg/g while that from clay loam was 10.21µg/g.

On sandy loam soil, significant differences ($p<0.05$; $LSD_{0.05} = 3.65$) between the exchangeable Cu fraction on control (FW) and CW75%. There were apparently no significant differences of Cu extracted in the exchangeable fraction contaminated water treatments. The highest extracted Cu on the exchangeable fraction on sandy loam was 6.2 µg on CW75%.

On clay loam soil, the exchangeable Cu fractions were only detected in clay soil when higher amounts of contaminants were in the water, from CW50%, CW75% and CW100%. There were significant ($p<0.05$; $LSD_{0.05} = 3.65$) differences among exchangeable Cu fractions on clay loam soil. The amount of extracted Cu on the exchangeable fraction increased from 5.3 µg with CW50% to 19.3 µg with CW100%.

On sandy loam soil, there were also significant differences ($p<0.05$; $LSD_{0.05} = 0.95$) in the exchangeable fraction of Co among treatments with the highest being 18 µg in CW100% although there was no definite pattern between the treatments. On clay loam soil, significant differences ($p<0.05$; $LSD_{0.05} = 0.95$) in exchangeable Co fractions were observed among irrigation water treatments. The exchangeable Co fractions in clay loam soils irrigated with CW50% and CW100% did not differ significantly and their mean values are 12.8 and 12.25 µg/g respectively. Exchangeable Co in clay loam soil irrigated using water with concentration below CW50% were largely undetected.

The exchangeable fraction is held onto the soil surface by weak electrostatic attractions, for by cation exchange. The cation exchange on clay soils is generally higher than on sandy soils thus the possibility of high amounts of Cu on the exchangeable fraction on clay loam than sandy loam soils.

At low concentration of contaminants in irrigation water (below 50%), clay loam did not adsorb either Cu or Co on the exchangeable fraction. The extracted amounts exchangeable fractions were higher on the clay loam soils than for those treatments that were irrigated with CW50% and above. The absence of extracted Cu and Co in clay loam soils irrigated with low concentrations of contaminants in irrigation water was because all the Cu and Co could have been held on the organic and other fractions.

Exchangeable fractions elements held in soils are those that are weakly adsorbed on exchange sites due to negative charges that exist on the soil surfaces and the positive charges (cations) for metal elements (Fedotov and Spivakov, 2008). Their behaviour in the soil will largely depend upon the pH of the soil and the composition of the ions in solution. For instance a decrease in pH would result in the metal to be mobilized in solution and vice versa when the pH increases. The exchangeable fraction is largely composed of trace elements in the form of mineral salts.

4.4.2.3 **Water soluble fractions**

Significant differences ($p<0.05$; $LSD_{0.05} = 0.95$) in water soluble Co were observed between treatments in sandy loam soil. Concentration of water soluble fraction of Co extracted in sandy loam declined with increasing contaminants in irrigation water. The water soluble Co extracted declined from 2.8 µg in FW to 0.9 µg in CW100%. The water soluble Co fraction in clay loam soil showed significant differences ($p<0.05$; $LSD_{0.05} = 0.95$) among irrigation water treatments. There was an apparent significant decline in the extracted water soluble Co fraction when contaminated water was used on the soil. The decline in the water soluble fraction in clay loam soil ranged from 0.94, 0.68 and 0.01 µg/g in FW, CW25% and CW100% respectively.

Water soluble fraction appeared to be more important in soil for Co than Cu. Irrigating the soils with increasing concentrations of contaminants resulted in a decrease in amounts of recovered water soluble fractions of Co in both sandy loam and clay loam soils. The water soluble fraction contains the most mobile and plant available forms of elements in soil (Leita and De Nobili, 1991). The elements in this form are largely salts, water soluble and exchangeable fractions. The low extractions of water soluble fractions from the soils when irrigated with higher concentrations of contaminated water was probably due to these fractions' being in the form of mineral salts with high positive charges on them. These mineral salts are highly attached to other fractions on the soil such as organic and clay colloids hence reduced amounts of water soluble fractions on soils.

4.4.2.4 **Carbonate fractions**

The carbonate fraction of Cu and Co in soil was only recovered when concentrations of contaminants in irrigation water were high. On sandy loam soil, the amount of carbonate Cu recovered was 0.7 µg and 1.33 µg for CW 75% and CW 100% respectively.

Carbonate fractions are composed of metal elements that are adsorbed on mineral salts that are adsorbed to the carbonates (Salomons and Forstner 1980). The carbonate forms are released in the water phase when there is a change in the pH of the soil solution. The release of the carbonate forms into solution happens when the pH changes from neutral to acid phase.

4.4.2.5 **Residual fractions**

Only Cu was extracted on the residual fraction. On sandy loam, the residual Cu fractions extracted were in the range between 3 to 7 µg and did not differ significantly among the water treatments. On clay loam, the residual Cu fractions among the treatments ranged from 3.9 to 7.3 µg but did not show any regular pattern with increasing levels of contaminants in irrigation water. There was generally no Co extracted in the residual fractions on both sandy loam and clay loam used in the experiment. The residual fraction is that which is fixed to the primary or secondary minerals in the mineral lattices. It is therefore, not released in the water phase under normal conditions in the environment as such the amounts extracted would remain the same in the different treatments used in the experiment (Fedotov and Spivakov, 2008).

4.5 Evaluation of pumpkin and Chinese cabbage grown on clay loam and sandy loam soils and irrigated with heavy metal contaminated water

The results and discussion presented in this section relate to objective 3of the study that was intended to determine the accumulation of Cu and Co in pumpkin and Chinese cabbage. It was also meant to evaluate the influence of soil types and concentration of contaminants in irrigation water on the uptake of Cu and Co by pumpkin and Chinese cabbage.

The following assumptions were made when this experiment was set up. First; it was assumed that increasing loads of contaminants in irrigation water would result in increased uptake by crops. In addition increasing loads of contaminants of heavy metals (of Cu and Co) in water for irrigation would simulate situations in which there are varying levels of heavy metals in irrigation water. Secondly; it was assumed that pumpkin and Chinese cabbage would be different in their uptake characteristics of the heavy metals as they belong to different plant families and because of their different growth habits. Responses by pumpkin and Chinese cabbage to increasing levels of heavy metal contamination would be observed in (a) metal contents of above and below ground tissues, and (b) biomass yields of the two crops. Thirdly; it was assumed that there would be differences in the uptake of Cu and Co by the crops when grown on two different soils which would be irrigated contaminated water. It was assumed that uptake of heavy metal elements by plants would be influenced by the differences in physical and chemical properties between sandy loam and clay loam soils. The differences in sand and clay content, cation exchange capacity, and organic matter content between the two soil types would ultimately influence the adsorption and desorption of heavy metals and ultimately uptake by crops grown on them.

4.5.1 **Concentration of Cu and Co in pumpkin and Chinese cabbage**

Figure 4.4 shows the concentration of Cu and Co in total plant tissues of Pumpkin and Chinese cabbage. There were significant differences ($p < 0.05$; $LSD_{0.05} = 53.7$ mg/kg) in Cu content in total plant tissue between pumpkin and Chinese cabbage. The Cu concentration was significantly higher in Chinese cabbage than in pumpkin plants. The mean Cu content in Chinese cabbage was 180.0 mg/kg while in pumpkin it was 116.3 mg/kg. Generally, there was higher Cu and Co uptake into the below ground tissue than the aboveground tissue. However, the discussion will focus on the aboveground tissue which represents portion consumed for food.

There were apparently no statistical differences in Co concentration in the plant tissues between pumpkin and Chinese cabbage where the mean element content was around 66.0 mg/kg.

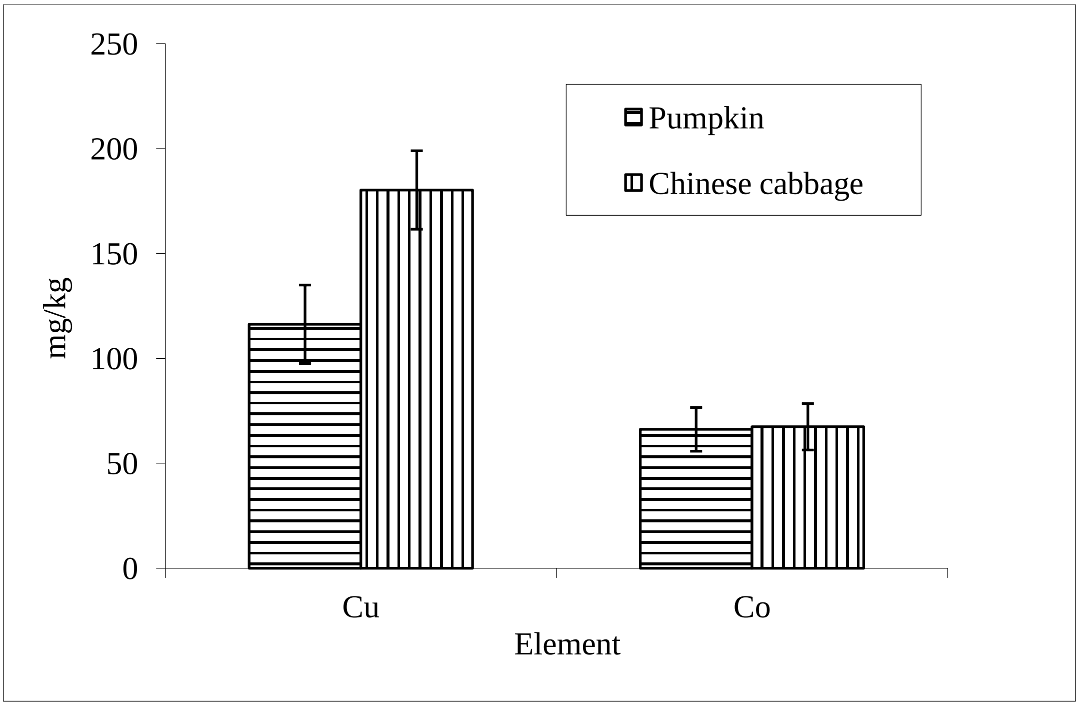


Figure 4.4 The mean concentration of Cu and Co in plant tissue of Pumpkin and Chinese cabbage irrigated with contaminated water. The error bars are experimental standard errors of means.

Results of above ground plant tissues for Chinese cabbage and pumpkin are presented in figure 4.5. Results of above ground plant tissue of Chinese cabbage had significantly higher ($p<0.05$; $LSD_{0.05} = 84.7$) levels of Cu than pumpkin. The concentration of Cu in Chinese cabbage was 160.0 mg/kg while that of pumpkin was 73.0 mg/kg. There were apparently no statistically significant differences in concentrations of Co in the two crops. The concentration of Co in Chinese cabbage was 102.0 mg/kg while that of pumpkin was 75.4 mg/kg.

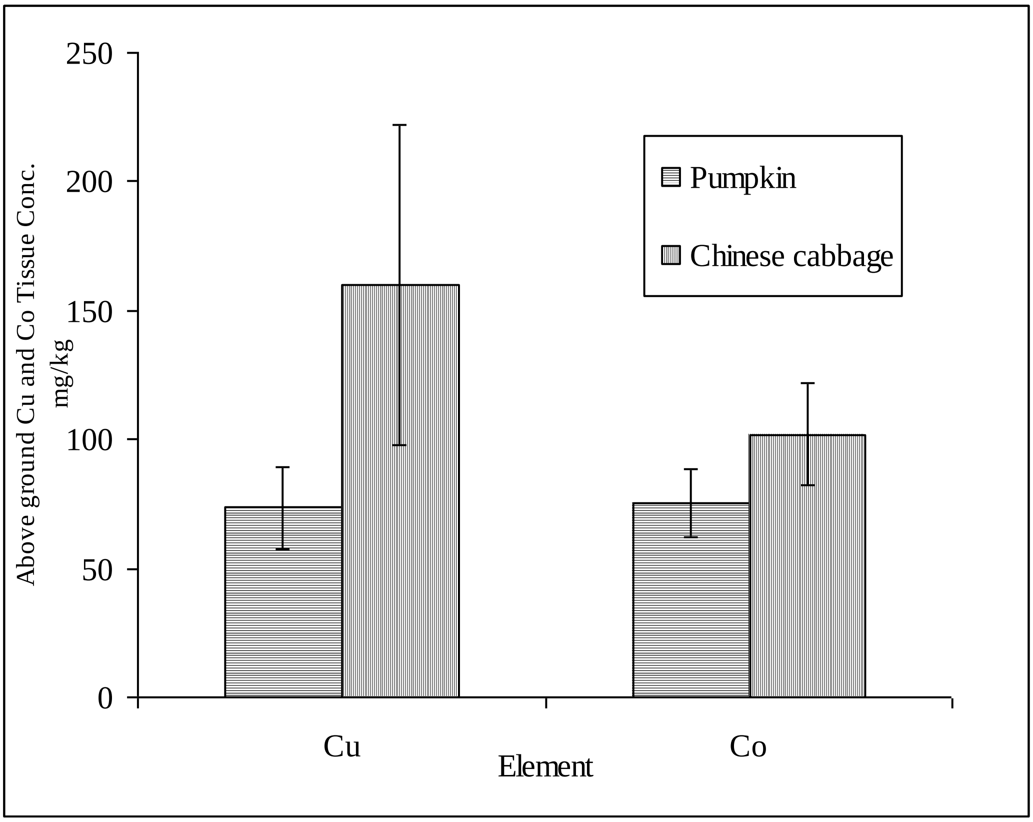


Figure 4.5 Mean Concentration of Cu and Co in above ground plant tissue of Pumpkin and Chinese cabbage. The error bars are standard errors of means.

Results on Figure 4.6 depict the mean concentrations of Cu and Co in below ground tissue of pumpkin and Chinese cabbage. The levels of concentration of Cu by both pumpkin and Chinese cabbage in below ground tissues were generally higher than that of Co. The concentration of Cu in below ground tissues of pumpkin was 159.0 mg/kg while that of Chinese cabbage was 201.0 mg/kg. There was however, no statistically significant difference in the uptake of Cu between pumpkin and Chinese cabbage in their below ground tissue. Pumpkin however, showed significantly higher ($p<0.05$, $LSD_{0.05} = 2.2$ mg/kg) Co uptake than Chinese cabbage. The mean Co concentration was 56.0 mg/kg and 32.8 mg/kg for pumpkin and Chinese cabbage respectively in their below ground tissue.

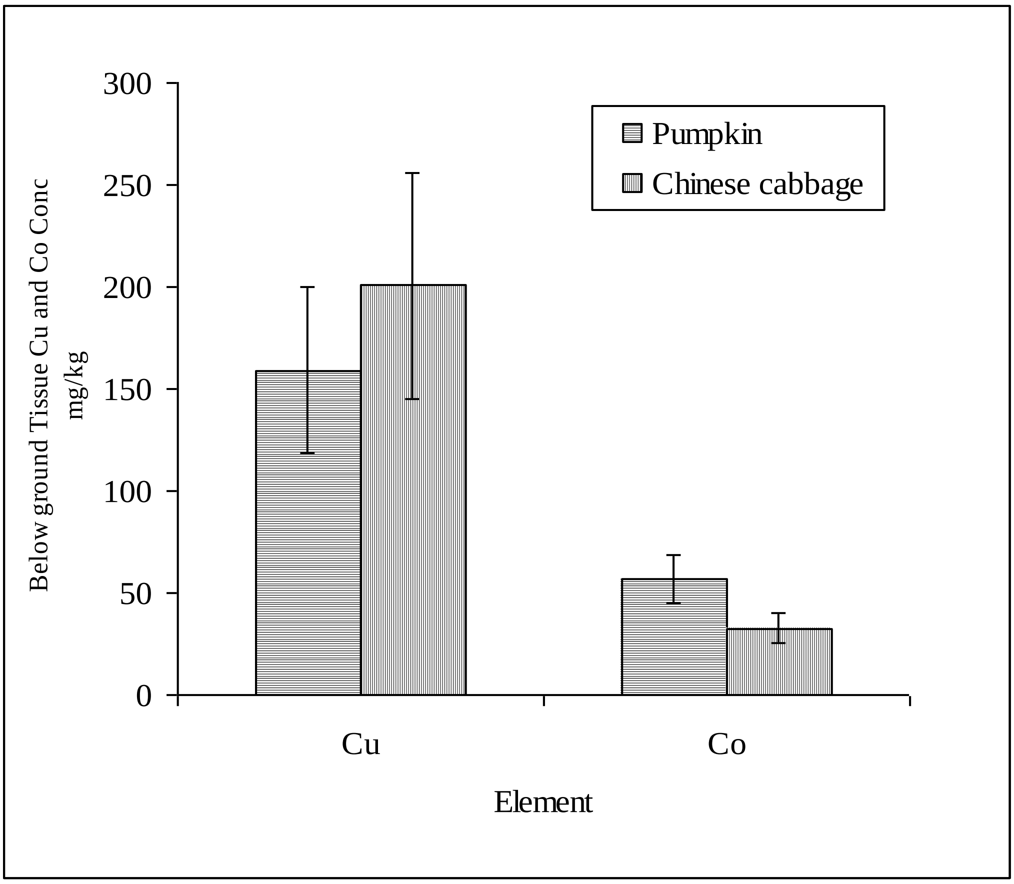


Figure 4.6 The mean concentration of Cu and Co in below ground plant tissue in Pumpkin and Chinese cabbage irrigated with contaminated water. The error bars are standard errors of means.

The study established that, Chinese cabbage had a much higher uptake of Cu than pumpkin. The concentration of Cu in Chinese cabbage of 180 mg/kg was 50% more than in pumpkin. The amount of Cu concentrated by Chinese cabbage in its above ground tissue was 160 mg/kg compared to 73 mg/kg in pumpkin. The same picture is observed in below ground tissue where the concentration of Co in Chinese cabbage was 102 mg/kg compared to 75.4 mg/kg in pumpkin.

There were however, no statistically significant differences in the concentration of Cu in below ground tissues of pumpkin and Chinese cabbage. Below ground pumpkin tissue showed higher concentration of Co than in Chinese cabbage (56.0 and 32.8 mg/kg for pumpkin and Chinese cabbage respectively). The concentrations of cobalt in below ground tissues however, were below those in above ground for both crops.

From the foregoing presentation, the ratios of below to above ground concentrations of Cu and Co in Chinese cabbage are higher (1:0.8 for Cu and 1:3.11 for Co) compared to pumpkin (1:0.46 for Cu and 1:1.35 for Co). Chinese cabbage is well known to be a hyperaccumulator plant (Arora *et. al.*, 2008; Liu *et al.*, 2010; Szczygłowska *et al.*, 2011). Among the characteristics of hyperaccumulators is that plants should accumulate higher metals in the above ground parts than below (Jabeen *et al.*, 2009). It is possible that pumpkin is not a hyperaccumulator bearing in mind that the ratio of cobalt in root to shoot is only 1:1.35 compared to Chinese cabbage with 1:3.11.

Farooq *et al. (*2008) and Kumar *et al.* (2009) showed that different plant species growing in the same environment have different uptake of heavy metals. In this study Chinese cabbage and pumpkin were exposed to the some soil and irrigation water conditions. At 180 mg Cu/kg in Chinese cabbage, the value is more than 3 times the permitted Cu in green vegetables in Zambia. The legal limit for Cu in green vegetables in Zambia is 50 mg/kg (GRZ, 1995). In pumpkin, where the mean uptake of Cu is 116 mg/kg was still higher than the permitted Cu in green vegetables by more than double. The concentration of Cu in both crops also exceeded the normal range for the element in vegetables which is cited as 8-20 mg/kg (Awofolu, 2005; Awofolu *et al.*, 2005).

4.5.2 **Effect of increasing concentration of contaminants in irrigation water on the uptake of Cu and Co by pumpkin and Chinese cabbage**

Figure 4.7 shows the effect of increasing concentration contaminants in irrigation water on Cu in pumpkin and Chinese cabbage. The concentration of Cu in pumpkin and Chinese cabbage increased significantly in both crops ($p< 0.05$, $LSD_{0.05} = 120.0$ mg/kg).

The concentrations of Cu in pumpkin increased from 8.33 mg/kg in control treatments (FW) to 230.9 mg/kg in treatments irrigated with undiluted contaminated water (CW 100%). In Chinese cabbage concentrations of Cu increased from 4.8 mg/kg in control treatments (FW) to 513.8 mg/kg in treatments irrigated with undiluted contaminated water (CW 100%).

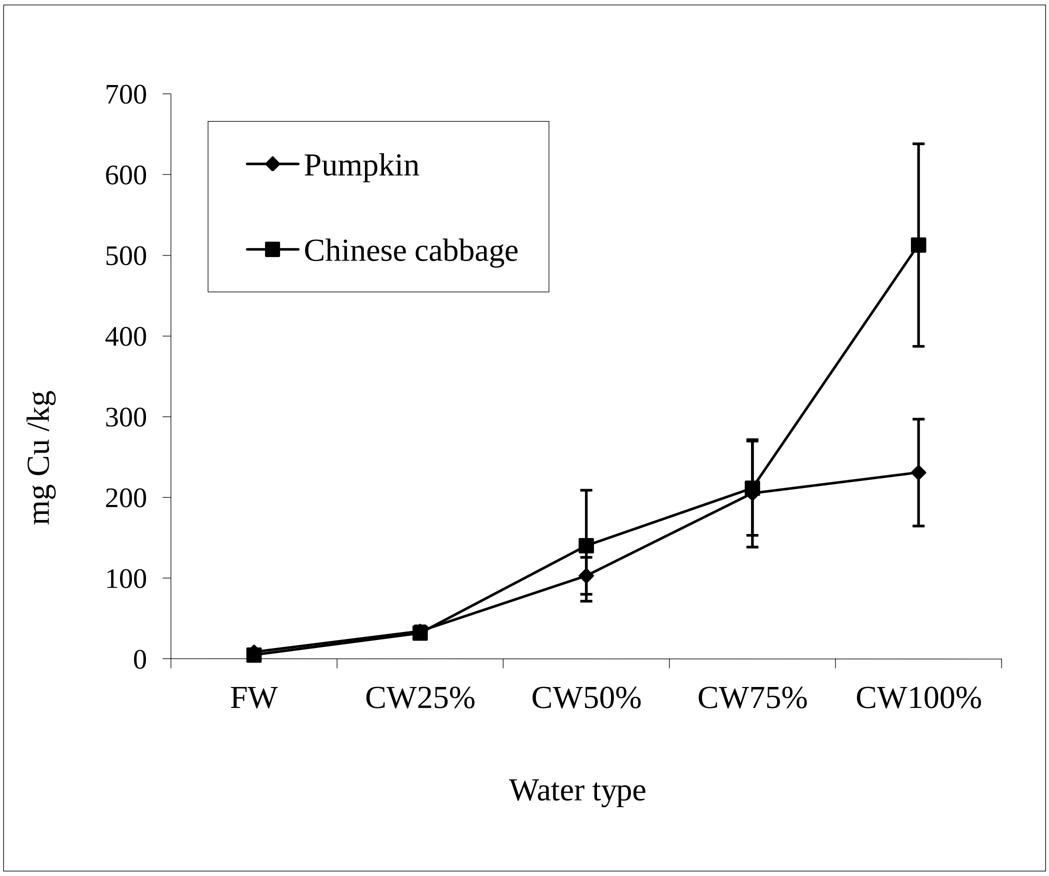


Figure 4.7 Concentration of Cu in pumpkin and Chinese cabbage irrigated with contaminated water. The error bars are standard errors of means.

Figure 4.8 shows the effect of increasing concentration contaminants in irrigation water on Cu in pumpkin and Chinese cabbage. There were apparently no statistically significant differences in the concentration of cobalt in plant tissue of crops with increasing concentrations of irrigation water.

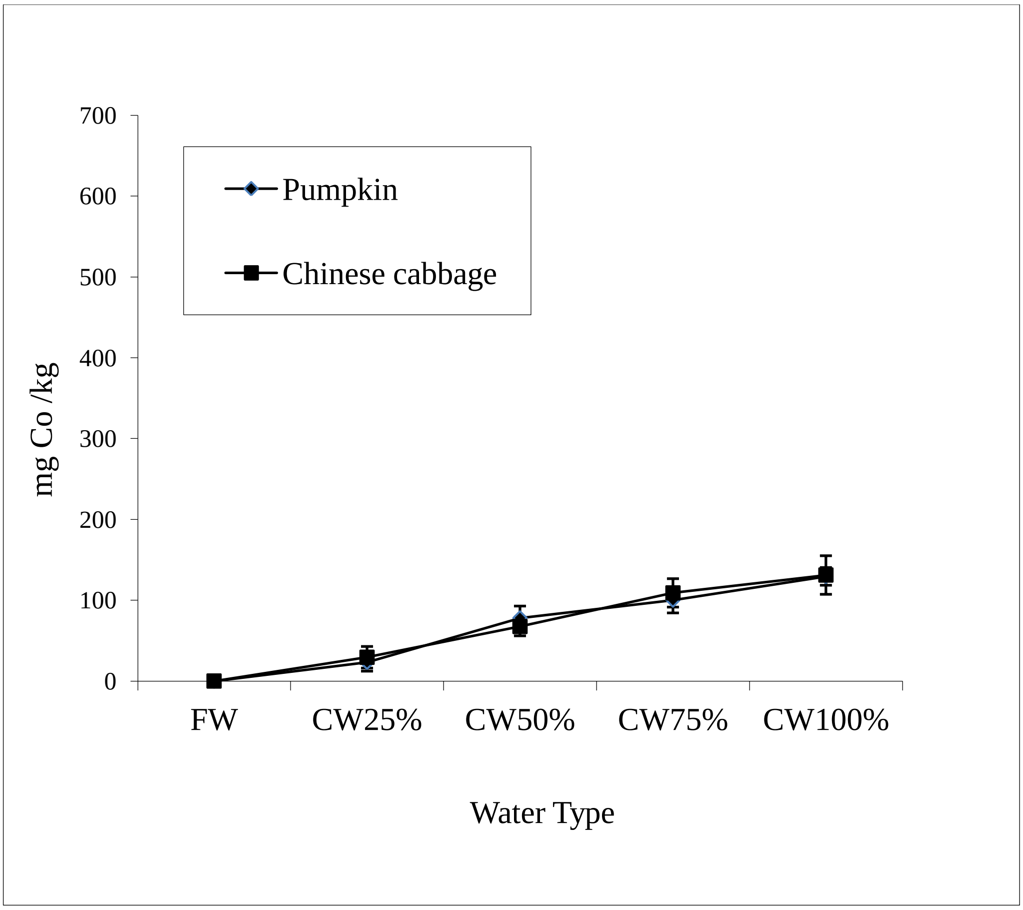


Figure 4. 8 Concentration of cobalt in pumpkin and Chinese cabbage irrigated with contaminated water. The error bars are standard errors of means.

Results in Figure 4.9, shows the concentration of Cu in above ground tissue of pumpkin and Chinese cabbage that was irrigated with contaminated water. Results show the concentration of Cu increased in plant tissues of both pumpkin and Chinese cabbage when irrigated with increasing levels of contaminants in water. For pumpkin, the levels of Cu ranged from 9.0 mg/kg when irrigated with fresh water to 142.0 mg/kg when irrigate with undiluted contaminated water. In Chinese cabbage the concentration of Cu ranged from 4.0 mg/kg when irrigated with fresh water to 539.0 mg/kg when irrigated with undiluted contaminated water. The uptake of Cu by Chinese cabbage exceeded that of pumpkin at levels above CW50%.

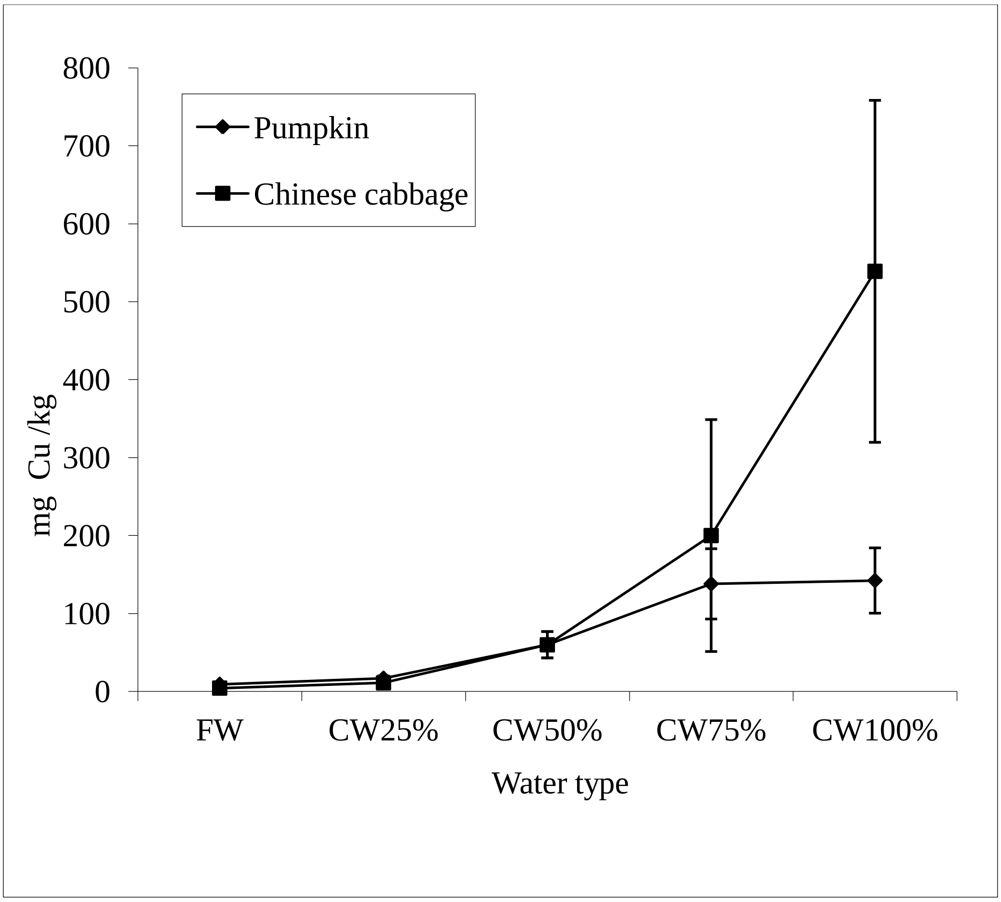


Figure 4. 9 Concentration of Cu in above ground plant tissue of Pumpkin and Chinese cabbage irrigated with contaminated water. The error bars are standard errors of means.

Results in Figure 4.10, shows the concentration of cobalt in above ground tissue of pumpkin and Chinese cabbage that was irrigated with contaminated water.

The concentration of Co was consistently higher in Chinese cabbage than in pumpkin at all levels of contamination of irrigation water. There were also significant differences ($p<0.05$; $LSD_{0.05} = 35.1$ mg/kg) in Co concentrations in above ground plant tissue of Chinese cabbage with different irrigation water contamination. The range of Co concentration in above ground pumpkin plant tissue ranged from below detection in FW (control) to 160.0 mg/kg with CW100% in plant tissue. The concentration of Co in Chinese cabbage ranged from 0 (in control) to 244.0 mg/kg with CW100%.

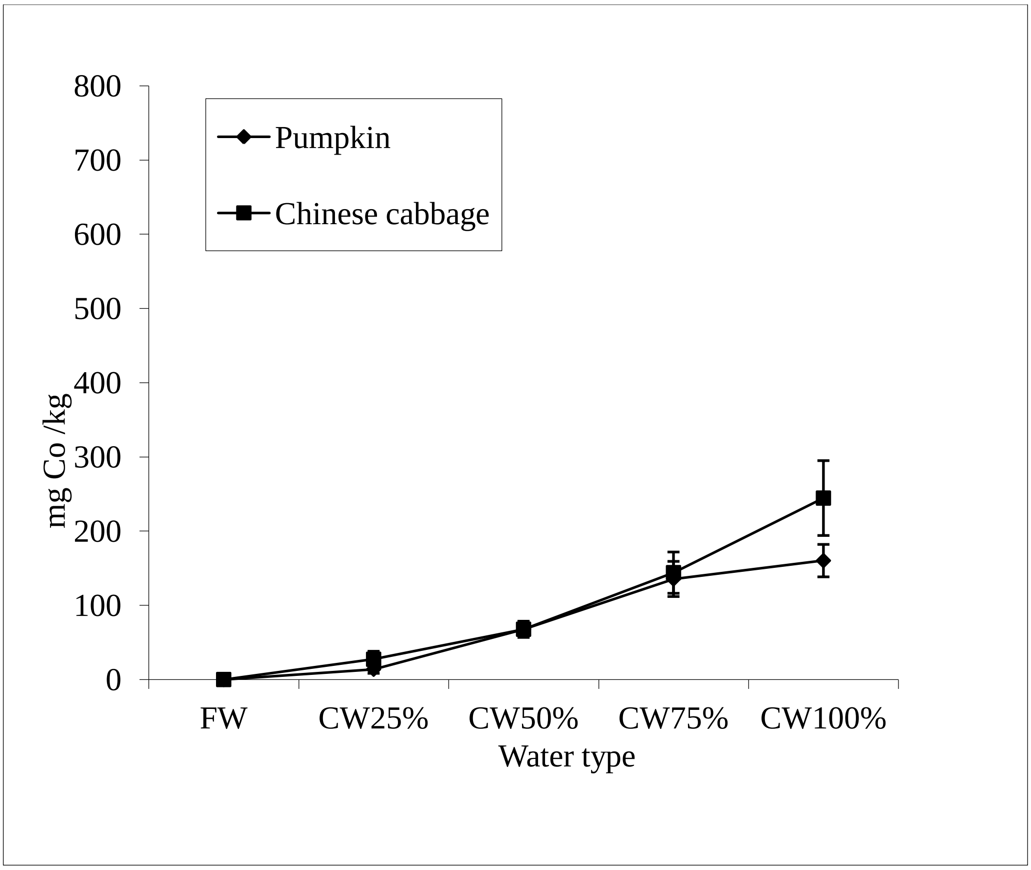


Figure 4. 10 Concentration of cobalt in above ground plant tissue of Pumpkin and Chinese cabbage irrigated with contaminated water. The error bars are standard errors of means.

Figure 4.11 shows the concentration of Cu in below ground plant tissue of pumpkin and Chinese cabbage that was irrigated with contaminated water. There were apparently no statistically significant differences in the tissue Cu in below ground plant tissues of pumpkin and Chinese cabbage with contaminated water used for irrigation.

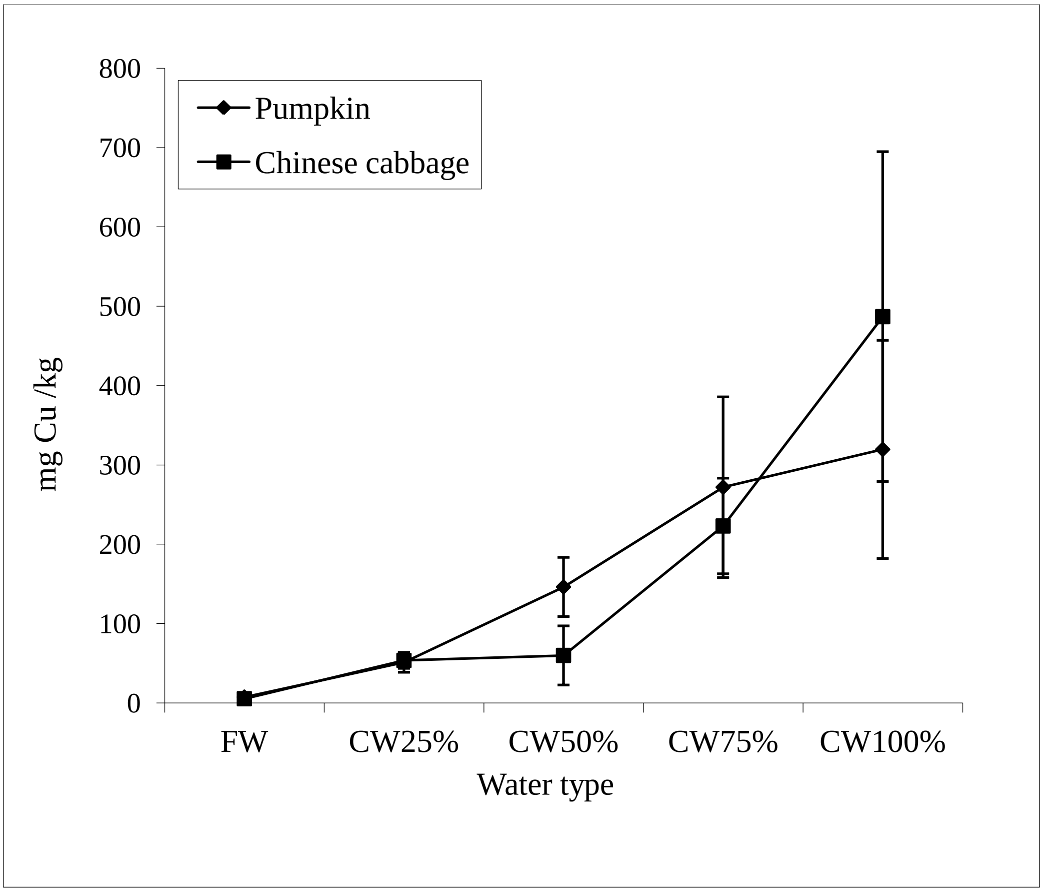


Figure 4. 11 Concentration of Cu in below ground tissue of Pumpkin and Chinese cabbage irrigated with contaminated water. The error bars are standard errors of means.

Figure 4.12 shows the concentration of cobalt in below ground plant tissue of pumpkin and Chinese cabbage that was irrigated with contaminated water. There were also no statistically significant differences in the tissue Co in below ground plant tissues of pumpkin and Chinese cabbage with contaminated water used for irrigation.

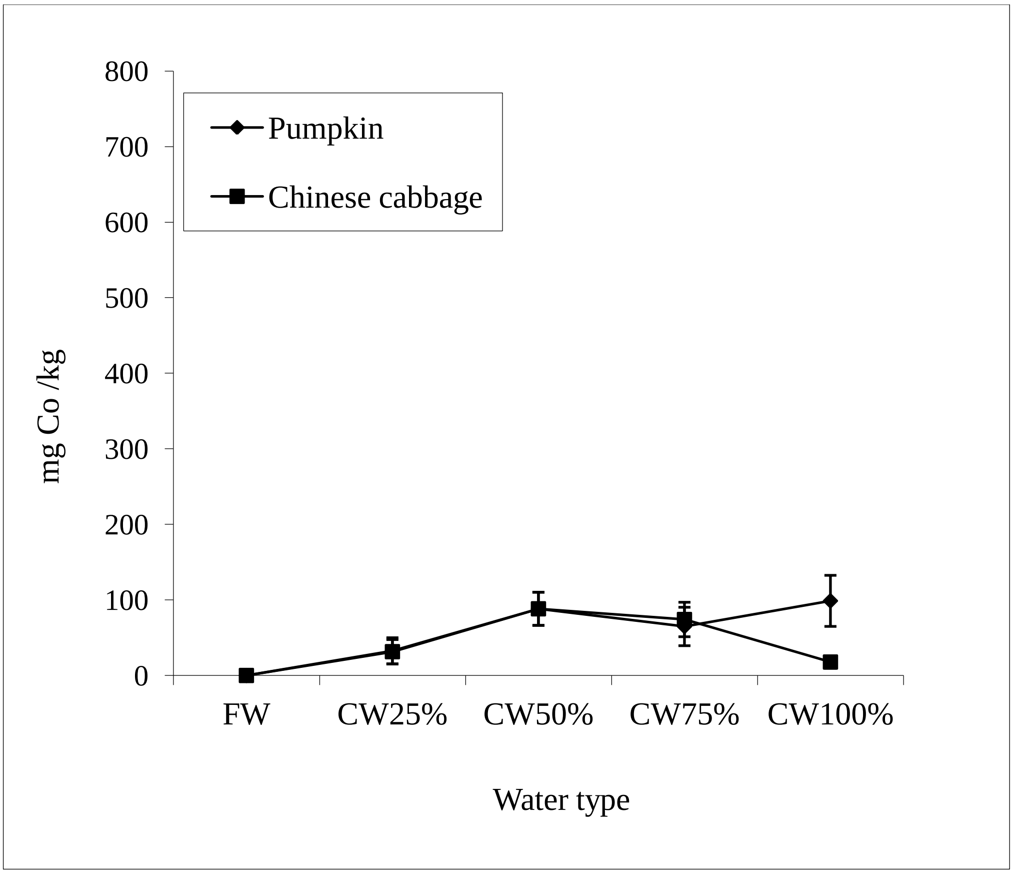


Figure 4. 12 Concentration of cobalt in below ground tissue of Pumpkin and Chinese cabbage irrigated with contaminated water. The error bars are standard errors of means.

The study revealed that increasing the concentration of contaminants in irrigation water resulted in increase in the uptake of Cu and co by plants. For Cu, the mean concentration in plants ranged from 6.6 mg/kg in control treatment (FW) to 372 mg/kg in plants irrigated with undiluted contaminated water. For Co, the mean concentration in plants was not detectable in control treatment (FW) to 130 mg/kg in plants irrigated with undiluted contaminated water. This component of the study therefore, established that increasing levels of Cu and Co in irrigation water resulted in increased uptake of the same metals in crops. These results are consistent with those obtained by Xiong and Wang (2005), where they observed that the levels of Cu in tissue of lettuce and spinach increased with increasing concentration of Cu in irrigation water. Jayakumar and Jaleel (2009).also obtained increasing uptake of Co by soyabean with increasing application of exogenous Co to the soil.

The study also showed that the uptake of Cu and Co varied between pumpkin and Chinese cabbage in above ground tissue. Chinese cabbage had the highest concentration of Cu and Co above ground at 539.0 mg Cu /kg and 244 mg Co /kg in undiluted contaminated water (CW100%). For pumpkin, the concentrations of the metals were 142.0 mg Cu /kg and 160 mg Co /kg in undiluted contaminated water (CW100%). Accumulation of heavy metals in plant tissues vary with species and even within species (Máthé-Gáspár and Anton, 2002; Arora *et. al.*, 2008; Mohsen and Mohsen, 2008) with *Brassicas* generally being the highest accumulators of heavy metals.

4.5.3 Influence of Soil Type on the uptake of Cu and Co from Contaminated Irrigation Water by Crops

The results depicted in Figure 4.13 show the concentration of Cu and Co in above plant tissue of pumpkin and Chinese cabbage. The results show that the above ground plant tissue concentrations of Cu were significantly higher ($p<0.05$; $LSD_{0.05} = 119.8$ mg/kg) for Chinese cabbage grown on sandy loam than clay loam soil. Uptake of Cu did not vary significantly between pumpkin grown on sandy loam and on clay loam soil. The mean concentration of Cu in aboveground tissue of Chinese cabbage on sandy loam was 277.0 compared to 44.0 mg/kg on clay loam.

The results also show that the above ground plant tissue concentrations of Co was significantly higher ($p<0.05$; $LSD_{0.05} = 22.2$ mg/kg) in both pumpkin and Chinese cabbage grown on sandy loam than clay loam soil. The mean concentration of Co in Chinese cabbage on sandy loam was 142.6 and 61.4 mg/kg on clay loam. The concentration of cobalt in pumpkin was 97.0 and 53.9 mg/kg on sandy loam and clay loam respectively.

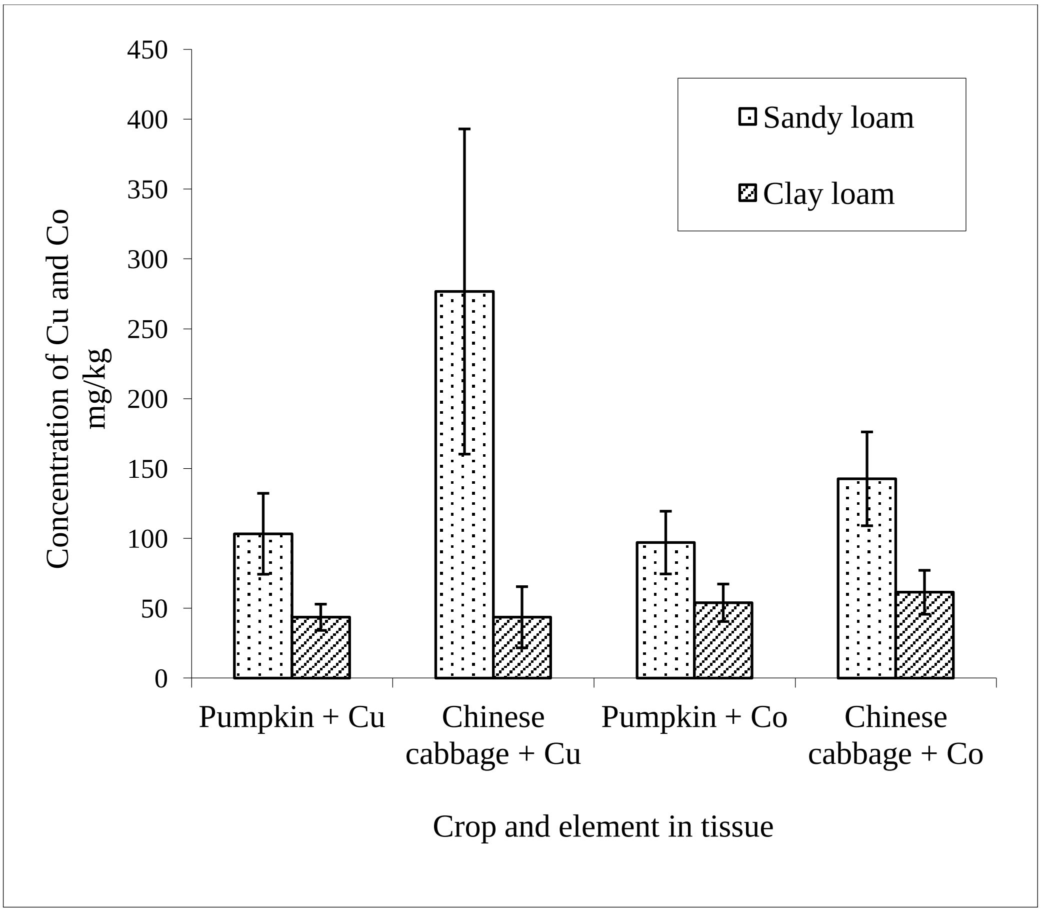


Figure 4.13 Mean concentration of Cu and Co in above ground plant tissue of Pumpkin and Chinese cabbage grown on sandy loam and clay loam soils and irrigated with contaminated water. The error bars are standard errors of means.

The mean concentration of Cu and Co in below ground tissue of Pumpkin and Chinese cabbage grown on sandy loam and clay loam and irrigated with contaminated water are depicted in Figure 4.14. There were no statistically significant differences in Cu or Co uptake by pumpkin or Chinese cabbage on sandy loam or clay loam soil in their below ground tissue. There was however, a general tendency by both crops to take up more Cu than Co in sandy loam and clay loam soil in their below ground tissue.

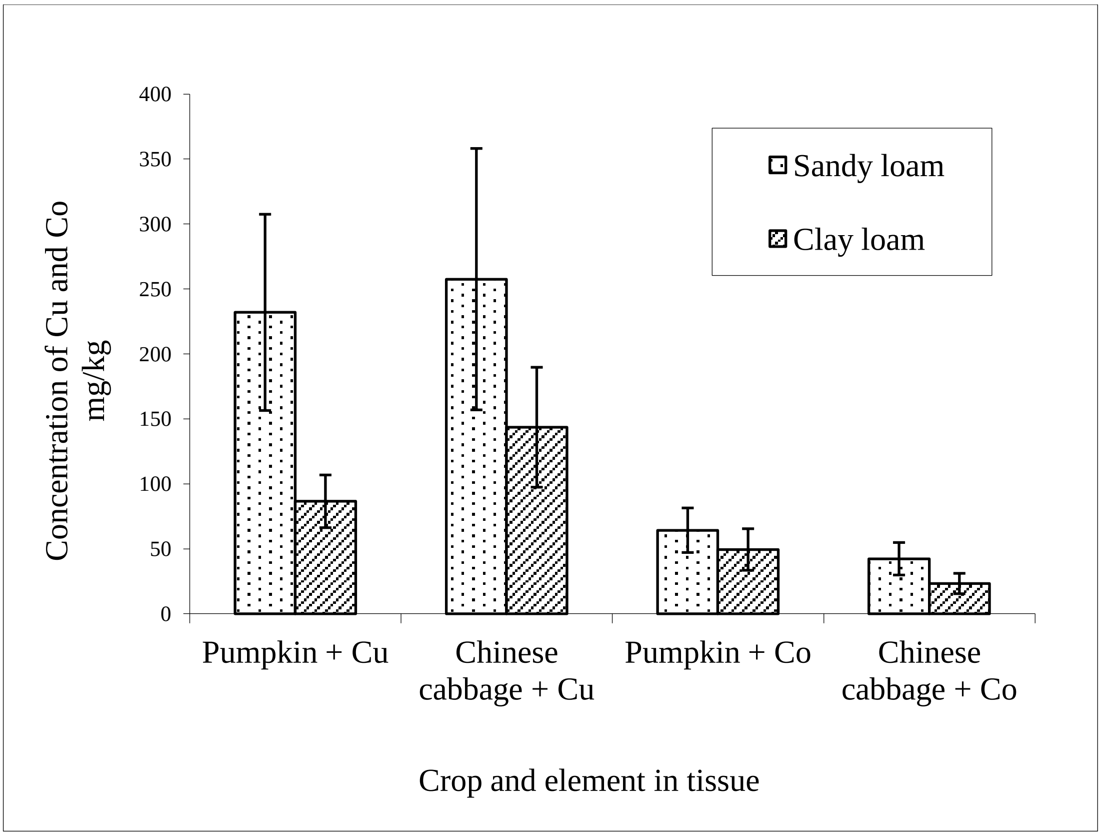


Figure 4.14 Concentration of Cu and Co in below ground tissue of Pumpkin and Chinese cabbage grown on sandy loam and clay loam and irrigated with contaminated water. The error bars are standard errors of means.

4.5.4 The effect of concentration of contaminated water on soils and uptake of Cu and Co by pumpkin and Chinese cabbage

Results in Figure 4.15 and Figure 4.16 present the average results from each treatment combination in the greenhouse study for Cu and Co in plant tissues. There is a general increase in Cu and Co uptake by pumpkin and Chinese cabbage at different levels of concentration of contaminants. For both Cu and Co, their uptake was higher when grown on sandy loam than clay loam soil. For example, the concentration of Cu in pumpkin when irrigated with undiluted contaminated water (CW100%) was 192.0 mg/kg in sandy loam and 93.0 mg/kg in clay loam soil (Figure 4.13).

The pattern is repeated again for Co (Figure 4.14), where most of the element was taken up when grown on sandy loam than clay loam soil. In addition, Chinese cabbage had higher accumulation of Co at the same contaminant concentration than pumpkin. These results however, did not show statistically significant effects of the combined effects of the three factors: soil type, water concentration of contaminants and crop type in a factorial analysis.

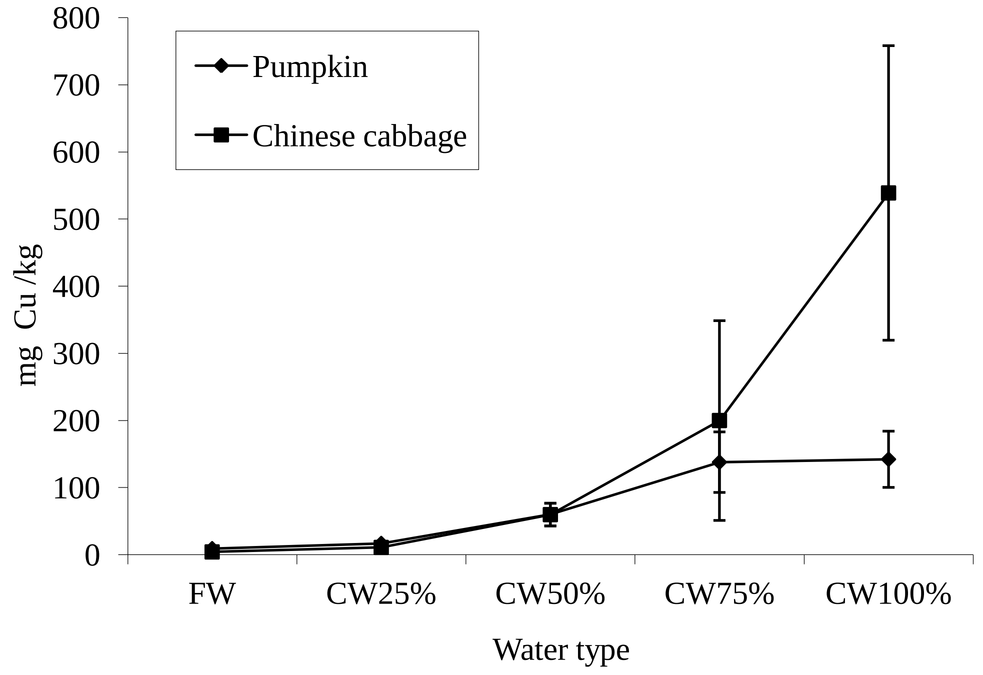


Figure 4. 15 Mean concentration of Cu in above ground plant tissue of Pumpkin and Chinese cabbage grown on sandy loam and clay loam soils and irrigated with contaminated water. The error bars are standard errors of means.

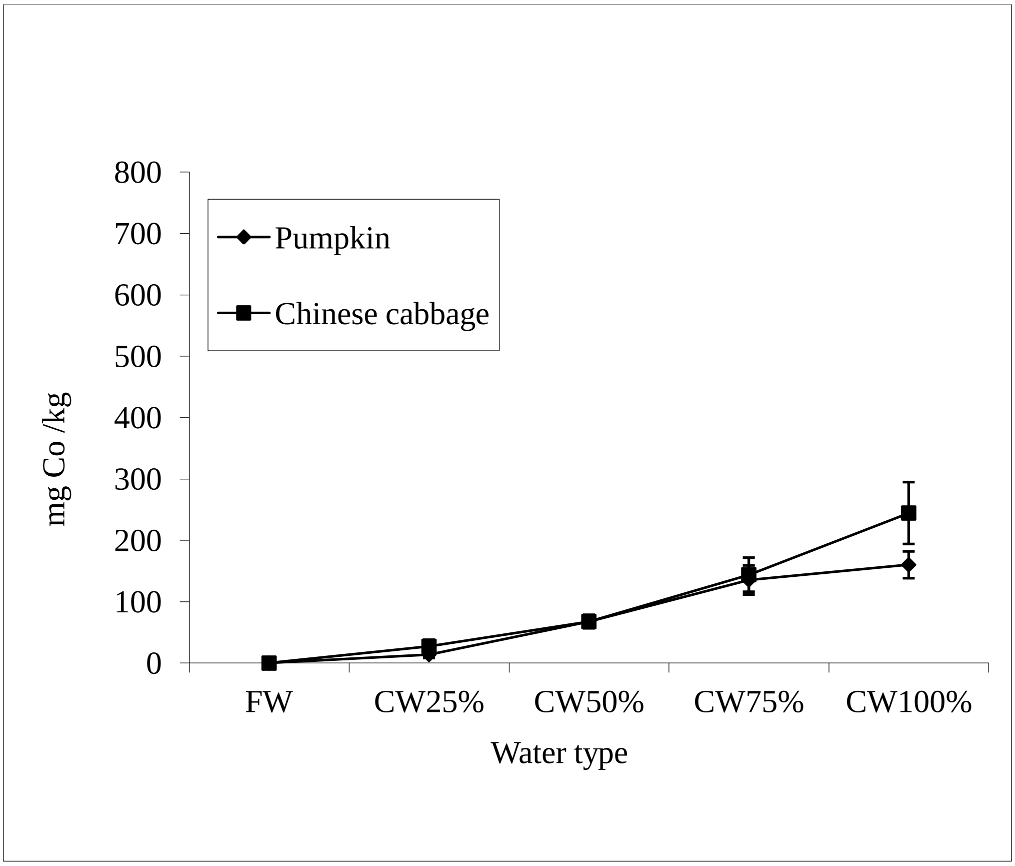


Figure 4. 16 Mean concentration of cobalt in above ground plant tissue of Pumpkin and Chinese cabbage grown on sandy loam and clay loam soils and irrigated with contaminated water. The error bars are standard errors of means.

Results of all the experimental treatment combinations are illustrated in Figure 4.17 for Cu and Figure 4.18 for Co concentrations in below ground plant tissue. The results show that there is a general tendency for the concentration of Cu and Co to increase with increasing concentration of irrigation water. However, statistically significant difference ($p<0.05$) in Cu or Co concentrations in below ground tissue was largely observed between control treatments (irrigated with FW) and those irrigated with contaminated water (CW). There were apparently no statistically significant differences among treatments using contaminated water.

On sandy loam soil, the uptake of Cu by pumpkin in below ground tissue increased from 8.0 mg/kg when irrigated with fresh water (FW or control) to 435.0 mg/kg when irrigated with undiluted contaminated water (CW100%). The uptake of Cu by pumpkin in below ground tissue on clay loam soil increased from 7.0 mg/kg when irrigated with fresh water (FW or control) to 205.0 mg/kg when irrigated with undiluted contaminated water (CW100%)

The concentration of Cu in below ground tissue of Chinese cabbage ranged from 5 mg/kg when irrigated with FW (control) to 589.0 mg/kg when irrigated with undiluted contaminated water (CW100%) on sandy loam soil compared to 6.0 mg/kg (when irrigated with FW) and 385.0 mg/kg (when irrigated with CW100%)

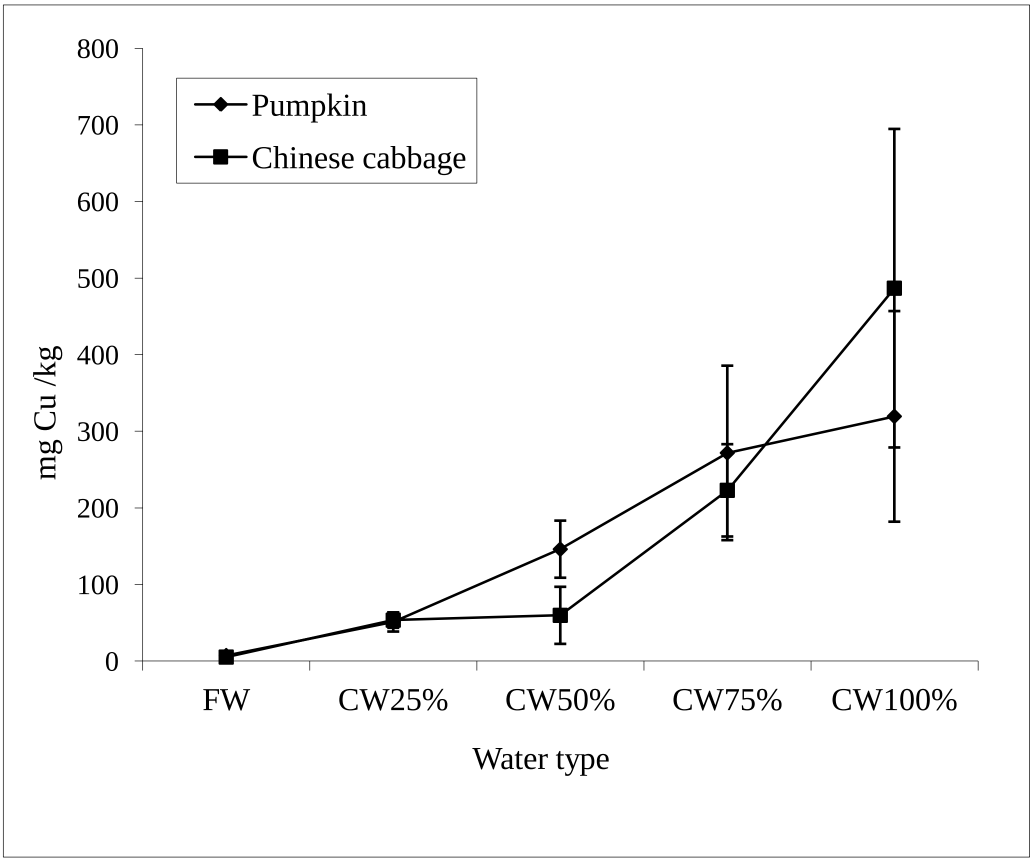


Figure 4.17 Concentrations of Cu in below ground tissue of Pumpkin and Chinese cabbage grown on sandy loam and clay loam and irrigated with contaminated water. The error bars are standard errors of means.

For Co, the uptake by pumpkin in below ground tissue increased from below detection when irrigated with fresh water (FW or control) to 76.4 mg/kg when irrigated with undiluted contaminated water (CW100%) on sandy loam soil. There was a somewhat lower Co uptake by below ground pumpkin tissue on clay loam soil from below detection (when irrigated with FW) to 121.0 mg/kg (when irrigated with CW100%). Again, these results did not show statistically significant effects of the combined effects of the three factors: soil type, water concentration of contaminants and crop type in a factorial analysis.

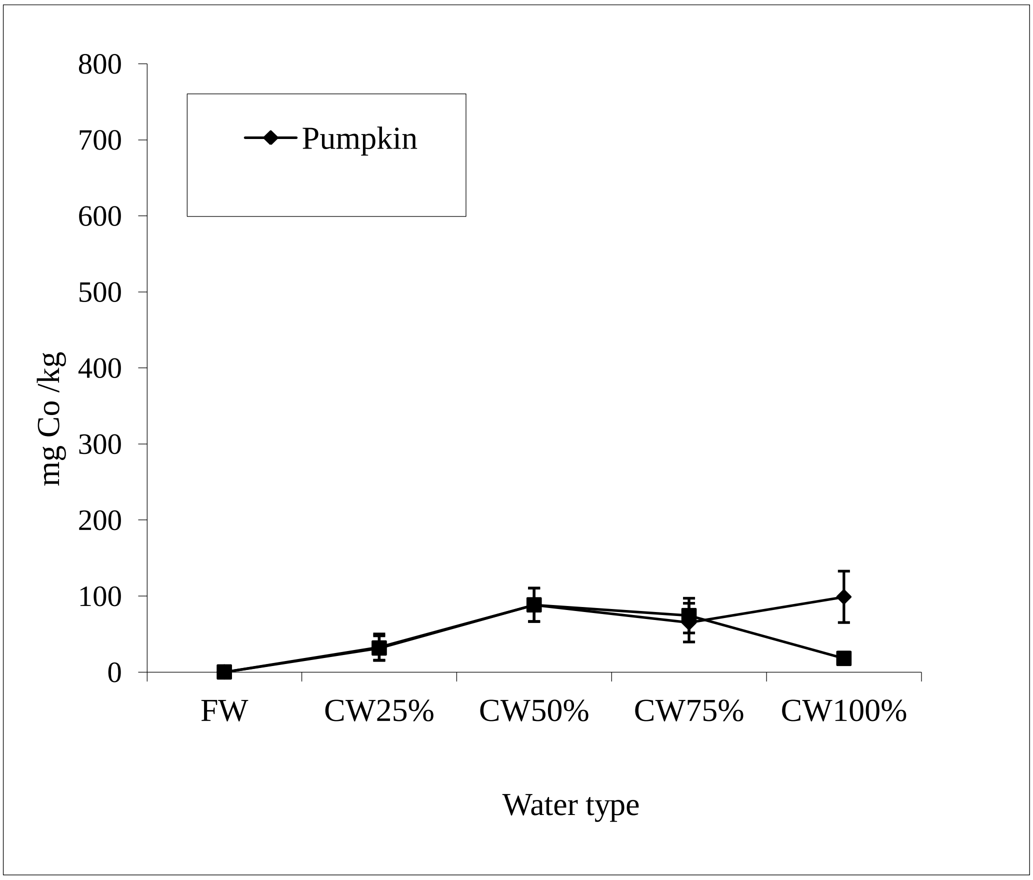


Figure 4.18 Concentrations of cobalt in below ground tissue of Pumpkin and Chinese cabbage grown on sandy loam and clay loam and irrigated with contaminated water. The error bars are standard errors of means.

The study showed that the concentration of Cu and Co in pumpkin and Chinese cabbage tissues was different when the crops are grown either on clay loam or sandy loam soil. The two crops in the study showed significantly higher concentrations of Cu and Co when grown on sandy loam than on clay loam soil. On average the concentration of Cu in plants grown on sandy loam soil was 217.4 mg/kg dry weight compared to 79.3 mg/kg when grown on clay loam. This observation was the same for Co, however, at reduced concentrations. The mean Co in plants grown on sandy loam soil was 86.6 mg/kg compared to 47.0 mg/kg on clay loam soil. These results show that the amount Cu and Co being transferred from contaminated water to plants were higher on sandy loam than on clay loam soil. These results are consistent with results obtained by Gharbi *et al.* (2005) who observed higher transfer of Cu on sandy soil than on clay soil.

Although both crop species exhibited significantly higher uptake of Cu and Co on sandy loam soil than clay loam, the magnitude of uptake by Chinese cabbage was higher than in pumpkin over the same treatments. The mean concentration of Cu in Chinese cabbage on sandy loam soil was 276.7 mg/kg compared to 43.6 mg/kg on clay loam. The mean concentration of Cu in pumpkin on sandy loam soil was 103.3 mg/kg compared to 43.5 mg/kg on clay loam.

When the concentration of plant available Cu and Co were measured in the soil after harvesting the crops, it was observed that their concentrations were higher in clay loam than sandy loam. These results show that clay loam removes higher amounts of Cu and Co from contaminated water than sandy loam. Subsequently, the concentration of the Cu and Co in water that is passed on to plants is lower on clay loam than sandy loam soil. It is for this reason that plants irrigated with the same type of contaminated water show differences in amount of contaminants absorbed in them when grown on different soil types.

The removal of higher quantities of Cu and Co from contaminated water contributed to the reduction of these elements from the irrigation water. This reduction of Cu and Co from water in the soil resulted in the subsequent low uptake of these elements by Chinese cabbage and pumpkin in the experiment.

The result indicates that uptake of heavy metals can vary when crops are grown on soils with different characteristics such as clay and organic matter even when the levels of heavy metals in them are the same from irrigation water. The study brings out evidence that soil types can have significant effect on the uptake of heavy metals by crops from irrigation water that is contaminated. Soils with higher clay and organic matter would result in the reduction of concentration of the heavy metals in irrigation water thereby reducing the availability of toxic ions to plants. On the other hand, plants would absorb higher amounts of heavy metals when grown on sandy soil as they will be exposed to higher concentrations of toxic ions from contaminated water.

This result has significant implications on the levels of standards for disposal of waste water with respect to soil types where the water would be disposed off. Waste water disposal standards on sandy soils should be lower as compared to clay soil. This is because even lower concentrations of heavy metals could be toxic on sandy soils than on clay soils.

4.6 Evaluation of the effect of Cu and Co in contaminated water on crop yield

The results and discussion presented in this section relate to objective 4 of the study that was intended to evaluate the effect of Cu and Co in tissue on crop yield when irrigated with contaminated water. It was assumed increasing contamination in water would result in plants taking up high amounts of contaminants in water such as Cu and Co and consequently have toxic effects that would result in yield reductions.

4.6.1 Effects contaminated water on plant growth and yield

The growth of plants was observed throughout the duration of the experiment. The most obvious differences observed during the experiment were the low growth of plants watered with CW100% and CW75% on sandy loam soil. The first signs of wilting of plants from the effects of treatments started appearing after 43 days from the date of planting when pumpkin and Chinese cabbage on sandy loam soil irrigated with CW 100% started wilting. Plants growing on sandy loam soil and irrigated with CW100% had stunted growth and appeared yellowish compared to those grown on clay loam soil and irrigated with CW100% (Figure 4.19)

Figure 4. 19 Chinese cabbage and pumpkin plants growing on sandy loam and clay loam soils and irrigated with CW 100% contaminated water. The codes are in the order: Plant-Soil-water.

4.6.2 The effect of irrigating contaminated water on sandy loam and clay loam soil on the biomass yield of pumpkin and Chinese cabbage

Figure 4.20 shows the results of above ground biomass yield of Pumpkin and Chinese cabbage grown on sandy loam and clay loam soils and irrigated with contaminated water. Above ground biomass yield decreased with increasing concentration of contaminated water in a linear manner. The negative linear relationships for the treatments are: pumpkin above ground biomass and contaminated water on sandy loam was: $y = -0.919x + 8.445$ ($R^2 = 0.6282$), on clay loam $y = -1.392x + 12.444$ ($R^2 = 0.963$). For Chinese cabbage above ground biomass, the relationship can be explained by the negative linear relationship $y = -1.505x + 9.865$ ($R^2 = 0.8434$) on sandy loam and $y = -0.306x + 9.282$ ($R^2 = 0.906$) on clay loam soil.

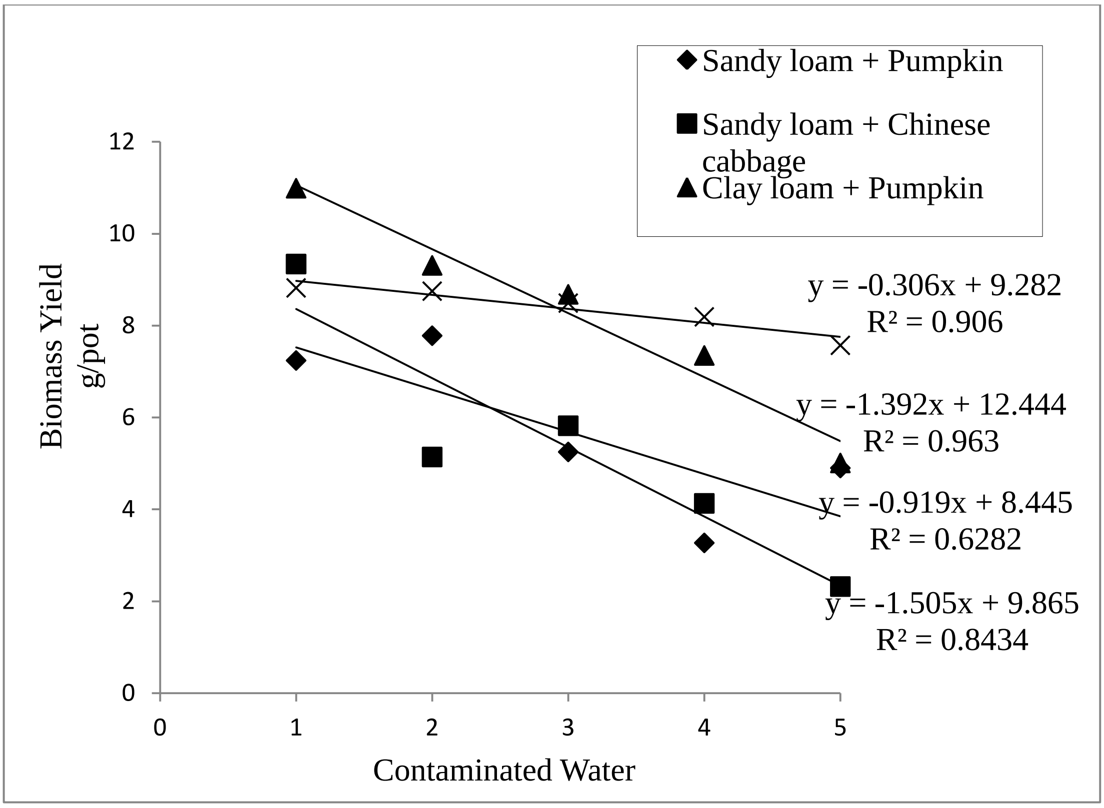


Figure 4. 20 Above ground biomass yield of Pumpkin and Chinese cabbage grown on sandy loam and clay loam soils and irrigated with contaminated water. The numbers on contaminated water are: 1 = FW, 2 = CW 25%, 3 = CW 50%, 4 = CW 75%, and 5 = CW 100%.

Figure 4.21 shows the results of below ground biomass yield of Pumpkin and Chinese cabbage grown on sandy loam and clay loam soils and irrigated with contaminated water. There was apparently some decrease in below ground biomass yield with increasing concentration of contamination of irrigation water on both sandy loam and clay loam soil. The mean yields were however, smaller on sandy loam than clay loam for both pumpkin and Chinese cabbage. The negative linear relationships for the treatments are: pumpkin above ground biomass and contaminated water on sandy loam was: $y = -0.3325x + 2.2161$ ($R^2 = 0.8593$), on clay loam $y = -0.1218x + 2.2026$ ($R^2 = 0.19$). For Chinese cabbage above ground biomass, the relationship can be explained by the negative linear relationship $y = -0.3118x + 1.9284$ ($R^2 = 0.7727$) on sandy loam and $y = -0.0571x + 1.0987$ ($R^2 = 0.4017$) on clay loam soil. The y is biomass yield and x is the fraction of element extracted in soil.

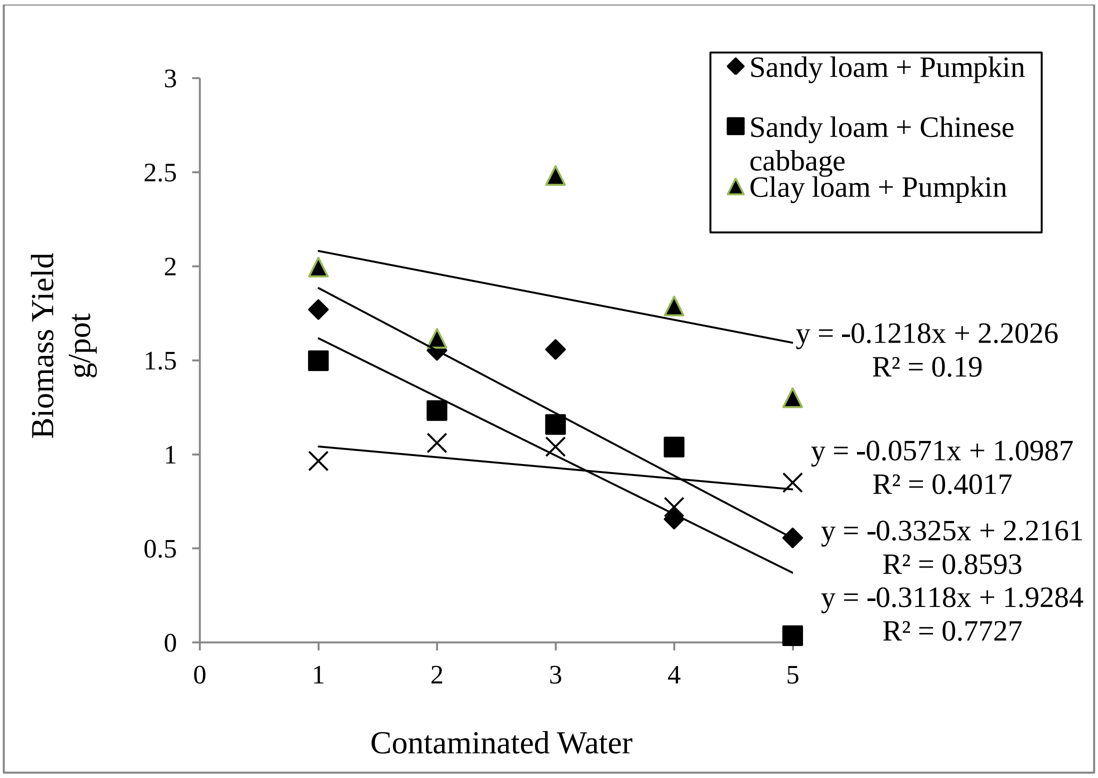


Figure 4. 21 Below ground biomass yield of Pumpkin and Chinese cabbage grown on sandy loam and clay loam soils and irrigated with contaminated water. The numbers on contaminated water are: 1 = FW, 2 = CW 25%, 3 = CW 50%, 4 = CW 75%, and 5 = CW 100%.

Chinese cabbage and pumpkin showed decline in both above and below ground biomass yield with increasing concentrations of contaminants (Cu and Co) in the irrigation water. Declines in yields with increasing concentration of contaminants observed indicates that the concentration of these contaminants in the irrigation water resulted in concentrations that were enough to induce toxicity that affected plant growth. Yield reduction begins only when the doses or intake has exceeded the tolerance levels of plants (Mertz, 1998). When the concentration of element reaches toxic level, the plant begins to show signs of contamination through colour changes or through yield reduction (Clarkson *et al.*, 1988; Mertz, 1998; Moolenaar and Lexmond, 1998). If the yields do not reduce or plants show no colour changes, then this could be an indication that plants are tolerant to contamination at that concentration of the heavy metal.

Normal Co concentration in plants have been cited to be between 0.1 and 10 mg/kg dry weight (Bakkus *et al.*, 2005) and that for Cu is 8-20 mg/kg dry weight (Shorrocks and Alloway, 1995). In this study, the lowest concentration of Cu in plant tissue was 17.0 mg/kg in above ground tissue of pumpkin while in Chinese cabbage it was 11.0 mg/kg in CW 25% treatments while the highest was 142 mg/kg for pumpkin and 539.0 mg/kg for Chinese cabbage in CW 100% treatments.

For Co, the lowest concentration in plant tissue was 13.8 mg/kg in above ground tissue of pumpkin while in Chinese cabbage it was 27.3 mg/kg in CW25% treatments while the highest was 160.2 mg/kg for pumpkin and 244.5 mg/kg for Chinese cabbage in CW100% treatments. Cu and Co are important for normal metabolic functions of plants but at higher concentrations, they can be toxic and severely interfere with biochemical and physiological functions of plants (El-Sheekh *et al.*, 2003; Parmar and Chand, 2005; Jayakumar *et al.*, 2008).

The effect of other elements and which were also found in the contaminated water in higher concentrations could also be important. The other heavy metal contaminants could also have confounding influences on the outcomes observed. Ginocchio *et al.* (2009) in their study of toxicity tests from Cu enriched mine wastes, observed that confounding effects from soil acidification had a confounding effect on results.

An important feature of the experiment that was examined in this study was combined effects of soil type, levels of contamination in irrigation water and crop type. A factorial analysis of the results of Cu and Co concentrations in above ground plant tissue showed that there were no statistically significant effects on combined effects of soil type, contaminated irrigation water and crop type on concentration of Cu and Co in plant tissue and above ground yield.

The most probable causes for the lack of combined effects are that there are likely to be interactions among these three treatments that cancel out the other significant effects from single treatment effects such as soil type and irrigation water and crop type effects that have been found to have statistically significant ($p<0.05$) effects on plant metal uptake and yield. This implies for example the factors that influence the differences in the levels of Cu and Co in above ground tissue due to soil differences are not the same as those due to concentration of irrigation water or crop species. The results however, imply there are several factors in the environment that can influence plant uptake of metals and these can be explained as single treatments or in pairs as was observed in other sections above. This finding illustrates the importance of including other variables during the examination of samples that could have several variables that can influence the outcomes.

For this study, it implies also that when crops are irrigated with contaminated water, the concentrations of metals that will be obtained in tissue can be influenced by soil type (Shorrocks and Alloway 1985; Baltrenaite and Butkus, 2004; Fedotov and Spivakov, 2008), level of concentrations of heavy metals in water (Khan *et al*., 2008) or crop species (Dogheim *et al*., 2004; Finster *et al*., 2004; El-Arby and Elbordiny, 2006). In this type of studies, analysing many variables at the same time to determine their combined effects may not yield satisfactory results to explain the interactions taking place. Using spiked water as control for elements of interest may help to improve understanding of what is taking place. Another way to understand the effect of confounding is to use multivariate analysis that would allow for adjustment of multiple variables simultaneously and used as “control” (Skelly *et al*., 2012)

4.6.3 **Effect of Cu concentration in plants on biomass yield**

The relationship between above ground plant Cu concentration in pumpkin and its plant biomass yield are shown in Figure 4.22. The experiment showed that there is a weak correlation between Cu content and above ground plant yield. The best fit-trend line fitted an exponential relationship. The data show that with increasing above ground Cu concentration, the biomass yield decreased exponentially according to the equation $Y=8.4895e^{-0.004x}$ with the model R^2 being 0.58. The parameter Y in the equation is above ground plant biomass Yield and X is the above ground plant Cu concentration.

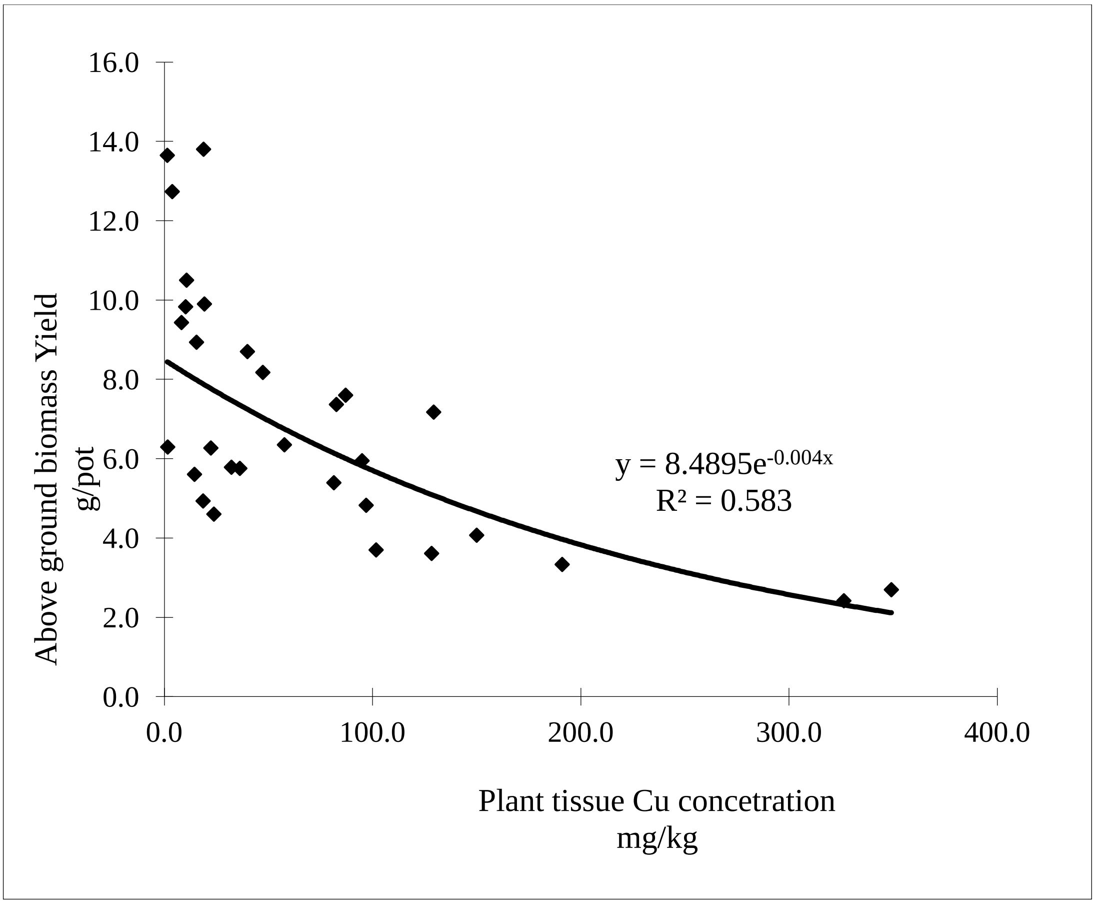


Figure 4. 22 Correlation between above ground plant biomass of pumpkin against the concentration of Cu in its above ground plant tissue.

The relationship between above ground plant Cu concentration in Chinese cabbage and its plant biomass yield are shown in Figure 4.23. The experiment showed that there is a fairly strong correlation between above ground plant Cu content and above ground plant yield per pot. The best fit-trend line fitted an exponential relationship. The data show that with increasing above ground Cu concentration, the biomass yield may decrease exponentially according to the equation $Y=7.624e^{-0.0016x}$ with the model R^2 being 0.737. The parameter Y in the equation is above ground plant biomass yield of Chinese cabbage and X is the above ground plant Cu concentration.

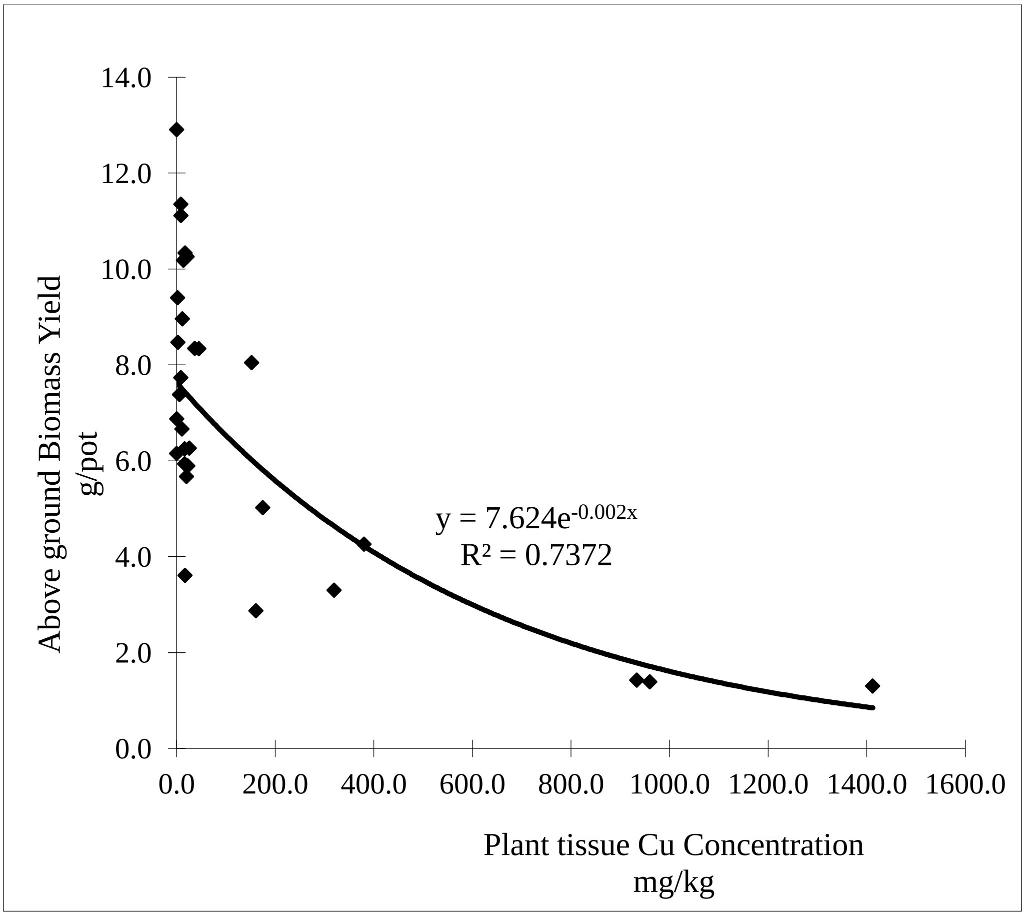


Figure 4. 23 Correlation between above ground plant biomass of Chinese cabbage against the concentration of Cu in its above ground plant tissue.

4.6.4 **Relationship of plant Co concentration and biomass yields**

The relationship between above ground plant Co concentration in Pumpkin and its plant biomass yield are shown in Figure 4.24. The experiment showed that there is a low correlation between above ground plant Co content and above ground plant yield per pot. The best fit-trend line fitted an exponential relationship. The data show that with increasing above ground Cu concentration, the biomass yield may be described by an exponential equation $y = 8.4803e^{-0.0039x}$ with the model R^2 being 0.3903. The parameter Y in the equation is above ground plant biomass yield of Pumpkin and X is the above ground plant Co concentration

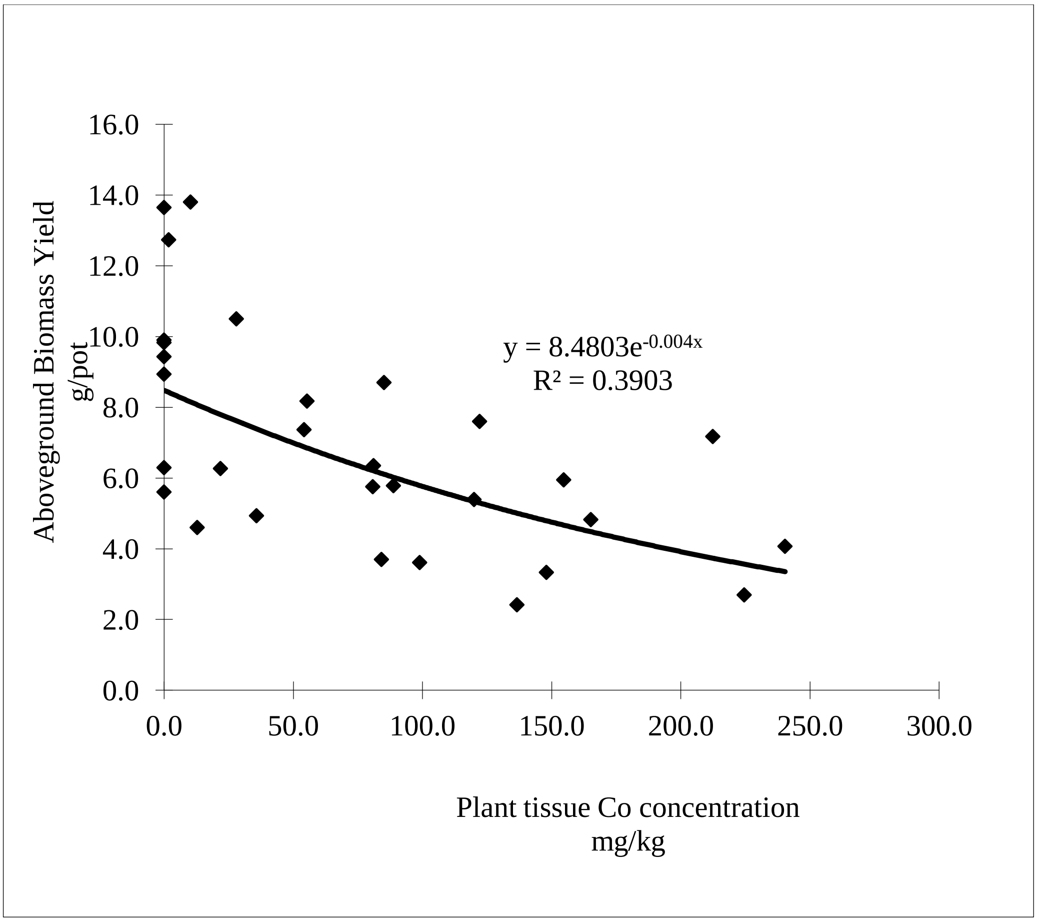


Figure 4. 24 Correlation between above ground plant biomass of Pumpkin against the concentration of Co in its above ground plant tissue.

The relationship between above ground plant Co concentration in Chinese cabbage and its plant biomass yield are shown in Figure 4.25. The experiment showed that there is a moderate correlation between above ground plant Co content and above ground plant yield per pot. The best fit-trend line fitted an exponential relationship. The data show that with increasing above ground Co concentration, the biomass yield may be described by an exponential equation $y=9.4289e^{-0.0045x}$ with the model R^2 being 0.63. The parameter Y in the equation is above ground plant biomass yield of Chinese cabbage and X is the above ground plant Co concentration.

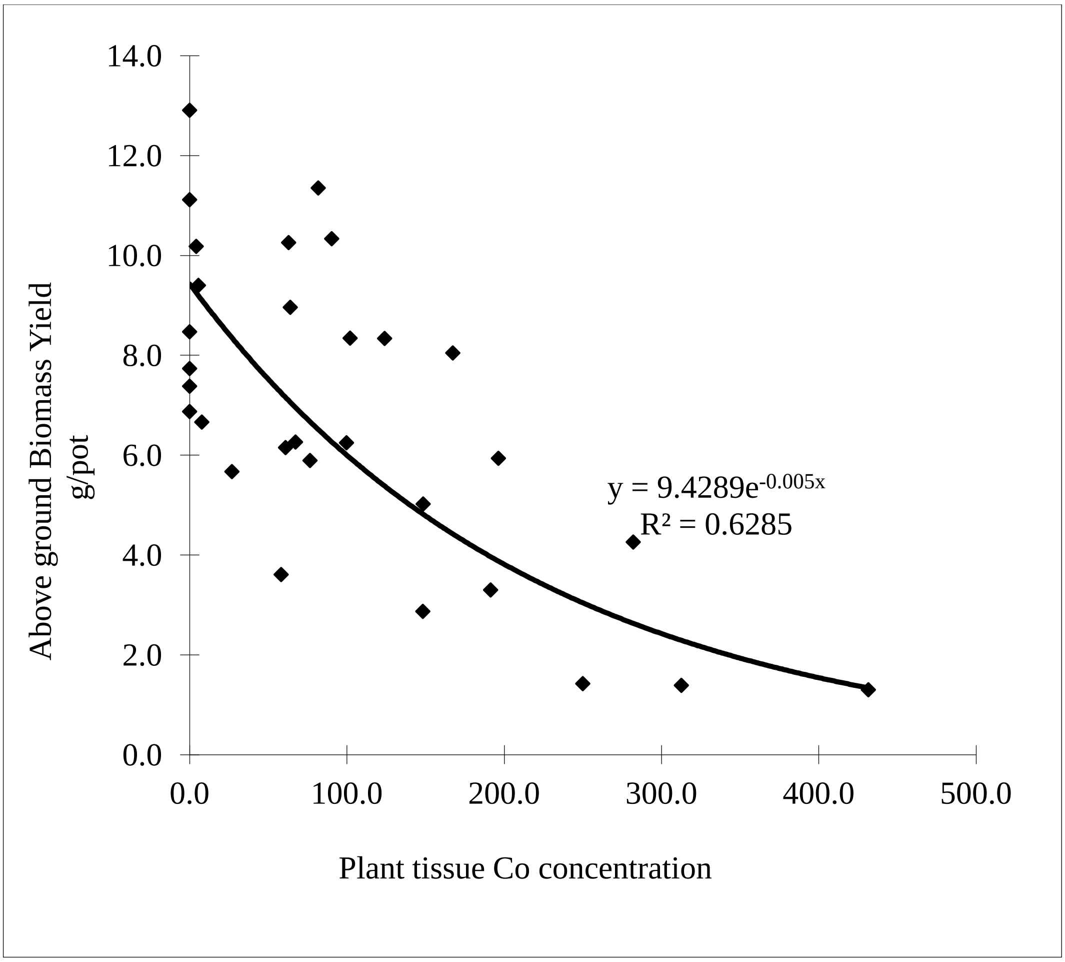


Figure 4. 25 Correlation between above ground plant biomass of Chinese cabbage against the concentration of Co in its above ground plant tissue.

The decline in above ground biomass for Chinese cabbage and pumpkin are consistent with results obtained by Jaleel *et al.* (2009) where they observed reduced yield parameters in maize when irrigated with water spiked with Co. Imtiyaz *et al.* (2014) also using Pb and Co spiked solutions for watering plants, observed reduced growth parameters such as root length and shoot weight of soyabean with increased uptake of Co.

These results show that any amount of Cu and Co beyond that of fresh water resulted in a decline in above ground yield of Chinese cabbage. The concentrations of Cu Chinese cabbage were in excess of 20 mg/kg the level of which has been found to cause toxicity and result in yield loss in crops (Tiller and Merry, 1981; Shorrocks and Alloway, 1985). Results in this study showing decline in dry matter yields are consistent with those found by Gharbi *et al.* (2005) where they obtained declining yields of Lettuce and spinach with increasing concentrations of Cu. In pumpkin, Co did not have a strong negative effect. It is possibly due to unknown roles of the element in the metabolic processes of pumpkin or perhaps the concentration in tissue was below the threshold of 100 mg/kg in plant tissue when toxicity effects for the metal are reported to be observed (Breckle, 1991; MOE., 2002; Lind, 2004).

4.6.5 **Relationship between the fractions of Cu and Co in soils and crop uptake of the elements**

Table 4.11 presents' results of the relations between pumpkin and Chinese cabbage above and below ground plant Cu and Co concentrations and the different fractions extracted in soils. The results presented are only for those whose R^2 values were greater than 50%. Some fractions like Cu in the water soluble, Co in the organic, Co in the sulphide and both Cu and Co in the carbonate were not detected.

The relationships were generally stronger with the organic fractions with R^2 values from 59.43 % to 89.95 %. In the exchangeable fractions the R^2 ranged from 53.42 % to 58.57 %. In the water soluble fractions against Pumpkin below ground fractions of Co in clay soil, the R^2 was 51.18 %.

In the water soluble fraction, only a moderate relationship between Co in pumpkin below ground parts and the fraction in clay loam soil was observed. The relationship can be explained by a linear equation $y = -101.44x + 91.301$ ($R^2 = 0.5118$).

Table 4.11. Relationship of Cu and Co in crops and fractions of the Cu and Co in soils

Relation	Equation	R^2
Pumpkin below ground Co Vs water soluble Co fractions in clay loam	$y = -101.44x + 91.301$	0.5118
Chinese cabbage above ground Cu Vs Organic Cu fractions in clay loam	$y = 0.8409e^{0.0631x}$	0.6132
Pumpkin above ground Cu Vs Organic fractions Cu in clay loam	$y = 3.4286e^{0.0462x}$	0.5943
Chinese cabbage below ground Cu Vs Organic Cu fractions in clay loam	$y = 2.7755e^{0.0709x}$	0.8995
Pumpkin below ground Cu Vs Organic Cu fractions in Sandy loam	$y = 5.613e^{0.1251x}$	0.6493
Pumpkin below ground Cu Vs Organic Cu fractions in clay loam	$y = 4.4297e^{0.0557x}$	0.8712
Chinese cabbage below ground Cu Vs Exchangeable Cu fractions in clay loam	$y = 19.882e^{0.1095x}$	0.5857
Pumpkin below ground Cu Vs Exchangeable Cu fractions in clay loam	$y = 24.389e^{0.0783x}$	0.5342
Chinese cabbage above ground Co Vs Exchangeable Co fractions in Sandy loam	$y = 13.233x + 82.305$	0.5461
Chinese cabbage above ground Co Vs Exchangeable Co fractions in clay loam	$y = 7.9226x + 9.9264$	0.5791
Pumpkin above ground Co Vs Exchangeable Co fractions in clay loam	$y = 6.499x + 11.633$	0.5343

The organic fractions on both soils tended to show stronger exponential relationship with plant Cu uptake by both crops. Figure 4.26 shows the relationship between the organic Cu fractions and below ground plant Cu concentrations in pumpkin on sandy loam soil. The relationship is explained by the exponential equation, $y = 5.613e^{0.1251x}$ ($R^2 = 0.6493$)

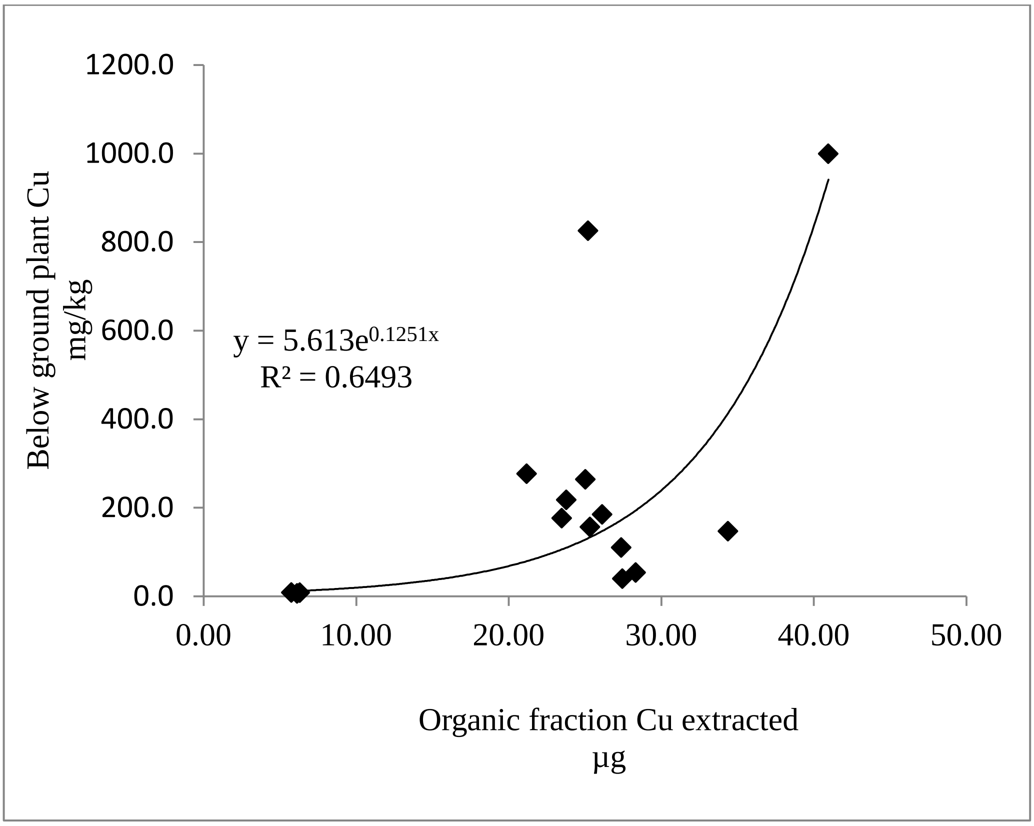


Figure 4.26 Relationship between the organic Cu fractions and below ground plant Cu concentrations in pumpkin on sandy loam soil.

Figure 4.27 shows the relationship between the organic Cu fractions and below ground plant Cu concentrations in pumpkin on clay loam soil. The relationship is explained by the exponential equation, $y = 4.4297e^{0.0557x}$ ($R^2 = 0.8712$). The y is below ground biomass yield and x is the organic fraction Cu extracted in soil.

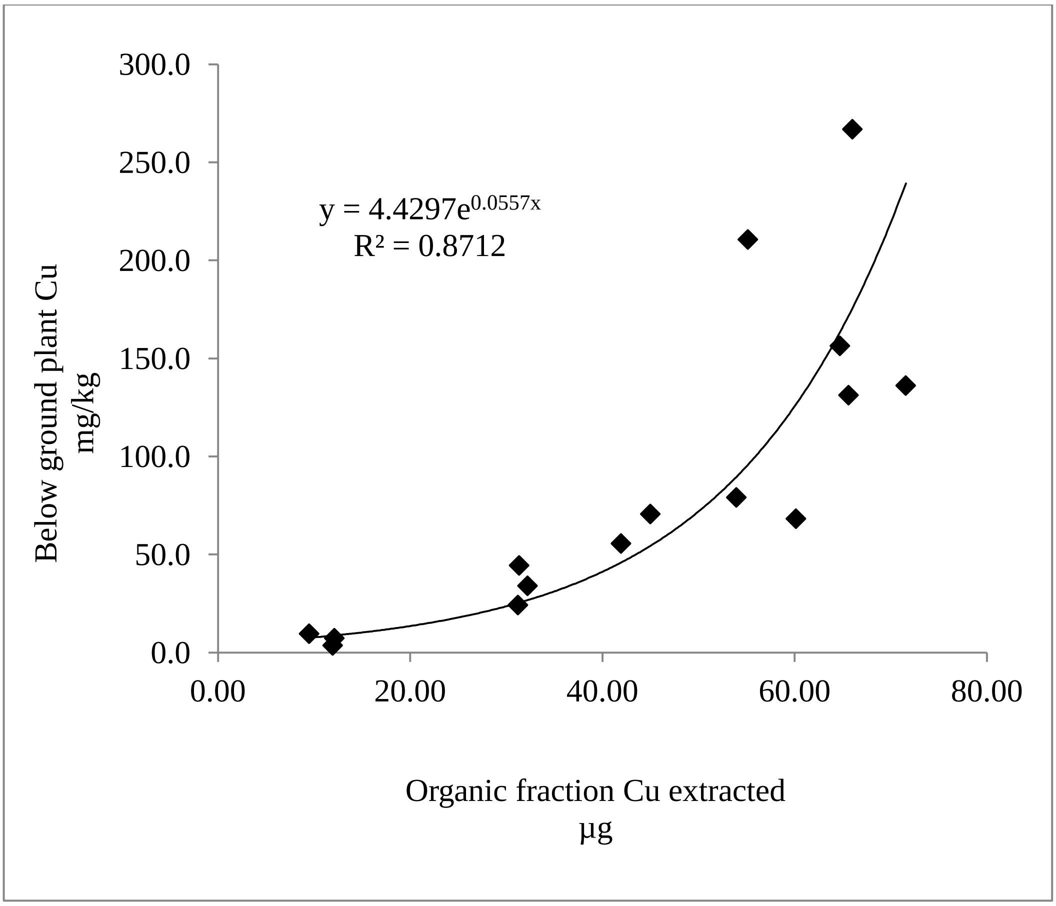


Figure 4.27 Relationship between the organic Cu fractions and below ground plant Cu concentrations in pumpkin on clay loam soil.

In both figures 4.26 and 4.27, as the amount of Cu extracted from the organic fraction increases, the concentration of Cu in plant tissue also increases exponentially. However, the proportion increase on sandy loam is greater than that on clay loam. This is attributed to the organic matter which is higher on clay loam soil, thus retaining more Cu. The organic matter content in the sandy loam soil was 2.13 % compared to 4.32 % in clay loam soil.

Figure 4.28 shows the relationship between the exchangeable Co fractions and above ground plant Co concentrations in Chinese cabbage on sandy loam soil. The relationship is explained by the linear equation, $y = 13.233x + 82.305$ ($R^2 = 0.5461$). The y is below ground biomass yield and x is the organic fraction Cu extracted in soil.

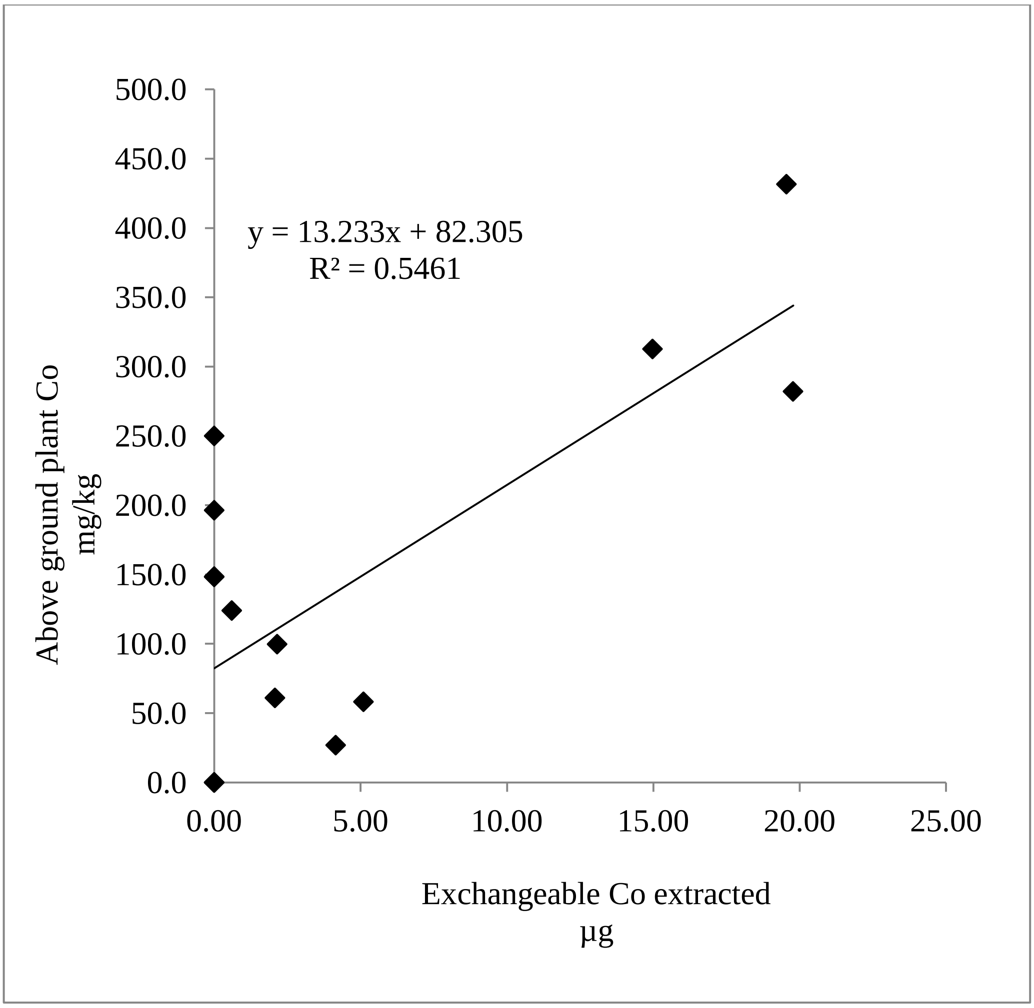


Figure 4.28 Relationship between the exchangeable Co fractions and above ground plant Co concentrations in Chinese cabbage on sandy loam soil.

Figures 4.29 and shows the relationship between the exchangeable Co fractions and above ground plant Co concentrations in Chinese cabbage on clay loam soil. The relationship is explained by the linear equation, $y = 7.9226x + 9.9264$ ($R^2 = 0.5791$). The y is above ground biomass yield and x is the organic fraction Co extracted in soil.

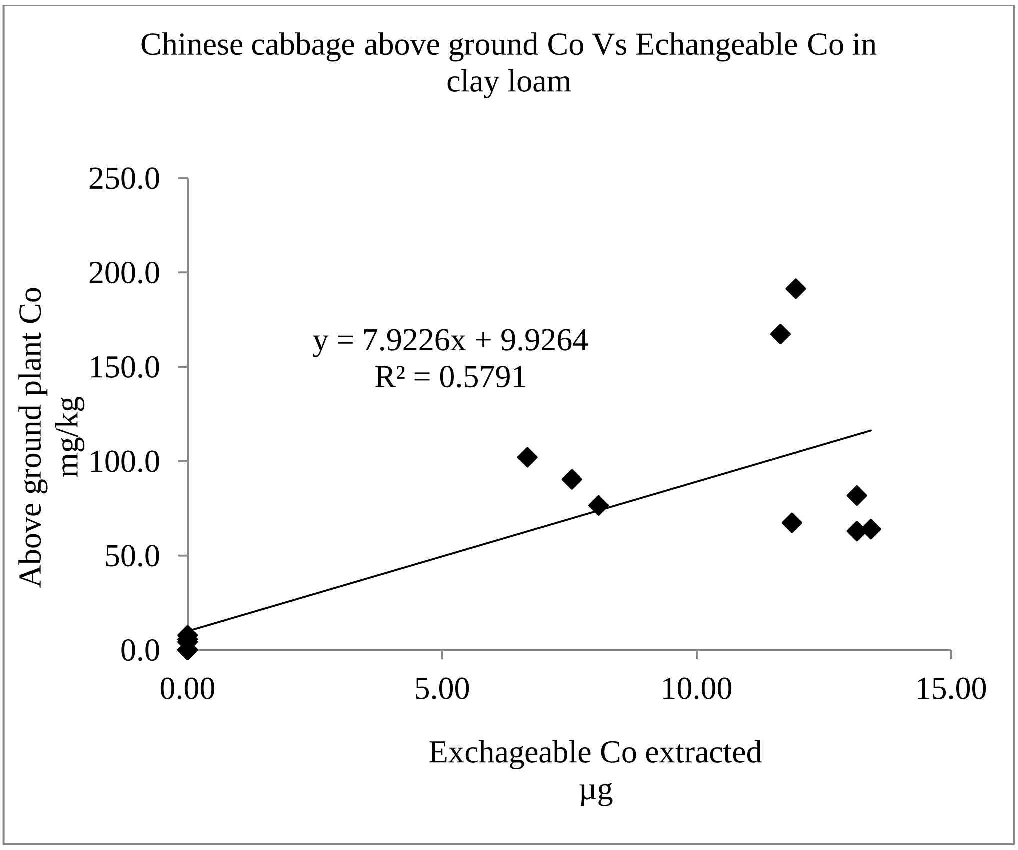


Figure 4.29. Relationship between the exchangeable Co fractions and above ground plant Co concentrations in Chinese cabbage on clay loam soil.

The relationship between concentrations of Cu and Co in crops with the exchangeable fraction shows a general positive relationship indicating that as the amount of the elements increase on the fractions, the uptake by plants tends to increase also. However, when observe the differences exhibited in Chinese cabbage, the crop takes up more Co from sandy loam (see Figure 4.28) than from clay loam (see Figure 4.29).

The exchangeable fractions are largely from the clay in soil, which in this study was higher in clay loam than sandy loam soil. This is also the possible observation of higher plant uptake of Cu and Cu from sandy loam than clay loam soil.

CHAPTER 5:CONCLUSIONS AND RECOMMENDATIONS

5.1 Main Conclusions

The conclusions are made bearing in mind the objectives (Chapter 1) of this study.

5.1.1 Cu and Co concentration on sandy loam and clay loam soils after they have been irrigated with contaminated water

The study established that concentration of plant available Cu and Co in soils at end of the experiment increased in all treatments where contaminated water was used for irrigating the crops.

It was also established that an increase in the concentration of the contaminants in irrigation water resulted in increased concentration of Cu and Co in clay loam and sandy loam soils. It was also established that the soil becomes saturated with the Cu and Co from the contaminated irrigation water at levels below the highest concentrations used in the experiment.

Heavy metals in soils increased with concentration of pollutants in irrigation water until a maximum level. It was established that the concentration of available Cu and Co in clay soil increased and levelled at 38 and 21 mg/kg soil, respectively. The concentration of available Cu and Co in sandy soil levelled at 16.7 and 15.3 mg/kg soil respectively. The maximum point of heavy metal concentration in soils may coincide with their saturation of the capacity to hold cations. The capacity to hold heavy metals was higher for clay loam soil than it was for sandy loam soil. The concentration of plant available Cu and Co on clay loam was 1.7 times than sandy loam soil for both elements. This result may result in high adsorption of heavy metals on soils thus reducing concentration of metals available to plants in soil. A further consequence of reduced concentration of metals to plants in soil results in plants growing in these soils taking up lower amounts of heavy metals. Plants growing in clay soil would have lower heavy metal content than those on sandy loam soil.

5.1.2 The fractions of Cu and Co in sandy loam and clay loam soils which had been irrigated with contaminated water

The general trend showed that Cu and Co was largely held on the organic and exchangeable fractions on both clay loam and sandy loam soil at the end of the experiment. The study also shows the amounts of Cu extracted from the exchangeable and organic fractions on clay loam were about double that on sandy loam soil.

It is apparent that organic and exchangeable fractions are the primary forms in which Cu is adsorbed in both sandy loam and clay loam soils. While the primary forms in which Co is held in sandy loam and clay loam soils was in water soluble and exchangeable fractions.

5.1.3 Uptake of Cu and Co by pumpkin and Chinese cabbage from contaminated water on sandy loam and clay loam soils

It was established from this study that Chinese cabbage has a higher uptake of heavy metals such as Cu and Co than pumpkin when irrigated with contaminated irrigation water. The magnitude of difference in the uptake of heavy metal uptake between Chinese cabbage and pumpkin was found to be about 50% or more.

The study also confirmed that differences in the uptake of heavy metals can occur when crops are grown on sandy or clay loam soils. Crops grown sandy soils had a higher susceptibility to take up heavy metals than on clayey soils. Clay loamy soil adsorbed high amounts of heavy metals than sandy loam. The differences in the uptake of heavy metals on different soils should provide explanations of observations in which differences have been observed in heavy metal concentrations in crops over different edaphic areas.

5.1.4 Effect on crop yield to concentration of Cu and Co in plant tissues

In this study, Cu and Co concentrations in plants were the only two elements measured in plant tissues. The biomass yields of both crops decreased with increasing concentrations of Cu and Co in both above and below ground tissues. Declines in yields with increasing concentration of Cu and Co observed indicates that the concentration of these elements in plant tissues resulted in concentrations that were enough to induce toxicity that affected plant growth. The concentrations of Cu and Co in the tissues were much higher than normal for plants.

5.2 Recommendations

The findings of study provide opportunities for further research in the areas of heavy metal in the environment. Some of these suggested recommendations are presented below:

5.2.1. In this study, the elements of interest were Cu and Co as the waste water used was from a smelter that processed these two metals. The interpretations of the results were done only with reference to these two elements. There is an opportunity to carry out similar research using a wider number of elements and assessing the contribution of each to crop observations.

5.2.2. The findings from this study identified the effects of different fractions on retention of Cu and Co in sandy loam and clay loams. Including the different fractions of heavy metal contaminants found in soils will give better understanding of the environmental implications. It is recommended that those studying soils and how they get contaminated by different materials should go beyond looking at the total and available forms by including the fractions in which they are found. A wider number of soil types could be used and a more direct relation of how fractions contribute to crop observations and ecology can be elucidated. The fractions of the elements give a better explanation of the environmental impacts of heavy metals in soils in the short and long term perspectives.

5.2.3. The findings will be disseminated to stakeholders through workshops on the Copperbelt and Lusaka among the stakeholders who have the responsibility for managing the environment, decision makers within local government, appropriate ministries of government, local leaders and affected farmers in areas where they use waste water for farming. Publications in peer reviewed journals have been done.

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APPENDICES

Appendix 8.1 **Refereed papers published from this study**

1. John Volk and Benson H. Chishala (2016). Response of Pumpkin and Chinese Cabbage to Increasing Copper and Cobalt Levels in Irrigation Water on Sandy and Clay Loam Soils. *Journal of Agricultural Science*; Vol. 8, No. 8; 98-111. doi:10.5539/jas.v8n8p98
2. John Volk and Olusegun Yerokun (2016) Effect of Application of Increasing Concentrations of Contaminated Water on the Different Fractions of Cu and Co in Sandy Loam and Clay Loam Soils. *Agriculture*, *6*(4), 64; doi:10.3390/agriculture6040064

Appendix 8.2 **Thesis contribution to knowledge**

The study of concentration of Cu and Co in crop plants under increasing concentration of contaminants in irrigation water between different soils is important to explain the transfer heavy metals under different edaphic conditions. It was observed from this study with increasing concentration of contaminants in irrigation water resulted in statistically significant increase ($p < 0.05$) in concentration of Cu and Co in plant tissues. However, it was also observed crops took up significantly higher ($p< 0.05$) levels of Cu and Co on sandy loam than on clay loam soil. The mean Cu content plants grown on sandy loam was 217.4 mg/kg for plants grown on sandy loam and 79.3 mg/kg for those grown on clay loam. The highest Co concentration in plant tissue on plants grown on sandy loam was 86.6 mg/kg compared to 47.0 mg/kg on clay loam soil. This finding implies that crops grown on sandy soils will accumulate higher levels of heavy metals than clay soils and thus recommendations for waste water use should include soil type. Current waste water use and disposal recommendations (GRZ, 2011) do not include the edaphic conditions on which this water can be discharged and thus posing higher risks to its use on sandy than clayey soils.

The general trend indicated that there was an increase in the amounts of Cu and Co extracted from the two soils at the end of the experiment when compared with the beginning. However, when the forms of Cu and Co were separated by fractionation to show the forms they were adsorbed, the general trend showed higher organic and exchangeable fractions from soils. This finding contributes to important knowledge gap that explains what happens to heavy metals in soil that would explain the conditions that would make the elements available at different time scales. Organically bound heavy metals may be made unavailable in the short term but could be desorbed over a long period as a result of decomposition and mineralisation.

This study provides evidence for importance of examination of crop plants for heavy metal levels in crops especially those grown in peri urban areas using waste water. It also provides evidence differences among crop species in their concentration of heavy metals and that *brassicas* are among the highest accumulators (Máthé-Gáspár and Anton, 2002; Mohsen and Mohsen, 2008; Arora *et. al.*, 2008). It was observed that when irrigated with contaminated water, Chinese cabbage concentrated significantly higher ($p < 0.05$) Cu than pumpkin in Chinese cabbage than in pumpkin plants. The mean Cu content in Chinese cabbage was 180.0 mg/kg while in pumpkin it was 116.3 mg/kg. The concentration of Cu was higher than the maximum legal limits of 50 mg/kg for Zambia (GRZ, 1995) or 40 mg/kg (FAO/WHO, 1995). The threshold for Cu toxicity to plants which is 20 mg/kg (Shorrocks and Alloway, 1985).

The mean Co content in pumpkin and Chinese cabbage was around 66.0 mg/kg with the highest being 160.2 mg/kg for pumpkin and 244.5 mg/kg for Chinese cabbage in CW100% treatments. The highest values for tissue Co are higher than the concentration threshold for toxicity in plants which is between 25-100 mg/kg (MOE, 2002). For the two crops, Chinese cabbage and pumpkin, despite higher concentrations of Cu and Co, marginal yield declines and no visible plant toxicity effects were observed. This observation from the research has significant implications that vegetables that would appear physically healthy may have higher levels of heavy metals that could be unhealthy for human and animal consumption.

The study established that the different treatments and their combinations were analysed, the responses and their magnitudes were different and explained different factors. This implies for example, that the factors which influence the differences in the levels of Cu and Co in above ground tissue due to soil differences are not the same as those due to concentration of irrigation water or crop species. With this observation, the study confirms that there are several factors in the environment which influence plant uptake of metals and these can be explained as single treatments or in pairs as was observed in other sections above. This finding illustrates the importance of including other variables during the examination of samples that could have several variables that can influence their outcomes.

Literature present on heavy metals problems in Zambia have only measuring their concentration in water, plants and soil. This study has gone beyond the measurement of heavy metals in water, plants and soil but established the differences in their retention between sandy loam and clay loam and identified soil factors that influenced transfer from soil solution to plants

Appendix 8.3 Water applications in the treatments during the conduct of green house experiment

Month-Year	Date	Cumulative days from planting	Cumulative weeks from planting	Cumulative days from germination	Cumulative days from germination	Amount Water applied /pot	Cumulative water applied /pot	
	day			Chinese cabbage	Pumpkin			
		Days	Weeks	Days	Days	ml	ml	
May-08	21	1 (Date Planted)				350	350	
May-08	22	2				350	700	
May-08	23	3		1 (Date First Germination)		0	700	
May-08	24	4		2		175	875	
May-08	25	5		3		0	875	
May-08	26	6		4		175	1050	
May-08	27	7	1	5		175	1225	
May-08	28	8		6		175	1400	
May-08	29	9		7	1 (Date First Germination)	0	1400	
May-08	30	10		8	2	175	1575	
May-08	31	11		9	3	175	1750	
Jun-08	1	12		10	4	0	1750	
Jun-08	2	13		11	5	175	1925	
Jun-08	3	14	2	12	6	0	1925	
Jun-08	4	15		13	7	175	2100	
Jun-08	5	16		14	8	0	2100	
Jun-08	6	17		15	9	175	2275	
Jun-08	7	18		16	10	0	2275	

Jun-08	8	19		17	11	175	2450	
Jun-08	9	20		18	12	175	2625	
Jun-08	10	21	3	19	13	175	2800	
Jun-08	11	22		20	14	0	2800	
Jun-08	12	23		21	15	175	2975	
Jun-08	13	24		22	16	175	3150	
Jun-08	14	25		23	17	0	3150	
Jun-08	15	26		24	18	175	3325	
Jun-08	16	27		25	19	175	3500	
Jun-08	17	28	4	26	20	175	3675	
Jun-08	18	29		27	21	0	3675	
Jun-08	19	30		28	22	175	3850	
Jun-08	20	31		29	23	350	4200	
Jun-08	21	32		30	24	175	4375	
Jun-08	22	33		31	25	175	4550	
Jun-08	23	34		32	26	175	4725	
Jun-08	24	35	5	33	27	175	4900	
Jun-08	25	36		34	28	175	5075	
Jun-08	26	37		35	29	175	5250	
Jun-08	27	38		36	30	175	5425	
Jun-08	28	39		37	31	175	5600	
Jun-08	29	40		38	32	175	5775	
Jun-08	30	41		39	33	175	5950	
Jul-08	1	42	6	40	34	175	6125	
Jul-08	2	43		41	35	175	6300	
Jul-08	3	44		42	36	175	6475	
Jul-08	4	45		43	37	175	6650	
Jul-08	5	46		44	38	175	6825	
Jul-08	6	47		45	39	175	7000	
Jul-08	7	48		46	40	175	7175	
Jul-08	8	49	7	47	41	175	7350	

Jul-08	9	50		48	42	175	7525	
Jul-08	10	51		49	43	175	7700	
Jul-08	11	52		50	44	175	7875	
Jul-08	12	53		51	45	175	8050	
Jul-08	13	54		52	46	175	8225	
Jul-08	14	55		53	47	175	8400	
Jul-08	15	56	8	54	48	175	8575	Last day for watering
Jul-08	16	57		55	49			All plants harvested
Jul-08	17	58		56	50			
Jul-08	18	59		57	51			
Jul-08	19	60		58	52			
Jul-08	20	61		59	53			
Jul-08	21	62		60	54			
Jul-08	22	63	9	61	55			
Jul-08	23	64		62	56			
Jul-08	24	65		63	57			
Jul-08	25	66		64	58			
Jul-08	26	67		65	59			

Appendix 8.4 Concentrations of Cu and Co in above and below ground tissue of plants.

Replicate 1 of the experiment.

Plot No	Treatment No.	Above ground Cu	Above ground Co	Below ground Cu	Below ground Co
		mg/kg	mg/kg	mg/kg	mg/kg
101	125	81.5	120.0	210.7	200.9
102	112	18.7	35.8	110.4	111.1
103	214	174.7	148.5	365.9	93.9
104	114	191.1	148.0	185.1	125.3
105	115	97.0	165.2	999.8	191.5
106	212	0.0	61.0	85.0	78.3
107	221	0.2	0.0	9.6	0.0
108	124	39.9	85.2	68.3	4.5
109	113	32.3	88.8	176.8	190.0
110	222	1.9	5.6	23.9	0.0
111	211	0.0	0.0	0.0	0.0
112	122	3.8	1.8	34.0	5.6
113	213	16.2	99.8	253.5	67.1
114	225	8.6	81.8	139.8	55.0
115	111	1.7	0.0	8.8	0.0
116	121	1.5	0.0	7.3	0.0
117	224	17.1	90.4	129.2	58.2
118	223	25.7	67.4	160.2	38.5
119	123	10.7	28.0	70.7	61.0
120	215	380.2	282.1	1438.5	0.0

Appendix 8.4 (continuation) Concentrations of Cu and Co in above and below ground tissue of plants from Replicate 2 of the experiment.

Plot No	Treatment No.	Above ground Cu	Above ground Co	Below ground Cu	Below ground Co
		mg/kg	mg/kg	mg/kg	mg/kg
201	123	47.3	55.4	79.1	42.9
202	212	20.0	26.9	74.5	29.3
203	111	10.3	0.0	8.5	0.0
204	211	5.8	0.0	5.6	0.0
205	223	21.3	63.0	107.5	9.7
206	215	1412.0	431.6	165.0	18.1
207	222	13.8	4.2	52.8	0.0
208	214	933.5	250.0	0.0	0.0
209	225	152.1	167.4	683.8	0.0
210	122	15.5	0.0	24.2	0.0
211	121	19.3	0.0	3.6	0.0
212	115	349.2	224.6	147.3	0.0
213	221	2.6	0.0	2.7	0.0
214	124	87.1	122.2	156.4	155.1
215	213	45.1	124.0	60.5	5.7
216	125	101.8	84.2	266.9	57.3
217	224	22.7	76.6	210.5	33.3
218	112	18.9	10.3	40.1	40.1
219	114	150.1	240.4	825.9	6.5
220	113	57.7	81.1	277.1	101.3

Appendix 8.4 (continuation) Concentrations of Cu and Co in above and below ground tissue of plants from Replicate 3 of the experiment.

Plot No	Treatment No.	Above ground Cu mg/kg	Above ground Co mg/kg	Below ground Cu mg/kg	Below ground Co mg/kg
301	214	16.1	196.4	395.2	158.5
302	124	36.3	80.8	131.3	53.6
303	213	160.9	148.3	789.3	83.6
304	122	22.4	21.9	44.4	0.0
305	211	8.5	0.0	8.7	0.0
306	212	17.0	58.2	57.4	81.5
307	223	11.6	64.0	32.2	39.1
308	113	128.4	99.0	218.0	78.3
309	115	129.4	212.4	156.8	37.8
310	221	8.7	0.0	5.5	0.0
311	112	23.8	12.9	53.9	38.5
312	125	95.0	154.7	136.2	105.2
313	114	326.4	136.6	264.4	44.3
314	225	319.5	191.4	330.6	14.6
315	111	14.5	0.0	6.7	0.0
316	123	82.7	54.2	55.6	56.4
317	121	8.3	0.0	9.6	0.0
318	222	10.8	7.8	27.8	0.0
319	224	36.7	102.1	237.5	100.8
320	215	959.9	312.7	164.0	19.4

Appendix 8. 5 Measurements from above and below ground tissues of plants

Replicate 1 of the experiment

Plot No	Treatment No.	No of harvested plants	Leaf Length. cm	Leaf width .cm	No of leaves	Total leaf dry wt. g	Root length. cm	Total Root dry wt. g	Total Plant Dry wt. g	Notes
101	125	4	20	9	23	5.4	23	1.4	6.8	
102	112	4	20	9	21	4.9	14	1.2	6.1	
103	214	4	15	6	28	5.0	13	0.8	5.8	
104	114	4	8	6.5	22	3.3	11	0.7	4.0	
105	115	4	8	6	18	4.8	8	0.5	5.4	
106	212	4	18	6	31	6.2	13	1.0	7.2	
107	221	4	18	6	36	6.9	15	1.2	8.1	
108	124	4	24	11	24	8.7	23	2.5	11.2	
109	113	4	23	10	21	5.8	20	2.5	8.3	
110	222	4	22	8	35	9.4	13	0.4	9.8	
111	211	4	23	7	46	12.9	11	1.4	14.3	
112	122	4	27	11	25	12.7	35	2.2	14.9	
113	213	4	18	7	31	6.2	18	1.4	7.6	
114	225	4	26	11.5	38	11.4	16	1.9	13.2	
115	111	4	20	9	24	6.3	19	1.3	7.6	
116	121	4	25	12	36	13.6	23	2.6	16.3	
117	224	4	20	7	32	10.3	11	1.1	11.4	
118	223	4	17	5	33	6.3	15	1.1	7.3	
119	123	4	21	10	23	10.5	20	3.2	13.7	
120	215	4	15	5	28	4.3	8	0.1	4.4	

Appendix 8.5 (continuation) Measurements from above and below ground tissues of plants. Replicate 2 of the experiment

Plot No	Treatment No.	No of harvested plants	Leaf Length cm	Leaf width cm	No of leaves	Total leaf dry wt g	Root length cm	Total Root dry wt g	Total Plant Dry wt g	Notes
201	123	4	22	9.5	24	8.2	30	1.9	10.1	
202	212	4	16	6.5	28	5.7	15	1.5	7.2	
203	111	4	22	9.5	28	9.8	22	2.3	12.1	
204	211	4	16	6	28	7.4	15	1.3	8.7	
205	223	4	21	6	34	10.3	15	1.1	11.3	
206	215	4	3	2.5	11	1.3	8	1.0	2.3	Wilted plants
207	222	4	19	7	36	10.2	19	1.6	11.7	
208	214	4	7	3	15	1.4	6	0.3	1.7	
209	225	4	16	6.5	36	8.0	12	0.2	8.3	
210	122	4	22	9	25	8.9		1.9	10.8	
211	121	4	25	10.5	28	9.9	25	1.7	11.6	
212	115	4	7	6	14	2.7	12	0.4	3.1	
213	221	4	22	8.5	36	8.5	22	1.2	9.6	
214	124	4	20	7	20	7.6	30	1.3	8.9	
215	213	4	19	6	38	8.3	23	1.7	10.1	
216	125	4	14	6	21	3.7	24	1.0	4.7	
217	224	4	16	5.5	33	5.9		0.3	6.2	
218	112	4	21	10	30	13.8	22	2.6	16.4	
219	114	4	10	10	17	4.1	19	0.7	4.7	
220	113	4	21	10	23	6.4	23	1.4	7.8	

Appendix 8.5 (continuation) Measurements from above and below ground tissues of plants

Replicate 3 of the experiment.

Plot No	Treatment No.	No of harvested plants	Leaf Length cm	Leaf width cm	No of leaves	Total leaf dry wt g	Root length cm	Total Root dry wt g	Total Plant Dry wt g	Notes
301	214	4				5.9	38	2.0	7.9	Wilted plants
302	124	4	19	9	20	5.8	34	1.5	7.3	
303	213	4	13	5	18	2.9	13	0.4	3.2	
304	122	4	21	9	21	6.3	33	0.8	7.1	
305	211	4	16	6	32	7.7	22	1.8	9.6	
306	212	4	12	4	30	3.6	24	1.1	4.8	
307	223	4	18	7	34	9.0	20	1.0	10.0	
308	113	4	12	8	17	3.6	8	0.7	4.3	
309	115	4	20	10	30	7.2	10	0.8	7.9	
310	221	4	21	7.5	44	11.1	18	0.6	11.7	
311	112	4	17	8	30	4.6	32	0.9	5.5	
312	125	4	20	10	24	5.9	26	1.5	7.4	
313	114	4	7	5	12	2.4	9	0.6	3.0	
314	225	4	15	4	17	3.3	8	0.4	3.7	
315	111	4	17	8.5	24	5.6	28	1.8	7.4	
316	123	4	19	9	24	7.4	43	2.4	9.8	
317	121	4	24	6	31	9.4	35	1.7	11.1	
318	222	4	15	5.5	31	6.7	28	1.2	7.9	
319	224	4	16	5	29	8.3	32	0.8	9.1	
320	215	4	6	3.5	14	1.4	6	1.0	2.4	Wilted plants

Appendix 8.6 Cu and Co fractions recovered from sandy loam and clay loam soils in a sequential extraction. Before and after they were used in the greenhouse experiment. Nd denotes not detected

Element	Soil Type	Water type	Total	Exchangeable	Water soluble	Organic	Carbonate	Residual
						µg		
Co	Sandy loam	Initial	9.60	0.22	6.37	Nd	Nd	Nd
Co	Sandy loam	FW	5.2	Nd	2.795	Nd	Nd	Nd
Co	Sandy loam	CW25%	40.4	3.78	1.65	Nd	Nd	Nd
Co	Sandy loam	CW50%	32.2	0.92	1.691	Nd	Nd	Nd
Co	Sandy loam	CW75%	13.8	Nd	1.169	Nd	Nd	Nd
Co	Sandy loam	CW100%	116.6	18.1	0.902	Nd	2.417	Nd
Co	Clay loam	Initial	9.17	Nd	0.92	Nd	Nd	Nd
Co	Clay loam	FW	26.8	Nd	0.937	Nd	Nd	Nd
Co	Clay loam	CW25%	57.5	Nd	0.678	Nd	Nd	Nd
Co	Clay loam	CW50%	123.5	12.82	0	Nd	Nd	Nd
Co	Clay loam	CW75%	84.4	7.43	0.437	Nd	Nd	Nd
Co	Clay loam	CW100%	116.2	12.25	0.01	Nd	Nd	Nd
Cu	Sandy loam	Initial	0.38	3.93	1.82	7.73	Nd	5.96
Cu	Sandy loam	FW	0.21	2.36	0.618	6.06	Nd	6.92
Cu	Sandy loam	CW25%	1.62	4.31	Nd	27.72	Nd	3.76
Cu	Sandy loam	CW50%	1.29	3.16	Nd	22.81	Nd	4.13
Cu	Sandy loam	CW75%	0.55	6.2	Nd	25.45	Nd	3.72
Cu	Sandy loam	CW100%	4.67	5.09	Nd	33.55	0.26	5.54
Cu	Clay loam	Initial	0.37	Nd	Nd	8.16	Nd	4.50
Cu	Clay loam	FW	1.07	Nd	Nd	11.19	Nd	5.68
Cu	Clay loam	CW25%	2.3	Nd	Nd	31.6	Nd	7.3
Cu	Clay loam	CW50%	4.94	5.36	Nd	46.97	Nd	3.93
Cu	Clay loam	CW75%	3.38	26.36	Nd	63.5	0.69	4.35
Cu	Clay loam	CW100%	4.65	19.32	Nd	64.25	1.33	5.3

Appendix 8.7 Cu and Co fractions recovered from sandy loam and clay loam soils in a sequential extraction.

The Percent values of the different fractions are calculated by getting the extracted amount and dividing by the sum of extracted fractions

			Fractions				
Element	Soil Type	Water type	Exchangeable	Water soluble	Organic	Carbonate	Residual
					%		
Co	Sandy loam	Initial	3.29	96.71	0.00	0.00	0.00
Co	Sandy loam	FW	0.00	100.00	0.00	0.00	0.00
Co	Sandy loam	CW25%	69.61	30.39	0.00	0.00	0.00
Co	Sandy loam	CW50%	35.24	64.76	0.00	0.00	0.00
Co	Sandy loam	CW75%	0.00	100.00	0.00	0.00	0.00
Co	Sandy loam	CW100%	84.50	4.21	0.00	11.28	0.00
Co	Clay loam	Initial	0.00	100.00	0.00	0.00	0.00
Co	Clay loam	FW	0.00	100.00	0.00	0.00	0.00
Co	Clay loam	CW25%	0.00	100.00	0.00	0.00	0.00
Co	Clay loam	CW50%	100.00	0.00	0.00	0.00	0.00
Co	Clay loam	CW75%	94.45	5.55	0.00	0.00	0.00
Co	Clay loam	CW100%	99.92	0.08	0.00	0.00	0.00
Cu	Sandy loam	Initial	20.24	9.35	39.75	0.00	30.66
Cu	Sandy loam	FW	14.79	3.87	37.97	0.00	43.36
Cu	Sandy loam	CW25%	12.04	0.00	77.45	0.00	10.51
Cu	Sandy loam	CW50%	10.50	0.00	75.78	0.00	13.72
Cu	Sandy loam	CW75%	17.53	0.00	71.95	0.00	10.52
Cu	Sandy loam	CW100%	11.45	0.00	75.50	0.59	12.47
Cu	Clay loam	Initial	0.00	0.00	64.45	0.00	35.55
Cu	Clay loam	FW	0.00	0.00	66.33	0.00	33.67
Cu	Clay loam	CW25%	0.00	0.00	81.23	0.00	18.77
Cu	Clay loam	CW50%	9.53	0.00	83.49	0.00	6.99
Cu	Clay loam	CW75%	27.78	0.00	66.91	0.73	4.58
Cu	Clay loam	CW100%	21.42	0.00	71.23	1.47	5.88

Appendix 8.8 Relationship between metal concentration in plants and biomass yield.

Interaction	n	DF	R^2	R	MS Regression	MS Residual	Calc F	Critical F0.01	F Signif	T Stat	Model	Model R^2
Cu Vs Whole plant Yield	60	59	0.43	0.65	315.72	7.33	43.07	7.31	*	*	$y=9.826e^{-0.0021x}$	0.55
Co Vs Whole plant Yield	60	59	0.40	0.63	295.43	7.68	38.47	7.31	*	*	$y=10.918e-^{0.0062x}$	0.43
Root Cu Vs Leaf Yield	60	59	0.14	0.37	74.84	8.18	9.15	7.31	*	*	$y=-0.0043x+7.68$	0.14
Root Co Vs Leaf Yield	60	59	0.07	0.26	36.83	8.84	4.17	7.31	*	ns	$y=-0.0145x+7.57$	0.07
Leaf Cu Vs Leaf Yield	60	59	0.33	0.58	182.79	6.32	28.91	7.31	*	*	$y=7.4647e^{-0.0017x}$	0.61
Leaf Co Vs Leaf Yield	60	59	0.42	0.65	230.52	5.50	41.92	7.31	*	*	$y=-0.0213x+8.8114$	0.42
Pumpkin Cu Vs Whole plant Yield	30	29	0.37	0.61	146.43	8.95	16.36	7.6	*	*	$y=10.012e^{-0.0022x}$	0.42
Pumpkin Co Vs Whole plant Yield	30	29	0.33	0.58	132.86	9.44	14.07	7.6	*	*	$y=-0.0376x+10.994$	0.33
C/cabbage Cu Vs Whole plant Yield	30	29	0.53	0.73	179.35	5.61	31.97	7.6	*	*	$y=9.7401e^{-0.002x}$	0.61
C/cabbage Co Vs Whole plant Yield	30	29	0.48	0.69	161.70	6.24	25.91	7.6	*	*	$y=11.3274e^{-0.0076x}$	0.54
Pumpkin Leaf Cu Vs Leaf Yield	30	29	0.41	0.64	112.98	5.80	19.49	7.6	*	*	$y=8.4895e^{-0.004x}$	0.58
Pumpkin Leaf Co Vs Leaf Yield	30	29	0.37	0.60	100.74	6.23	16.16	7.6	*	*	$y=8.4803e^{-0.0039x}$	0.39
C/cabbage Leaf Cu Vs Leaf Yield	30	29	0.47	0.69	129.03	5.18	24.92	7.6	*	*	$y=7.624e^{-0.0016x}$	0.74
C/cabbage Leaf Co Vs Leaf Yield	30	29	0.50	0.70	135.56	4.94	27.42	7.6	*	*	$y=9.4289e^{-0.0045x}$	0.63

Appendix 8.8 (continuation) Relationship between Metal Concentration in plants and Biomass Yield

Interaction	n	DF	R^2	R	MS Regression	MS Residual	Calc F	Critical F0.01	F Signif	T Stat	Model	Model R^2
Pumpkin Root Cu Vs Root Yield	30	29	0.24	0.49	3.88	0.45	8.66	7.6	*	*	$y=1.651e^{-0.0013x}$	0.29
Pumpkin Root Co Vs Root Yield	30	29	0.02	0.13	0.29	0.58	0.51	7.6	ns	ns	y=-0.0016x+1.6178	0.02
C/cabbage Root Cu Vs Root Yield	30	29	0.17	0.41	1.61	0.29	5.56	7.6	ns	ns	y=-0.00018x+1.116	0.17
C/cabbage Root Co Vs Root Yield	30	29	0.05	0.22	0.47	0.33	1.43	7.6	ns	ns	y=-0.0031x+0.8576	0.05
Water Cu Vs pumpkin Whole Plant Cu	30	29	0.44	0.66	229536.61	10347.44	22.18	7.6	*	*	Y=10.623e-0.1207X	0.80
Water Cu Vs pumpkin Leaf Cu	30	29	0.41	0.64	91646.81	4631.90	19.79	7.6	*	*	Y=6.665e-0.1178X	0.68
Water Cu Vs Chinese cabbage Leaf Cu	30	29	0.25	0.50	844358.03	89579.22	9.43	7.6	*	*	Y=17.42X-86.056	0.25
Water Cu Vs Chinese cabbage total Cu	30	29	0.46	0.67	780692.78	33289.78	23.45	7.6	*	*	Y=16.743X-56.371	0.46
Water Co Vs All plants total Co	60	59	0.66	0.81	131344.65	1193.04	110.09	7.3	*	*	Y=11.794X-10.165	0.66
Water Co Vs pumpkin Whole Plant Co	30	29	0.69	0.83	64468.63	1056.45	61.02	7.6	*	*	Y=11.685X-10.084	0.69
Water Co Vs pumpkin Leaf Co	30	29	0.68	0.83	107581.14	1787.75	60.18	7.6	*	*	Y=15.095X-23.061	0.68
Water Co Vs Chinese cabbage Plant Co	30	29	0.63	0.79	66887.16	1413.60	47.32	7.6	*	*	Y=11.902X-10.246	0.63
Water Co Vs Chinese cabbage Leaf Co	30	29	0.54	0.74	183652.35	5515.84	33.30	7.6	*	*	Y=19.723X-26.664	0.54
Water Cu Vs All plants total Cu	30	29	0.40	0.64	928432.03	23535.34	39.45	7.6	*	*	Y=12.911X-34.192	0.40