

# Response of Pumpkin and Chinese Cabbage to Increasing Copper and Cobalt Levels in Irrigation Water on Sandy and Clay Loam Soils

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## Abstract

A study where Chinese cabbage and pumpkin crops were grown on sandy loam and clay loam soils and irrigated with water contaminated with five levels of contamination from 0 (control), 25, 50, 75 and 100% of smelter water was carried out in a greenhouse. Copper concentration in Chinese cabbage ranged from 4.0 mg/kg in control treatments to 539.0 mg/kg in 100% contaminated water. In pumpkin, Cu ranged from 9.0 mg/kg in control to 142.0 mg/kg when irrigate with 100% contaminated water. Mean Cu in plants grown on sandy loam soil was 217.4 mg/kg dry and 79.3 mg/kg when grown on clay loam. Co in plants grown on sandy loam soil was 86.6 mg/kg and 47.0 mg/kg on clay loam soil. Increasing above ground tissue Cu in Chinese cabbage results in biomass yield decrease according to the equation  $Y = 7.624e^{-0.0016x}$ , ( $R^2 = 0.74$ ) and in pumpkin decreased according to the equation  $Y = 8.4895e^{-0.004x}$  ( $R^2 = 0.58$ ). This study suggests that Chinese cabbage tends to accumulate higher amounts of Cu and Co than pumpkin. It can be concluded from this study that irrigating crops with contaminated results in higher uptake of contaminants and reduced biomass on sandy loam than clay loam soils.

**Keywords:** Chinese cabbage, cobalt, copper, contaminated water, pumpkin, soil texture

## 1. Introduction

Copper (Cu) and Cobalt (Co) are commonly present in soil in trace amounts. Copper and cobalt are important in metabolic processes and synthesis of vitamins in human and plant life. For example Cu ions are used as catalytic cofactors in redox chemistry of proteins that are used in important biological systems (Lind, 2004). Similarly, Co is a vital element in the synthesis of vitamin B<sub>12</sub> which is important in a number of reactions in the human body including production of red blood cells (Lind, 2004). However, for Cu and Co, the very properties that make them indispensable to the body functions become toxic when they are present in excess (Centano et al., 2004; Lind, 2004; Nordberg & Cherian, 2004). Excess ingestion of Cu has been found to cause anaemia, liver and kidney damage, stomach and intestinal irritation and diarrhea (Nordberg & Cherian, 2004). In some case Cu toxicity has been found to cause Wilsons or Menkes diseases and Indian childhood cirrhosis (Centano et al., 2005). When cobalt sulphate is added to beer as stabilizer, it has been found to cause endemic problems of cardiomyopathy among beer drinkers and resulted in fatalities (Nordberg & Cherian, 2004; Centano et al., 2004).

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. The major problems arising from accumulation of Cu and Co in the environment include the toxic effects to plants and also to 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 water and food (Nordberg & Cherian, 2004). The processes of growing food crops on contaminated land or irrigating with water contaminated with Cu or Co can provide a pathway in which the metals find themselves in the human food pathway (Fuge, 2004).

Although there are numerous reports on the high levels of elements like Cu and Co in water and soil especially on the Copperbelt in Zambia (e.g. Chishala et al., 2006; ECZ, 2000; ECZ, 2001; Kasonde, 1995; Nkandu, 1999;

Sinkala et al., 1998), there is still lack of information on the accumulation of these metals in common crops on many locations where crops are grown on contaminated soil or irrigated with contaminated water. 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 factors according to Dube, Zbytniewski, Kowalkowski, Cukrowska, and Buszewski (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 (Dube et al., 2001; Birley & Lock, 1999; Alloway, 1995). In this view, uniform guidelines on the use of waste water on soils with different characteristics may not provide adequate protection against contamination by heavy metals.

Vegetables like pumpkin (*Cucurbita maxima*; family: *cucubitaceae*) and Chinese cabbage (*Brassica oleracea var. chinensis*; family: *cruciferae*) are widely grown in peri urban areas of Zambia. Farmers in these peri urban areas usually use waste water from poorly functioning sewer plants or industrial discharges because it is available during the long dry periods that last from April to around November. Pumpkins and Chinese cabbage are commonly grown in this dry season (summer) for their leaves which are sold at local markets and supermarkets. Leafy vegetables have been found to accumulate more heavy metals than non leafy vegetables or fruits grown on heavy metal polluted soils (Alam, Snow, & Tanaka, 2003; Boamponsem, Kumi, & Debrah, 2012; Finster, Gray, & Binns, 2004; Flores-Magdaleno, Mancilla-Villa, Mejia-Saenz, Olmedo-Bolanos, & Bautista-Olivas, 2011; Mathur, Bhatnagar, & Verma, 2006; Sharma & Kansal, 1986).

It is also important to investigate whether high concentrations of heavy metals such as Cu and Co in pumpkins and Chinese cabbage can have any effects on leaf yield. This is important because if there is no effect on crop yield and yet has high concentration of heavy metal, then the plants are hyperaccumulators (Mertz, 1998; Moolenaar & Lexmond, 1998). If the two crops are high accumulators of Cu and Co, then a crop that appear healthy may actually have concentration of these heavy metals higher than recommended for consumption by humans. Thus, consumption of these food crops contaminated with Cu and Co, would affect the health of those who regularly consume them. In a country like Zambia where heavy metals are not examined in diseases, misdiagnosis may occur and people affected may not get correct treatment.

The first objective of the study was to determine the levels of Cu and Co in pumpkin and Chinese cabbage irrigated with water contaminated with heavy metals on sandy loam and clay loam soil. The second objective was to determine the effect on crops yield in response to Cu and Co concentrations in plant tissues when crops are irrigated with increasing concentrations of contaminated water on sandy loam and clay loam soil.

It was hypothesized that there would be significant differences in the uptake of Cu and Co between pumpkin and Chinese cabbage and among the different concentrations of contaminated irrigation water on sandy loam and clay loam soil. It was also hypothesized that significant yield declines would be observed on crops grown if high concentrations of Cu and Co were taken up by plants when irrigated with increasing concentrations of contaminated irrigation water than on sandy loam and clay loam soil.

## 2. Materials and Methods

### 2.1 Study Site

The study was conducted in a greenhouse that is located at the School of Agricultural Sciences of the University of Zambia. The GPS position of the greenhouse is 15°23'39.2"S, 28°20'3.9"E, and altitude of 1266 m above sea level. The glasshouse measures 5.7 m by 8.3 m. It is made of aluminium frame with sides 1.9 m. It has two gables which are 0.8 m above the height of side. On each roof gable, there are 3 window openings for ventilation. There are two sliding doors which open in opposite directions. Daily dry and wet bulb thermometer readings inside the greenhouse were recorded each morning during 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 meteorological mean temperatures during the study period were obtained from the meteorological station at the University of Zambia. The mean maximum temperatures were 23.1 °C (±1.7 °C) and the mean minimum temperatures were 11.2 °C (±1.0 °C). No external temperature or humidity controls were used in the greenhouse for this study.

### 2.2 Experimental Design

The study used a Randomized Block Design with 3 replicates. The study had three factors comprising: (a) two crop species, (b) two soil types and (c) five irrigation water treatments. The experiment had thus 20 treatment combinations that were randomized in each block.

### 2.3 Plant Materials Used in the Study

The two crop types used in this study were Pumpkin (*Cucurbita maxima*) and Chinese cabbage (*Brassica oleracea* variety: Sjiniese Kool). A local variety of pumpkin called 'chibwabwa' which is grown for its leaves was bought from Kalingalinga township market in Lusaka. For Chinese cabbage, the variety used was Sjiniese kool (Chihili) which is produced by Mayford Seed Company of South Africa. The seed was bought from the Livestock Center Cooperative in the show grounds of Lusaka. Pumpkin and Chinese cabbage are commonly grown by farmers in the peri-urban areas for their edible leaves during the dry periods that last from April to October.

### 2.4 Soils Used in the Study

Two different soil types were used in this study: (a) sandy loam (Classified by Veldkamp (1984) as fine loamy isohypethermic udic (Kandi) ustalf: Choma soil series) was collected from University of Zambia Field Station at Liempe Farm. The latitude and longitude position the sandy loam soil was collected was 15°15'15.4"S and 023°8'25.8"E. The soil was collected from the surface between 0 and 15 cm. (b) the clay loam soil (Classified by Veldkamp (1984) as Clayey, montimorillonitic isohyperthermic Udic Paleustert: Kafue soil series). The latitude and longitude position for the site the clay loam soil was collected was: 15°22'47.9"S, 028°27'51.8"E within the Great East Road campus of the University of Zambia.

### 2.5 Soil Preparation

The soils were first sieved using a 0.5 mm mesh and mixed with D compound fertilizer (N:P:K = 10:20:10) at the rate of 2 g for every 3.0 kg of soil. The pots used in the study had a volume of 2.6 litres, The top of the pot had a diameter 17 cm and thus exposing a surface area of 230 cm<sup>2</sup>. Three kilograms of either sandy loam or clay loam soil were filled in each plastic pot that had been labeled with the treatment which was to be used. Two centimeters remained between the soil surface and pot opening. The pots were perforated at the bottom with two 4 mm holes to drain any excess water that could have accumulated at the bottom and also to aid in aeration of the soil. Each pot was seated on a flat and side raised plastic plate to retain any water or soil that would have percolated through the perforations at the bottom.

### 2.6 Water Used in the Study

The contaminated water used in the study was obtained from the Nkana stream in Kitwe on the Copperbelt province of Zambia at the road bridge. The latitude and longitude position for the site the water was collected was: 12°50.266'S, 28°12.856'E. The water is effluent from the Nkana Copper Smelter. The Nkana stream is a tributary of the Kafue River. Farmers use the water from the Kafue River to irrigate crops during the dry season of the year from April to October.

The contaminated water was diluted with rain water to create a gradient of concentrations of Cu, Co and other contaminants. Five levels of contamination from 0 (control) to 100% contamination (undiluted) were made. Table 1 shows the irrigation water types and how the dilutions were made. Depending on the combination of rain (FW) and contaminated water, the treatments were labeled FW (Control was rain water), CW 25% (ratio of contaminated to fresh water was 1:3), CW 50% (ratio of contaminated to fresh water was 2:2), CW 75% (ratio of contaminated to fresh water was 3:1) and CW 100% (not diluted contaminated water). The concentrations of Cu and Co that was 26.29 and 10.52 mg/l, respectively, were the original concentrations of these elements in contaminated water.

The water treatments were analyzed for concentration of copper and cobalt in them. The concentrations of Cu and Co in the water types used in the study are presented in Table 1.

Table 1. Water types that were used for irrigation and the mean concentrations of Cu and Co contained in them

Water Type	Water dilutions	Cu (mg/l)	Co (mg/l)
FW	Control (Fresh water only)	bd	bd
CW 25%	Diluted (Contaminated : Fresh water, 1:3)	7.18 (0.03)	4.33 (0.03)
CW 50%	Diluted (Contaminated : Fresh water, 2:2)	14.83 (0.07)	7.44 (0.02)
CW 75%	Diluted (Contaminated : Fresh water, 3:1)	22.43 (0.17)	10.33 (0.06)
CW 100%	Not Diluted (Contaminated water only)	26.29 (0.17)	10.52 (0.15)

Note. The numbers in parenthesis are standard errors (n = 4). bd denotes that the elements were below detection.

### *2.7 Management of Plants in the Experiment*

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 later thinned to 4 plants per pot.

All pots were irrigated every day with 175 ml of respective water treatment. All water applications were recorded so that the total amount of water applied up to the time of harvesting was known. On the first two days after planting, the amount of water applied was 350 ml and there after reduced to 175 ml. At the end of the experiment, each pot had received 8575 ml of water.

Plants were regularly checked for presence of pests. There were no pests or diseases that appeared on the crops. Weeds which germinated in the pots were removed by hand regularly. However, plant wilting was experienced on treatments where high concentrations of contaminated water were applied.

### *2.8 Plant Sample Collection and Preparation*

The plants were harvested from the experiment after growing for 57 days. At this stage the plants had reached a level of maturity where they could be harvested for consumption. The stem for Chinese cabbage at this stage was about 1 cm, while that of pumpkin was about 4 cm for larger plants but had not hardened. Plants on treatments with high concentration of contaminants (CW 100%) had low vegetative growth and thus collecting all above ground matter had to be done. The above ground plant components 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. The plant samples were placed in the oven where the temperature was set at 60 °C. The plant samples were left to dry for 48 hours until a constant weight was obtained. Weight of the plant samples were recorded for each treatment. After the plant samples had died, they were ground by hand and placed in a plastic bag.

### *2.9 Plant Analysis*

To determine the heavy metals such as Cu and Co in plants, a wet destruction method was used. This method mineralized the elements into solution and then the elements were determined by Atomic Absorption Spectrophotometer (AAS). The method was described by Cottenie, Verloo, Kikens, Velghe, and Camerlynck (1982).

A 1.0 g of plant material was placed in a 250 ml conical flask. Then 25 ml of concentrated Nitric acid (HNO<sub>3</sub>) 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 Perchloric acid (HClO<sub>3</sub>). 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 (manufactured by Perkin Elmer, Waltham, Massachusetts, USA). 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. In this case, Cu and Co used specific lamps for their analysis. For samples whose reading was 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.

### *2.10 Data Analysis*

Variations measured on sampled plants such as yield and metal content in above ground plant tissues were subjected to the analysis of variance (ANOVA) to test the significance of differences among the treatments. Significant differences were tested using the least significant differences (LSD) when significant ( $p < 0.05$ ) F ratio was observed.

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. Statistical analyses in this study were conducted using Excel<sup>®</sup> (manufactured by Microsoft, Redmond, Washington, USA) and Genstat<sup>®</sup> (manufactured by VSNI, Oxford, UK) software programs.

### 3. Results

#### 3.1 Concentration of Cu and Co in above Ground Tissues of Pumpkin and Chinese Cabbage

Results of above ground plant tissue of Chinese cabbage showed significantly higher ( $p < 0.05$ ;  $LSD_{0.05} = 84.7$  and  $15.7$  mg/kg for Cu and Co respectively) concentrations of Cu and Co than that of pumpkin. Results in Figure 1 shows that the concentration of Cu in Chinese cabbage was  $87.0$  mg/kg more than that of pumpkin. The concentration of Co in Chinese cabbage was  $26.6$  mg/kg more than that of pumpkin.

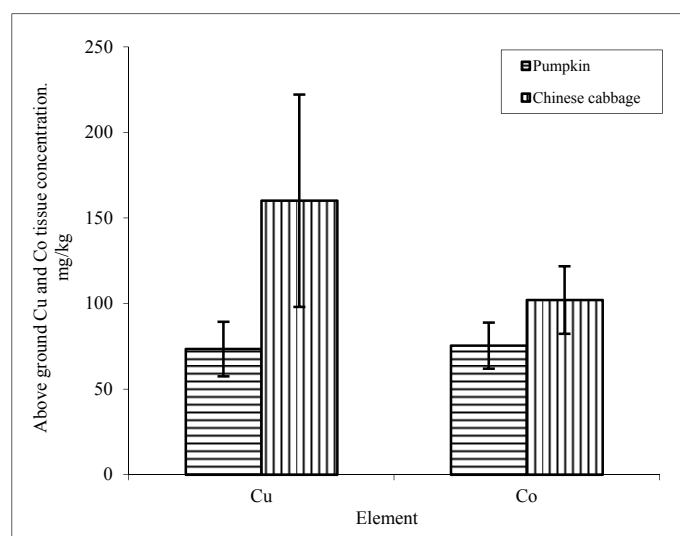


Figure 1. Mean concentration of Cu and Co in above ground plant tissue of pumpkin and Chinese cabbage. The error bars are standard errors of means

#### 3.2 Effect of Increasing Concentration of Contaminants in Irrigation Water on Cu and Co Measured in above Ground Tissues of Pumpkin and Chinese Cabbage

Results in Figure 2, shows the concentration of Cu and Co in pumpkin and Chinese cabbage that was irrigated with increasing concentrations of contaminated water. There was a general tendency by the two crops to increase their uptake of Cu and Co with increasing concentration of irrigation water.

Concentration of Cu in the above ground tissue were statistically significantly different ( $p < 0.05$ ,  $LSD_{0.05} = 189.5$  mg/kg) among water treatments only on Chinese cabbage. In Chinese cabbage the concentration of Cu ranged from  $4.0$  mg/kg in FW, to  $539.0$  mg/kg when irrigated with CW 100%. The concentration of Cu in Chinese cabbage increased significantly especially between CW 75% and CW 100%, with the former being also higher than CW 25% and FW. For pumpkin, the levels of Cu ranged from  $9.0$  mg/kg in FW to  $142.0$  mg/kg in CW 100%.

The Co concentration in the above ground tissue of Chinese cabbage and pumpkin exhibited statistically significant differences ( $p < 0.05$ ,  $LSD_{0.05} = 35.1$  mg/kg) among water treatments on both crops. The concentration of Co increased among all treatments with contaminated water for both crops. The concentration of Co in Chinese cabbage ranged from none detectable in FW to  $244$  mg/kg in CW 100%. In pumpkin, the concentration of ranged from none detectable in FW to  $160$  mg/kg in CW 100%.

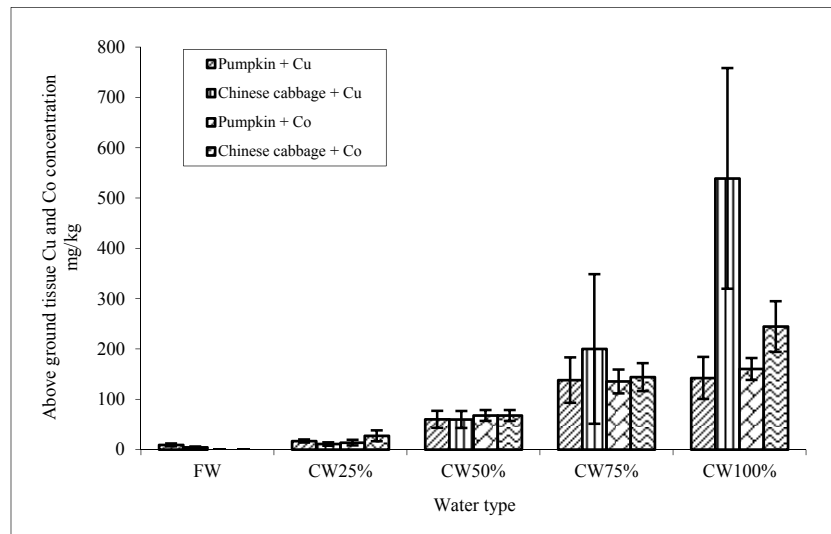


Figure 2. Mean Concentration of Cu and Co in above ground plant tissue of Pumpkin and Chinese cabbage irrigated with fresh and contaminated water. The error bars are standard errors of means

### 3.3 Effect of Soil Type on the Concentration of Cu and Co Measured in above Ground Tissue of Pumpkin and Chinese Cabbage Irrigated with Contaminated Water

Results in Figures 3 and 4 depict the average results from each treatment combination in the greenhouse study for Cu and Co in above ground 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 (CW 100%) was 192.0 mg/kg in sandy loam and 93.0 mg/kg in clay loam soil (Figure 3).

The pattern is repeated again for Co (Figure 4), 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 arrangement.

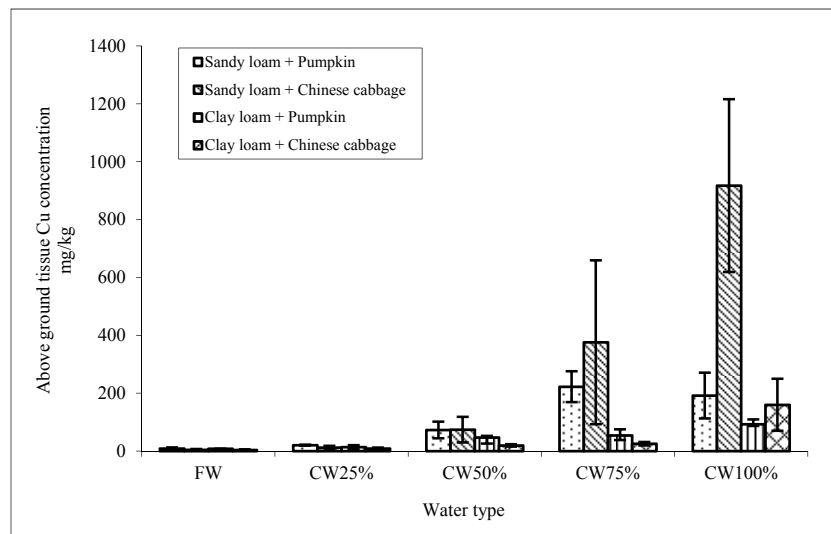


Figure 3. Mean concentration of Cu in above ground plant tissue of Pumpkin and Chinese cabbage grown on sandy loam and clay loam soils and irrigated with contaminated water. The error bars are standard errors of means

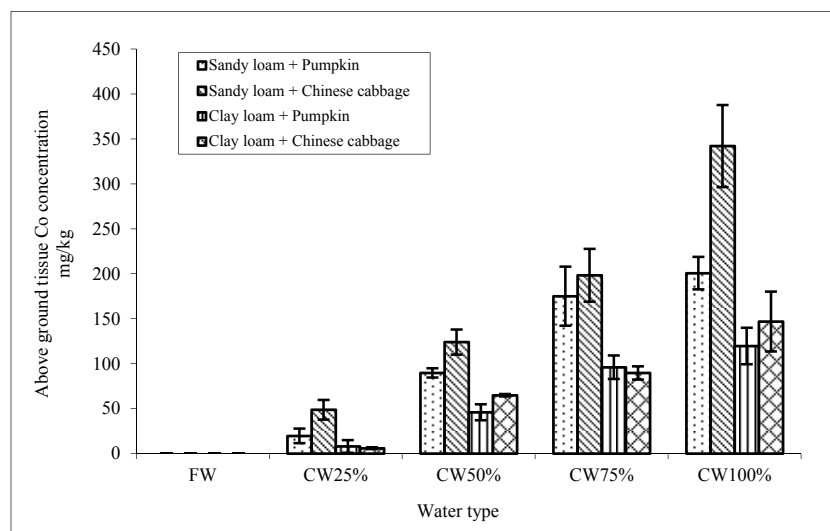


Figure 4. Mean concentration of Co in above ground plant tissue of Pumpkin and Chinese cabbage grown on sandy loam and clay loam soils and irrigated with contaminated water. The error bars are standard errors of means

The 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 shows that the amount Cu and Co being transferred from contaminated water to plants were higher on sandy loam than on clay loam 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.

#### 3.4 Effect of Plant Tissue Cu and Co Concentration On Biomass Yield

The relationship between above ground plant Cu concentration in pumpkin and its plant biomass yield are shown in Figure 5. The study showed that there was a weak correlation between above ground plant Cu content and above ground plant yield per pot. The study shows 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.

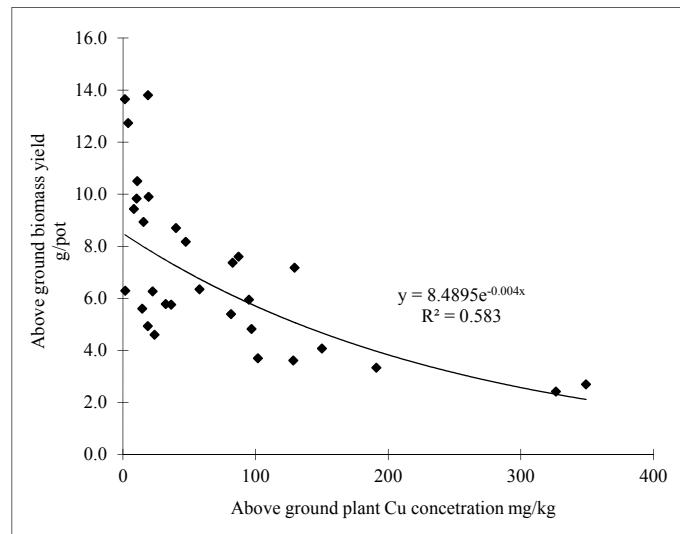


Figure 5. Relation between above ground plant biomass of pumpkin and the concentration of Cu in its above ground plant tissue

The relationship between above ground plant Cu concentration in Chinese cabbage and its plant biomass yield are shown in Figure 6. The experiment showed that there is a weak correlation between above ground plant Cu content and above ground plant yield per pot. The study shows that with increasing above ground Cu concentration, the biomass yield may decrease exponentially according to the exponential equation  $Y = 7.624e^{-0.0016x}$ , with the model  $R^2$  being 0.74.

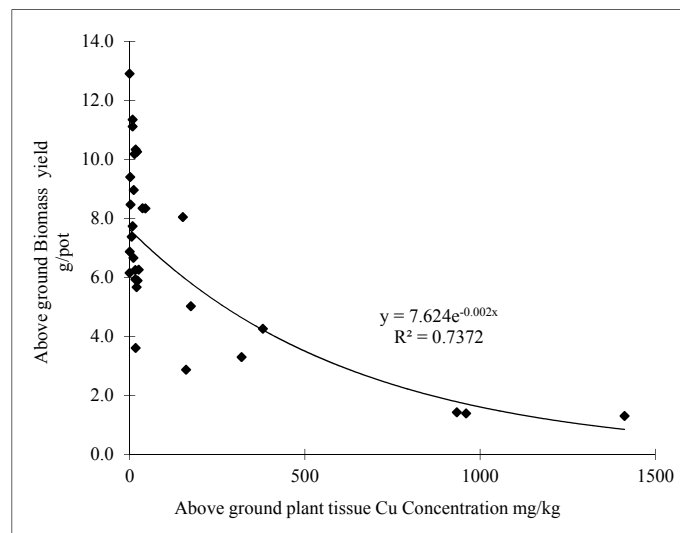


Figure 6. Relation between above ground plant biomass of Chinese cabbage and the concentration of Cu in its above ground plant tissue

The relationship between above ground plant Co concentration in Chinese cabbage and its plant biomass yield are shown in Figure 7. The experiment showed that there is a moderate correlation between above ground plant Co content and above ground plant yield per pot. The study shows that with increasing above ground Cu concentration, the biomass yield may be described by an exponential equation  $Y = 9.4289e^{-0.0045x}$  with the model  $R^2$  being 0.63.



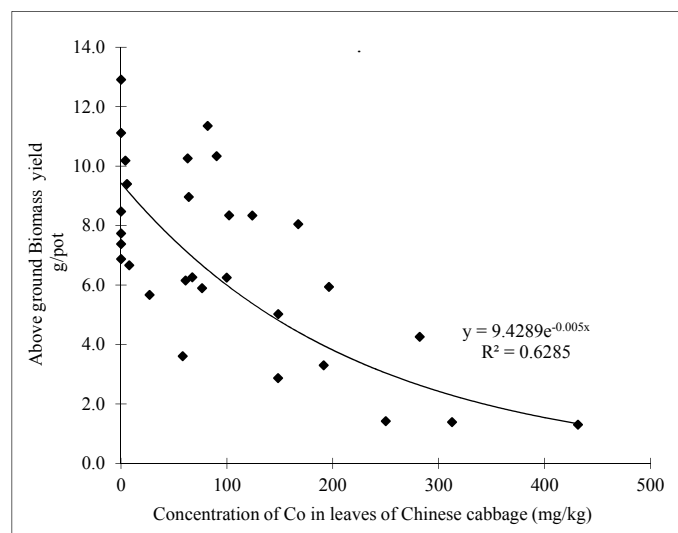


Figure 7. Relation between above ground plant biomass of Chinese cabbage against the concentration of Co in its above ground plant tissue

From this study it was observed that Chinese cabbage and pumpkin showed decline in above ground biomass yield with increasing concentrations of contaminants (Cu and Co) in the irrigation water. The study also shows that with increasing above ground tissue Cu and Co concentration, biomass yield decreased for both pumpkin and Chinese cabbage. Similar decreases in biomass production in plants as a result of high concentration of Cu were reported in green gram (Manivasagaperumal, 2011) and in Chinese cabbage (Xiong & Wang, 2005).

#### 4. Discussion

The study established that, Chinese cabbage accumulated much higher concentration of Cu in above ground tissue than pumpkin. Studies for example by Singh, Zacharias, Kalpana, and Mishra (2012) and Farooq, Anwar, and Rashid (2008) showed that different plant species growing in the same environment have different uptake of heavy metals.

Among the factors that determine the differences in accumulation of heavy metals among plants of different species are the rate at which plants transfer heavy metals from roots to shoot and the ability to detoxify and sequester these elements in leaves (Rascio & Navari-Izzo, 2011). These plants had only been growing for 57 days when they were harvested, Chinese cabbage may be a hyper accumulator whose reasons may be among those postulated by Boyd and Martens (1998) to include: (i) for metal tolerance or disposal where the HM are eliminated from the plant body by placing them in leaves which are later shade out (ii) drought resistance, where the heavy metal acts osmolytes inside cells of plants and thus act in water conserving role. (iii) allelopathy, which is the interference with neighbouring plants especially the susceptible ones to establish themselves near it, and (iv) defense latter e against natural enemies such as herbivores and pathogens. Pumpkin grows over a long period of time and produces more vegetative matter than Chinese cabbage. If allowed to grow for long, perhaps pumpkin would have removed more mass of Cu and Co due to the large overall mass per plant.

In this study Chinese cabbage and pumpkin were exposed to the some soil and irrigation water conditions. At 160 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). 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, Mbolekwa, Mtshemla, & Fatoki, 2005). Therefore, eating Chinese cabbage and pumpkin grown in such conditions would pose health risks from Cu and Co contamination.

The study revealed that increasing the concentration of contaminants in irrigation water apparently resulted in an increase in the concentration of Cu and Co in above ground plant tissue of Chinese cabbage and pumpkin. These results are consistent with those of Gharbi, Rajeb, Ghobal, and Morel (2005), and Xiong and Wang (2005) where Cu in tissue of lettuce and spinach (which are brassicas) increased with increasing concentration of Cu in irrigation water. The findings concur with conclusions that in addition to differences between species, variations have also occur within species (Máthé-Gáspár & Anton, 2002; B. Mohsen & S. Mohsen, 2008; Arora et al., 2008) with *Brassicac*s generally being the highest accumulators of heavy metals.

The apparent increase in the uptake of Cu and Co by both crops from contaminated water can be a result of these crops being hyper accumulators of heavy metals. The crops exhibited heavy metal uptake behavior that (Rascio & Navari-Izzo, 2011) suggested that some hyper accumulator species translocate heavy metals to the aerial parts.

The problem of consuming crops which show hyper accumulation of heavy metals is that those consuming them may be exposing themselves to high levels of heavy metals, which is beyond those accepted for consumption. This is because farmers and consumers may not see the effects as crops appear normal. In addition, most countries in the third world, like Zambia, do not carry out regular quality control in foods sold in the markets.

Soils with high clay content tend to have high specific surface and thus provide a large surface area for adsorption of metals on them (Du, Sun, Forsling, & Tang, 1997; Miller & Donahue, 1995; Zubillaga, Bressan, & Lavado, 2008). Clay minerals have a larger amount of negative charges on them in the range of 15 -30 cmol<sub>c</sub>/kg soil compared to sandy loam with about 5-10 cmol<sub>c</sub>/kg soil (Miller & Donahue, 1995). The other factor that governs adsorption of cations in soil is the organic matter (Logan & Traina, 1993). Organic matter adsorbs cations on negative charges that exist on their surfaces through a process called chelation. The higher amounts of clay and organic matter on clay loam could have contributed to the higher adsorption of Cu and Co than on 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 by clay loam soil, contributed to the reduction of these elements in the irrigation water. This reduction of Cu and Co from water in the clay loam soil resulted in the subsequent low uptake of Cu and Co 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 textural characteristics even when irrigated with similar type of irrigation water. The study brings out evidence that soils with higher clay content would result in the reduction of concentration of the heavy metals in contaminated 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.

Results from this study imply that farmers should be discouraged from growing crops on soil that are low in clay and organic matter when they are irrigating with contaminated water as the uptake of contaminants such as Cu and Co by plants can be high. In addition, the results have 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.

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 (Mertz, 1998; Clarkson, Friberg, Nordberg, & Sager, 1988). If the yields do not reduce or plants show no colour changes, perhaps this could be an indication that plants are tolerant to contamination at that concentration of 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 & Alloway, 1985). 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 CW 25% treatments while the highest was 160.2 mg/kg for pumpkin and 244.5 mg/kg for Chinese cabbage in CW 100% 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, El-Nagger, Osman, & El-Mazaly, 2003; Parmar & Chand, 2005; Jayakumar, Vijarengan, Chang-Xing, & Jaleel, 2008).

The effect of decline in yields with increased concentrations of heavy metals such as Cu and Co is that farmers using contaminated waste water obtain lower yields than they would if they had access to uncontaminated water. It is therefore, important that farmers should not use waste water where high concentrations of heavy metals such

as Cu and Co can affect the yields and quality of crops. Farmers in peri urban areas of third world countries like Zambia irrigate with waste water as it is available and cheapest source of water that they have access to during the long dry periods that last from April to November. Yield may not be the primary objective of farmers using waste water, but the long term health implication can be too high to the consumers of these food crops.

This study was conducted under controlled environments where the volume of water was uniformly applied in all pots, the soil volume was limited to the size of the pots, and thus limit the space for roots, the climate was controlled as opposed to environmental conditions where interferences from temperature, weeds, pest and diseases are difficult to control. Since the plant roots were restricted in pots, and always in contact with contaminated irrigation water, uptake of heavy metals, in this case, Cu and Co, it is possible that the results in this study could show higher uptake by plants. The benefits of using this pot experiment were that one could test different concentration levels on a smaller area and also help to simplify measurements of relevant parameters such concentrations of contaminants in water and kinds of soil. In addition it can be useful designing of field experiments after gaining some insights in possible results from controlled conditions.

## 5. Conclusions

It can be concluded from this study that Chinese cabbage tends to accumulate high amounts of Cu and Co than pumpkin if irrigated with contaminated water with these elements. It has also been shown in this study that both Chinese cabbage and pumpkin have a tendency to increase the uptake of Cu and Co in above ground tissue with increasing concentration of the metals in irrigation water. Uptake of Cu and Co was higher for both Chinese cabbage and pumpkin on sandy loam than on clay loam soil. Chinese cabbage and pumpkin show a decline in above ground biomass yield with increasing concentrations of Cu and Co in the irrigation water. The decline in above ground biomass with increasing concentrations of Cu and Co may be an indication that that Chinese cabbage and pumpkin are not bioaccumulators of these elements as they have a negative effect on yield. This study brings to light the importance of including edaphic characteristics when looking at heavy metal contamination on a landscape level as they can influence the uptake of these metals by different crops.

The results from this pot study however, could not provide the explanation that caused the differences in the concentration of Cu and Co in plants that were grown on the different soil types. It was thus logical that further examination of the soils different fractions be studied as they could explain the fate of the heavy metals in soil and how they become available to plants in soil. It is also recommended that further research on the cumulative effects of use of contaminated water on the uptake of heavy metals by crops indicating long term effects will improve our understanding of the long term ecological effects of contaminated waste water use.

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