

Article

Effect of Application of Increasing Concentrations of Contaminated Water on the Different Fractions of Cu and Co in Sandy Loam and Clay Loam Soils

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Abstract: This study aimed to establish the fate of copper (Cu) and cobalt (Co) in sandy loam and clay loam soils that had been irrigated with increasing concentrations of contaminated water. A sequential extraction procedure was used to determine the fractions of Cu and Co in these soils. The concentration of bioavailable Cu and Co on clay loam was 1.7 times that of sandy loam soil. Cu on sandy loam soil was largely in the organic > residual > exchangeable > water-soluble > carbonate fractions, whereas on clay loam soil the element was largely in organic > exchangeable > residual > carbonate > water-soluble fractions. Co was largely observed in the exchangeable, water-soluble, and carbonate fractions, but with no particular trend observed in both soil types. When crops are grown on sandy soils that have a low capacity to hold heavy metals, the resulting effect would be high uptake of the heavy metals in crop plants. Because the predominant forms of Cu and Co vary in soils, it is expected that the metals will behave differently in the soils.

Keywords: cobalt; copper; contaminated irrigation water; sequential extraction; soil texture

1. Introduction

The increased copper mining in Zambia has generally contributed to employment opportunities coupled with improvements in living standards of the population in these new mining areas. However, more people migrating to cities are still unable to find jobs in towns and end up in informal activities such as agriculture, which includes farming with any available water including water from mining and industrial sources for irrigation. Due to a lack of regular monitoring and regulatory frameworks in the use of wastewater, which could be polluted, contamination of soils and crops is likely to increase.

High levels of copper (Cu), cobalt (Co), lead (Pb), and zinc (Zn) have been recorded in the soils and in water on the Copperbelt in Zambia [1–8]. 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 only in Kitwe and Chambishi on the Copperbelt province of Zambia.

High levels of Cu have also been recorded in water and plants in other towns of Zambia where there are no copper mining and processing factories [3]. The likely sources of the high Cu content in plants and water in towns away from the Copperbelt province could be metallurgical and chemical industries, corrosion of copper pipes, and fossil fuel combustion activities (due to the high number of motor vehicles and industries burning coal) in the urban areas of Lusaka and Kafue. The 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 trace metal contamination is widespread.

Continuous application of wastewater loaded with heavy metals results in an increase in these toxic elements in the 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 the metals' behaviour in the environment and their mobilization ability [9,10]. It is thus necessary to assess the total contents of the hazardous substances, as well as the chemical forms in which they may be present.

A number of factors influence the adsorption of heavy metals like Cu and Co when applied to the soil through irrigation water. Immobilisation of heavy metals is due to the sorption properties of the components of soils such as clay minerals and organic matter, and can be affected by the soil pH and chemical properties of the metal ions [11–13].

Several studies have shown that total metal concentrations are generally a poor indicator of metal toxicity because the toxicity of elements is dependent upon the chemical form, mobility, degree of transformation, and bioavailability [10,14,15]. Heavy metals in soil have been found to exist in categories and these are based on primary accumulation mechanisms. These accumulation mechanisms can be classified into five categories. The categories are: (i) exchangeable; (ii) water-soluble; (iii) organic; (iv) carbonate; and (v) residual [9,10]. The first two forms are available for uptake by plants, whereas the rest may be potentially available in the long term [15–21]. 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.

It is thus important to determine the impact that these fractions have on the uptake of Cu and Co in soils where contaminated wastewater is used to irrigate crops. The effect of relationship between the fractions and uptake of Cu and Co, will provide reasons for the differences that may be observed over different edaphic areas on the concentration of heavy metals in plants. The objective of this study was to determine the chemical fractions of Cu and Co in sandy loam and clay loam soils that had been irrigated with contaminated water.

2. Materials and Methods

2.1. Study Site

The experiment was conducted in a greenhouse at the School of Agricultural Sciences at the University of Zambia. The greenhouse is located at latitude 15°23'39.2" S, longitude 28°20'03.9" E, and altitude of 1266 m above sea level. The glasshouse covers an area of 47.3 m² 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 °C and 11.2 °C ± 1.0 °C respectively.

2.2. Water Used in the Study

The contaminated water used in the study was obtained from the Nkana stream in Kitwe at the road bridge (12°50.266' S, 28°12.856' E, Elevation 1220 m). The water is believed to be contaminated with heavy metals because it has effluent from the Nkana Copper Smelter. The chemical characteristics of this water can be observed in the undiluted (100% CW) water in Tables 1 and 2. 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.

Table 1. Mean values for irrigation water chemical parameters. The numbers in parentheses are the standard error of the means of three replicates.

Irrigation Water Type	Parameter				
	pH	Total Acidity	Electrical Conductivity	Chlorides	Sulphates
		(meq/L)	(10 ³ µS/cm)	(meq/L)	(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)

µS, micro Siemens.

Table 2. Mean concentration (mg/L) of elements determined in fresh and contaminated water. Numbers in parentheses are the standard errors of the means of three replicates.

Element	Irrigation Water Type					Maximum Limit in Irrigation Water
	FW	25% CW	50% CW	75% CW	100% CW	
	mg/L					
Ca	nd	11.90 (0.29)	16.46 (5.19)	12.88 (5.02)	26.93 (0.20)	1.0
Co	nd	4.33 (0.03)	7.44 (0.02)	10.33 (0.04)	10.52 (0.15)	0.05 *
Cr	nd	nd	0.05 (0.04)	0.19 (0.04)	0.37 (0.04)	0.1 *
Cu	nd	7.18 (0.03)	14.83 (0.07)	22.43 (0.17)	26.25 (0.17)	0.2 *
Fe	nd	2.54 (0.03)	4.97 (0.03)	7.11 (0.03)	7.37 (0.78)	5.0 *
K	nd	47.91 (0.14)	69.07 (0.25)	84.33 (0.40)	92.08 (0.53)	2.0
Mg	nd	1.15 (0.03)	2.55 (0.17)	3.42 (0.05)	3.33 (0.02)	0.4
Mn	nd	0.26 (0.01)	0.51 90.01)	0.76 (0.02)	0.89 (0.01)	0.2 *
Na	nd	41.73 (0.24)	66.81 (0.47)	88.43 (0.47)	98.90 (0.82)	1.7
Ni	nd	0.44 (0.04)	1.04 (0.02)	1.55 (0.04)	1.83 (0.02)	0.2 *
Pb	nd	nd	nd	0.06 (0.03)	0.29 (0.04)	5.0 *
Zn	nd	1.55 (0.02)	2.99 (0.23)	5.29 (0.14)	8.46 (2.51)	2.0

nd = not detected in samples; * Maximum limit in irrigation water [22].

For the purpose of the study, the contaminated river water was diluted with rain water (fresh water, FW) so that a gradation of contamination strengths was created. The following levels of contamination were created: FW (this comprised only rain water only); 25% CW (comprised 1:3 ratio of river water to rain water); 50% CW (comprised 1:1 ratio of river water to rain water); 75% CW (comprised 3:1 ratio of river water to rain water); and 100% CW (this comprised only contaminated water without dilution).

Each of the above solutions was analysed for the following parameters: pH, total acidity, electroconductivity, sulphates, chlorides and elements including calcium (Ca), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb), and zinc (Zn). The mean values of these parameters are presented in Tables 1 and 2.

The electrical conductivity (EC) values for irrigation water are presented in Table 1. The EC value for 100% CW was $3.7 \times 10^3 \mu\text{S}\cdot\text{cm}^{-1}$ (micro Siemens), while FW had negligible EC. Dilution of contaminated water resulted in a decrease in the EC. The EC values were within the range for irrigation water, which is $0\text{--}300 \times 10^3 \mu\text{S}\cdot\text{cm}^{-1}$ [22].

The amount of sulphates in CW 100% was 13.58 meq/L, whereas FW had 0.03 meq/L. The concentration of sulphates in the CW 100% was within the usual range in irrigation water of 0–20 meq/L [22].

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 [22].

The concentrations of copper (Cu), cobalt (Co), calcium (Ca), chromium (Cr), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb), and zinc (Zn) in the irrigation water are presented in Table 2. Table 2 also shows the maximum permitted limits for selected elements in irrigation water [22]. All elements except for Pb indicated highly elevated concentrations in the contaminated water that exceeded the maximum permitted levels in irrigation water.

The concentrations of Cu in CW 100% were 26.25 mg/L. The concentrations of Cu in other water treatments were 22.43 mg/L for CW 75%, 14.83 mg/L for CW 50%, and 7.18 mg/L for CW 25%. Copper was not detectable in 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 fresh water.

2.3. Soils Used in the Study

Two soil types were selected for this study, a sandy loam and clay loam soil. 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 of the soil collection site are 15°15'15.4" S and 23°08'25.8" E, 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 fine loamy isohypethermic Kandiusstalf (Choma soil series) [23]. 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 for the soil collection site are: 15°22'47.9" S, 28°27'51.8" E. The soil was collected from a grassland that floods seasonally. The soil is classified as Clayey, montmorillonitic isohyperthermic Udic Paleustert (Kafue soil series) [23]. The soil was collected from the surface between 0 and 15 cm.

Initial Physical and Chemical Parameters of the Sandy Loam and Clay Loam Soils Used in the Study

The soil was characterised in terms of its physical and chemical parameters. The parameters analysed included: particle size composition (texture), pH, cation exchange capacity, concentration of exchangeable trace elements, and organic matter content. The soil particle size was determined using the hydrometer method that was described by Anderson and Ingram [24]. The soil pH was read in 0.01 M CaCl₂ solution using a Radiometer PHM82 standard pH meter that had a single glass and calomel electrode (van Ranst et al. [25]). The cation exchange capacity was calculated using the summation of cations in soil (Jackson [26], Hesse [27]). The exchangeable trace elements were determined using the method that was described by van Ranst et al. [25]. The Walkely and Black method as described by van Ranst et al. [25]) was used to determine the soil organic matter content in soil.

Textural analysis of the soils that were used in this study showed that the particle size composition in sandy loam was 65.65%, and 7.6% and 26.75% for sand clay and silt, respectively. Whereas clay loam had 46.4%, 27.6% and 26.0%, sand clay and silt respectively. The clay fraction content of clay loam was apparently more than three times that of sandy loam soil. The silt fraction content was almost the same in both sandy loam and clay loam soils.

The mean pH values of the soils were 5.91 for sandy loam and 5.85 for clay loam. The organic matter content in the soils showed that clay loam had more than double the amount of sandy loam. The amount of organic matter in clay loam soil was above 4.0% and in sandy loam it was about 2.13%.

Table 3 shows the concentrations of the trace elements in the soils use in this study. The initial total concentrations of Cu in soils were 0.7 mg/kg in sandy loam and 13.5 mg/kg, while for Co it was 17.7 mg/kg in sandy loam and 16.1 mg/kg in clay loam. The initial soil bioavailable concentrations of Cu in soils were 0.2 mg/kg for sandy loam and 0.7 mg/kg for clay loam, and for Co it was 0.6 mg/kg in sandy loam and 0.3 mg/kg in clay loam.

The CEC of the soils were calculated by summation of the measured bases in soil. The CEC for sandy loam soil was 3.54 meq/100 g and clay loam had 8.0 meq/100 g.

Table 3. The trace element contents of soils used in the pot experiment. The DTPA and Aqua regia extractions were used to determine bioavailable and total trace elements, respectively. Numbers in parentheses are the standard errors of the means of three replicates.

Element	Clay Loam Soil		Sandy Loam Soil	
	Total	Bioavailable	Total	Bioavailable
	mg/kg	mg/kg	mg/kg	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 (.00)
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)

nd = not detected in samples.

These concentrations of bioavailable Cu and Co in clay loam and sandy loam soils were still well below the levels of these two elements that would be considered toxic for plant uptake in soil. The average elemental soil content of Cu is around 30 mg/kg, while that of Co is 8 mg/kg [28].

2.4. Soils Used in the Fractionation Study

The soils used in the fractionation experiment were obtained from an experiment that was carried out to study the uptake of Cu and Co by pumpkin (*Cucurbita maxima*) and Chinese cabbage (*Brassica oleracea*) when grown on a sandy loam and a clay loam soil and irrigated with increasing concentrations of contaminated water from a copper smelter. After the crops were harvested, all similar soil types with water corresponding irrigation water treatments were bulked. Thus, after bulking the soil, there were 10 treatments of soil that were used in the fractionation study (sandy loam + FW, sandy loam + CW 25%, sandy loam + CW 50%, sandy loam + CW 75%, sandy loam + CW 100%, clay loam + FW, clay loam + CW 25%, clay loam + CW 50%, clay loam + CW 75% and clay loam + CW 100%). Three replicate samples were obtained from each soil treatment and analysed for bioavailable and the different fractions Cu and Co.

2.5. Concentration of Bioavailable Cu and Co

A subsample was collected from the main sample that had been dried in an oven at about 60 °C for 24 h. The analysis of bioavailable Cu and Co was determined following the procedures elaborated by Lindsay and Novell [29] and van Ranst et al. [25]. A solution of Diethylene Triamine Penta Acetic Acid (DTPA) was added to the soil as an extracting solution. The mixture was shaken on a mechanical shaker and then filtered, after which the filtrate was taken for reading on an Atomic Absorption Spectrophotometer (AAS).

2.6. Determination of Total Metal Concentrations in Soils

Two grams of air dry soil samples were placed in a 50-mL flask. To the soil 12.5 mL of 4 M HNO₃ solution was added and shaken briefly. The suspension was then placed on a hot plate for digestion at 80 °C for 12 h. After digesting, the solution was cooled down and then filtered. 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 HNO₃. Triplicate soil samples were used for this part of the experiment.

2.7. Experimental Arrangement for the Fractionation of Cu and Co in Soils

Composite soil samples from the treatments, which comprised soil types with the 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 subsamples that represented the replications. The soils comprised the following types and treatments: (a) sandy loam soil with five water types (FW, CW 25%, CW 50%, CW 75% and CW 100%); (b) clay loam soil with five water types (FW, CW 25%, CW 50%, CW 75% and CW 100%); (c) initial sandy loam soil that was not irrigated with any of the contaminated or fresh water; and (d) initial clay loam soil that was not irrigated with any of the contaminated or fresh water. The initial soils were examined for the same fractions in order to observe any variations that would have taken place during the use of the soil in the greenhouse experiment.

The separation of the Cu and Co and fractions in the soils was carried out using the sequential extraction technique described by Sposito et al. [30]. The five different fractions of Cu and Co evaluated comprised the following: exchangeable, water-soluble, organic, carbonate, and the residual forms.

The procedure involved carrying out the following steps: (a) determination of the total metal fraction; and (b) carrying out the sequential extraction procedure.

2.8. The Sequential Extraction Procedure

The extraction was carried out in the sequence as illustrated in Table 4. 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 also used for this part of the experiment.

Table 4. Steps showing the extractant, reaction time, temperature, and fraction extracted at each step in the sequential extraction procedure.

Step	Extractant	Time (h)	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 °C	Residual

2.8.1. Determination of Exchangeable Cu and Co Fractions in the Soils

Two grams of air dry soil were placed in a 50-mL flask. Then 25 g of 0.5 M KNO₃ was added to the flask. The suspension was then placed on the shaker and shaken for 12 h. The suspension was then left overnight to settle 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 water and the excess water decanted. The weight of the remaining solution was measured (in grams) and recorded. The extracted solution was read on the AAS for Cu and Co. Standard solutions of Cu and Co were prepared in a solution of KNO₃.

2.8.2. Determination of Water-Soluble Cu and Co Fractions in the Soils

To the sample that remained from Step 1, 25 g of deionised water were added. The suspension was placed on the shaker and shaken for 6 h. The suspension was then left overnight to settle 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 the remaining solution was measured (in grams) and recorded. The procedure was repeated two more times, making a total of three 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.

2.8.3. Determination of Organic Cu and Co Fractions in the Soils

To the sample that remained from Step 2, 25 g of 0.5 M NaOH solution were added. The suspension was placed on the shaker and shaken for 12 h. The suspension was then left overnight to settle 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 water and the excess water decanted. The weight of the 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.

2.8.4. Determination of Carbonate Cu and Co Fractions in the Soils

To the sample that remained from Step 3, 25 g of 0.05 M Na₂ EDTA solution were added. The suspension was placed on the shaker and shaken for 12 h. The suspension was then left overnight to settle 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 water and the excess water decanted. The weight of the 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 Na₂ EDTA.

2.8.5. Determination of Residual Cu and Co Fractions in the Soils

To the sample that remained from Step 4, 25 g of 4 M HNO₃ solution were added. The suspension was placed on the shaker and shaken for 12 h. The suspension was then left overnight to settle 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 the 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 HNO₃.

The amount of metal extracted by a given extracting reagent was calculated using Equation (1) (below), as recommended by Sposito et al. [30]:

$$\mu\text{g extracted} = C \times 25\text{g} - C' \times M. \quad (1)$$

In Equation (1), C is the concentration in ($\mu\text{g/g}$) of metal in the solution.

C' is the concentration in ($\mu\text{g/g}$) of metal in the preceding step of sequence.

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

Equation (1) contains a correction for the amount of metal in the solution entrained in the soil sample after the shaking that 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) [31], because washing may lead to metal losses and therefore low recovery of metal from the soil in a given step.

3. Results

3.1. Concentration of Bioavailable Cu and Co in Sandy Loam and Clay Loam at the End of the Greenhouse Experiment

The concentration of bioavailable Cu and Co in soil at the end of the pot experiment increased in all treatments where contaminated water was used for irrigating the crops. Figure 1 shows that the mean concentration of available Cu in soil was 16.5 mg/kg in sandy loam and 27.7 mg/kg in clay loam. The concentration of bioavailable 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 ($p < 0.05$; $\text{LSD}_{0.05} = 4.4$ and 0.8 mg/kg for Cu and Co respectively) concentration of available Cu and Co in clay loam than in sandy loam soil. The amount of Cu and Co in clay loam soil was about 1.7 times the amount in sandy loam soil.

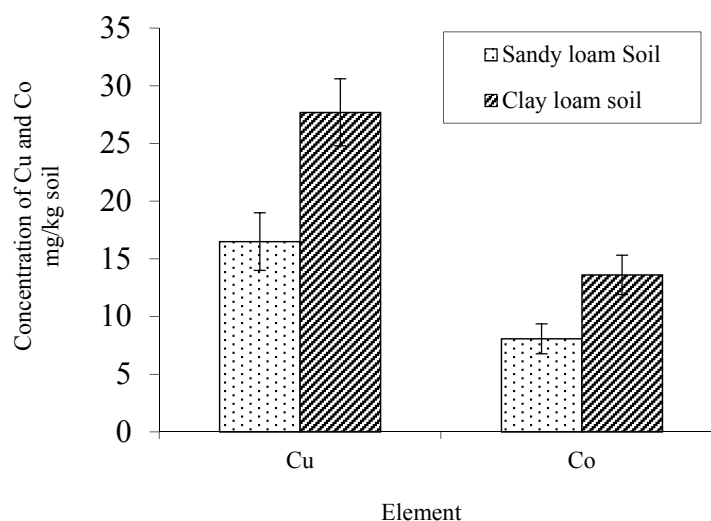


Figure 1. Means of concentration of Cu and Co in the soil after the crops were harvested. The error bars shown are the standard error of means.

3.2. The Effect Contaminated Irrigation Water on Cu and Co in Sandy Loam and Clay Loam Soils

Figure 2 shows the concentration of Cu and Co in sandy loam and clay loam soils that were irrigated with increasing levels of contaminants. The concentration of bioavailable Co in soils significantly ($p < 0.05$; $LSD_{0.05} = 5.88$ mg/kg) increased with increasing levels of contamination in irrigation water. There were marginally statistically significant differences ($p = 0.05$; $LSD_{0.05} = 9.93$ mg/kg) in available Cu between sandy loam or clay loam soils.

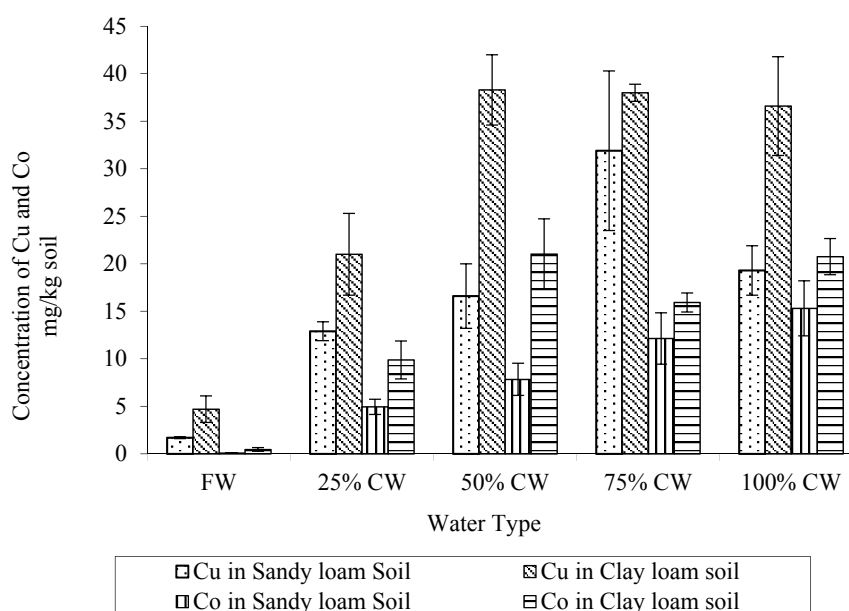


Figure 2. Concentration of Cu and Co in sandy loam and clay loam soils, which were used for growing pumpkin and Chinese cabbage and irrigated with increasing levels of contaminants in irrigation water. The error bars shown are the standard error of means.

In sandy loam, the concentration of Co increased from 0.09 mg/kg soil with FW to 15.3 mg/kg when irrigated with 100% CW. In clay loam, the concentration of Co increased from 0.5 mg/kg soil with FW to 15.3 mg/kg when irrigated 50% CW. There were, however, no significant differences in Co concentrations in clay loam among the 50% CW, 75% CW, and 100% CW water treatments.

The concentration of bioavailable Cu and Co in clay soil increased and levelled off at the point where the concentration of irrigation water was 50% CW or the concentration of Cu and Co was about 38 and 21 mg/kg soil, respectively. On the other hand, the concentration of available Cu in sandy soil levelled off when the concentration of irrigation water was 75% CW or the concentration of Cu was about 16 mg/kg soil.

3.3. Total Cu and Co in Soil Samples before and after Irrigation with Contaminated Water in the Greenhouse Experiment

The amounts of total Cu and Co extracted from the soils before and after the greenhouse experiments are shown in Table 5. The data depict the changes in total elemental amounts in the soil and not their specific fractions. 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 more Cu and Co extracted from clay loam than from sandy loam soil.

Table 5. Mean amounts of total Cu and Co extracted from sandy loam and clay loam soils before and after the soils were used in the greenhouse experiment. The numbers in parentheses are standard errors of means. Detection limits for Cu = 0.001 mg/L and Co = 0.006 mg/L.

Soil Type	Cu				μg	Co			
	Before		After			Before		After	
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 (<i>p</i> < 0.05)	ns		45.4			ns		12.5	

ns, not statistically significant.

There were statistically significant differences ($p < 0.05$, $\text{LSD}_{0.05} = 45.4 \mu\text{g}$ and $12.5 \mu\text{g}$ for Cu and Co, respectively) in the total Cu and Co recovered between sandy loam and clay loam soils. Clay loam soil had more than twice as much Cu and Co as sandy loam.

Results show that before the experiment, the amount of total Cu extracted from sandy loam was $21.58 \mu\text{g}$ and $39.32 \mu\text{g}$ from clay loam. At the end of the greenhouse experiment the mean amounts of Cu extracted from sandy loam increased to $137 \mu\text{g}$ and from clay loam to $304 \mu\text{g}$. The difference in the amount of extracted Cu from the soil before and after the experiment was $115 \mu\text{g}$ for sandy loam and $264 \mu\text{g}$ for clay loam.

For Co the amount extracted from sandy loam before the experiment was $9.60 \mu\text{g}$ and $9.17 \mu\text{g}$ from clay loam. The amounts of Co extracted from the soil after the greenhouse experiment increased to $41.6 \mu\text{g}$ from sandy loam and $81.7 \mu\text{g}$ from clay loam soil. The difference in the amount of extracted Co from the soil before and after the experiment was $32 \mu\text{g}$ for sandy loam and $72 \mu\text{g}$ for clay loam.

3.4. Fractions of Cu and Co in Sandy Loam and Clay Loam Soils before and after Irrigation with Contaminated Water in the Greenhouse Study

Table 6 shows the different fractions of Cu and Co on soil samples before (initial soil) and after use in the greenhouse.

In the soil before the experiment, it was found 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 amounts of Cu fractions extracted in sandy loam soil were 7.7, 6.0, 3.9, and $1.8 \mu\text{g}$ 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 $4.5 \mu\text{g}$, respectively.

Table 6. Mean amounts of Cu and Co fractions recovered from sandy loam and clay loam soils in a sequential extraction before and after the soils were used in the greenhouse experiment. Detection limits for Cu = 0.001 mg/L or 1 µg/L and Co = 0.006 mg/L or 6 µg/L.

Fraction	Soil before Use in Greenhouse				Soil after Use in Greenhouse				
	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								
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

For Co on sandy loam soil, the element was only extracted on exchangeable and water-soluble fractions with the highest recovery in the latter 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 the water-soluble fraction. 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 (Table 6). 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 ($p < 0.05$, $\text{LSD}_{0.05} = 1.63$) were observed in Cu fractions. Recovered amounts of Cu fractions on sandy loam soils after use in the greenhouse 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 the greenhouse study (final). The highest Cu fraction in final clay loam (10.2 μg had organic (43.5 μg) as the highest followed by exchangeable). In the final clay loam soil, statistically significant differences ($p < 0.05$, $\text{LSD}_{0.05} = 1.63$) were observed in Cu fractions. The pattern of the different Cu fractions extracted decreased in the following order: organic > exchangeable > residual > carbonate > water-soluble.

In the final clay loam soil statistically significant differences ($p < 0.05$, $\text{LSD}_{0.05} = 0.423$) were observed in Co fractions. 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 ($p < 0.05$, $\text{LSD}_{0.05} = 0.423$) 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 in 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.

3.5. Effect of Increasing Concentration of Contaminants in Irrigation Water on the Fractions of Cu on Sandy Loam Soil

Figure 3 shows the various fractions of Cu in sandy loam soil from the treatments where increasing concentrations of contaminated water were applied. 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.

In all the treatments with contaminated water, the amount of organic Cu extracted was higher than 22 μg , while that of the control treatment (FW) was around 6 μg . Significant differences ($p < 0.05$; $\text{LSD}_{0.05} = 3.65$) in organic Cu fractions were thus observed between FW treatment and those of contaminated water. There were apparently some marginal differences of Cu extracted in the organic fraction among the CW treatments. The highest extracted Cu on the organic fraction on sandy loam was 33.5 μg on 100% CW.

There were significant differences ($p < 0.05$; $\text{LSD}_{0.05} = 3.65$) in the extracted exchangeable Cu fraction between FW and the following CW treatments: 25% CW, 75% CW, and 100% CW. There were apparently some marginal differences of Cu extracted in the exchangeable fraction among the CW

treatments. The highest extracted Cu on the exchangeable fraction on sandy loam was 6.2 μg on 75% CW.

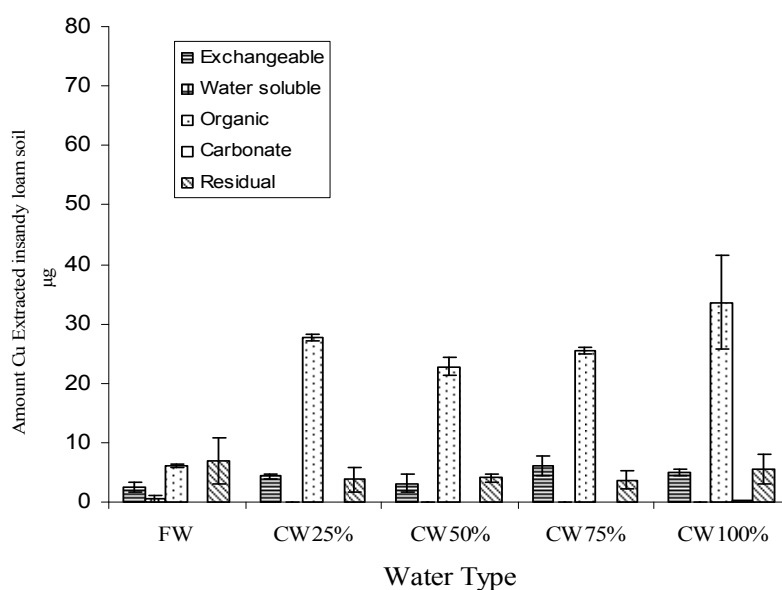


Figure 3. Mean amounts of recovered fractions of Cu (in μg) in sandy loam soil that had been irrigated with different concentrations of contaminated water.

The amount of water-soluble Cu fractions recovered was 0.6 μg in control treatment but none detectable in all treatments where contaminated water was used. The carbonate Cu fraction was also non-detectable in all treatments except for the 100% CW, where the amount extracted was 0.26 μg .

The residual Cu fractions extracted were in the range of 3 to 7 μg and did not differ significantly among the water treatments.

3.6. Effect of Increasing Concentration of Contaminants in Irrigation Water on the Fractions of Cu on Clay Loam Soil

Figure 4 shows the various fractions of Cu in clay loam soil from the treatments where different dilutions of contaminated water were used. 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.

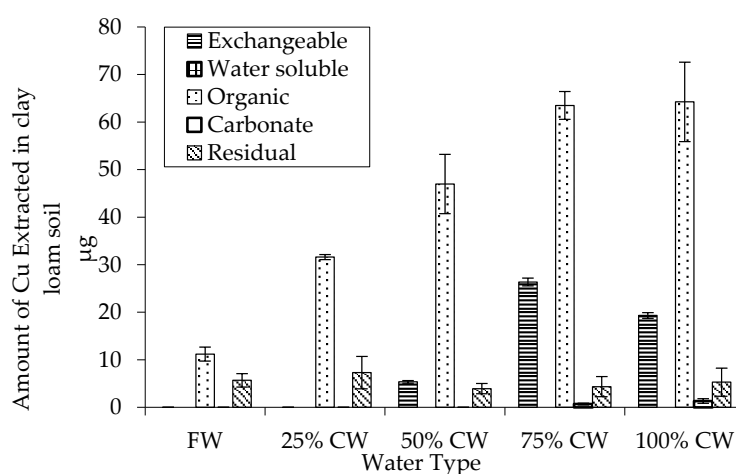


Figure 4. Mean amounts of fractions of Cu (in μg) recovered in clay loam soil that had been irrigated with different concentrations of contaminated water.

Organic Cu fractions increased significantly ($p < 0.05$; $LSD_{0.05} = 3.65$) between FW and all CW treatments. There were, however, no significant differences observed in the organic Cu fraction between 75% CW and 100% CW. The organic Cu fraction on clay loam soil increased from 11 μg in FW to 64 μg in 100% CW. The highest extracted Cu on the organic fraction on clay loam was 64 μg on 100% CW.

Exchangeable Cu fractions were only detected in clay soil when higher amounts of contaminants were in the water, from 50% CW, 75% CW, and 100% CW. 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 50% CW to 19.3 μg with 100% CW. The highest extracted Cu on the exchangeable fraction on clay loam was 19.3 μg on 100% CW.

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.

Water-soluble Cu fractions were not detected in clay loam from all treatments.

3.7. Effect of Increasing Concentration of Contaminants in Irrigation Water on the Fractions of Co on Sandy Loam Soil

Figure 5 shows the mean recovered amounts of Co in sandy loam soil where varying concentrations of contaminated water were applied.

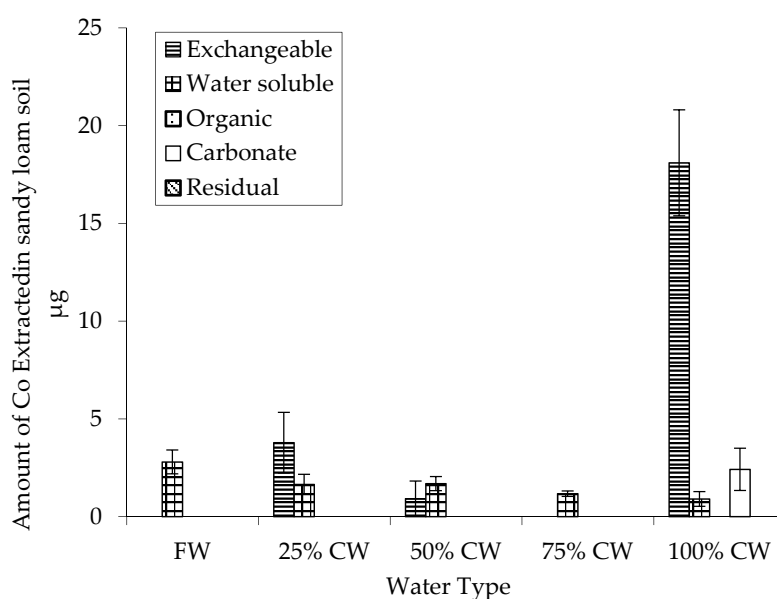


Figure 5. Mean recovered amounts of fractions of Co (in μg) in sandy loam soil that had been irrigated with different concentrations of contaminated water.

Significant differences ($p < 0.05$; $LSD_{0.05} = 0.95$) in water-soluble Co were observed between treatments in sandy loam soil. The concentration of the 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 100% CW.

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 100% CW, although there was no definite pattern between the treatments.

The carbonate fraction in sandy loam soil was only extracted where 100% CW was applied. There was no Co extracted from the organic and residual fractions in sandy loam soil.

3.8. Effect of Increasing Concentration of Contaminants in Irrigation Water on the Fractions of Co on Clay Loam Soil

Figure 6 shows the mean recovered amounts of Co in clay loam soil where varying concentrations of contaminated water were applied. There was no Co detected in other fractions such as organic, carbonate or residual in clay loam soil. Co in clay loam soils were only detected in significant ($p < 0.05$; $LSD_{0.05} = 0.95$) amount from exchangeable and water-soluble fractions.

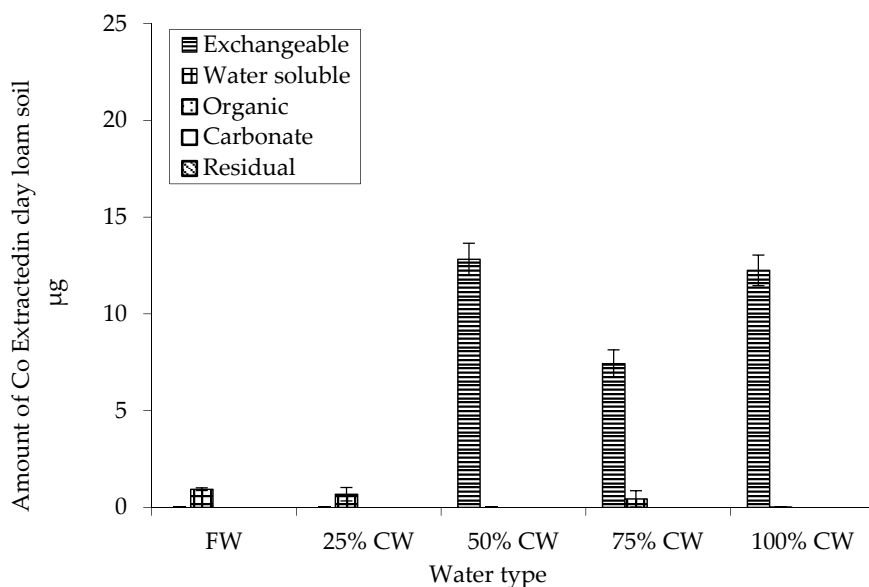


Figure 6. Mean recovered amounts of fractions of Co (in µg) in clay loam soil that had been irrigated with different concentrations of contaminated water.

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 50% CW and 100% CW 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 50% CW were largely undetected.

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 between 0.94, 0.68, and 0.01 µg/g in FW, 25% CW and 100% CW, respectively.

4. Discussion

4.1. Concentration of Bioavailable Cu and Co in Sandy Loam and Clay Loam at the End of the Greenhouse Experiment

In this study the concentration of bioavailable Cu and Co in clay soil was 1.7 times higher than in sandy loam soil. There was also an increase in the concentration of the two elements in irrigation water that resulted in increased concentration of Cu and Co in clay loam and sandy loam soils up to the saturation point.

The concentrations of bioavailable Cu and Co were higher in clay loam than in sandy loam. The concentrations of Cu were 27.7 mg/kg in clay loam and 16.5 mg/kg in sandy loam; on the other hand, concentrations of Co were 13.6 mg/kg in clay loam and 8.1 mg/kg in sandy loam.

The higher concentrations of Cu and Co in clay loam than in sandy loam soil can be attributed to the higher cation exchange capacity, surface complexation, and electrostatic interaction on the

surfaces [15,32]. 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 in clay soil is organic matter and humus [30,31]. 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 were used in the experiment could have contributed to the higher adsorption of Cu and Co than was observed on sandy loam soil.

4.2. Total Cu and Co in Soil Samples before and after Irrigation with Contaminated Water in the Greenhouse Experiment

The study showed that when both sandy loam and clay loam soils were irrigated with contaminated water, total extracted Cu and Co increased for both soils. For example, total extracted Cu in sandy loam increased from 21.58 μg to 137.0 μg , and in clay loam from 39.32 μg to 304.0 μg . Total Co extracted increased from 9.6 μg in sandy loam to 41.6 μg . The magnitude of increase of Cu and Co in sandy loam soil was four times, while in clay loam it was almost nine times. These results indicate that clay loam soils can adsorb more than double the amount of heavy metals from contaminated water. The consequent effect of this finding is that when crops are grown on the two soils, they will be exposed to higher concentrations on sandy loams than on clay loam.

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 about 27.6% clay and 4% organic matter, while sandy loam had 7.6% clay and 1.5% organic matter. These properties could largely influence the adsorption of Cu and Co observed.

Soils with high clay content, especially smectites (or montmorillonites) tend to have a high specific surface and thus provide a large surface area for adsorption of metals [33–37]. Clay minerals have a larger amount of negative charges on them in the range of 15–30 $\text{cmol}_\text{c}/\text{kg}$ soil compared to sandy loam with about 5–10 $\text{cmol}_\text{c}/\text{kg}$ soil [37]. The difference between the two soils indicates that clay loam has about three times more surface area to hold metals than sandy loam soil.

The other factor that governs adsorption of cations in soil is the organic matter [38]. 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 on sandy loam.

4.3. Effects of Increasing Loads of Heavy Metals from Contaminated Irrigation Water on Various Fractions of Soil Cu and Co Recovered from Sandy Loam and Clay Loam Soils

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.

4.3.1. Organic Fractions

The organic fraction is where the metal element is bound on the organic and sulphide fractions [39,40]. 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.

The study established that the organic fractions in both sandy loam and clay loam soils preferably attracted more Cu than Co. The higher preference for Cu of organic colloids in soils was reported by Sposito et al. [30]. The attraction of metals to colloids is usually affected by their ionic radii [11,41]. The attraction of the organic colloids with the metal ions is electrostatic. The atomic radius of Cu is 0.73 Å and Co is 0.745 Å [42]. The smaller atomic radius of Cu thus affects its higher affinity by organic fraction in soil than Co.

The other observation is that the organic Cu fraction extracted from clay loam was more than double that obtained from 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 of sandy loam and this ratio conforms closely to the amounts of Cu recovered from the two soils on the organic fractions.

The increasing concentration of contaminants in the water that was used for irrigation resulted in an increase in extracted Cu in the organic fraction until it reached a peak of around 65 µg in clay loam and 33 µg in sandy loam. Upon saturating the organic colloids, the remaining ions probably occupied other fractions. This pattern is seen from the increasing amounts of Cu extracted from the organic fraction from treatments with high concentrations of contaminants.

4.3.2. Exchangeable Fractions

Exchangeable fraction elements are weakly adsorbed on exchange sites due to negative charges that exist on the soil surfaces and positive charges (cations) for metal elements [39]. 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 being mobilized in solution and immobilized when the pH increases. The exchangeable fraction is largely composed of trace elements in the form of mineral salts.

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 more than twice as high on clay loam as on sandy loam soil. The mean Cu extracted from clay loam was 10.21 µg/g, while that from sandy loam was 4.22 µg/g. The exchangeable fraction is held onto the soil surface by weak electrostatic attractions, or by cation exchange. The cation exchange on clay soils is generally higher than on sandy soils, thus the possibility of higher amounts of Cu on the exchangeable fraction on clay loam than on sandy loam soils.

At a 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 of exchangeable fractions were higher on the clay loam soils than on sandy loam for those treatments that were irrigated with 50% CW 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.

4.3.3. Residual Fractions

Only Cu was extracted on the residual fraction. There were no differences between the different levels of concentrations of contaminants in the water used for irrigation. There generally was no Co extracted in the residual fractions on either sandy loam or clay loam. 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 [39].

4.3.4. Water-Soluble Fractions

This fraction contains the most mobile and bioavailable forms of elements in soil [42]. The elements in this form are largely salts, or water-soluble or exchangeable fractions.

The low extractions of water-soluble fractions of Co 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 there are reduced amounts of water-soluble fractions on soils.

Water-soluble fractions appeared to be more important in soil for Co than for Cu. Therefore, irrigating the soils with increasing concentrations of contaminants resulted in a decrease in the amounts of recovered water-soluble fractions of Co in both sandy loam and clay loam soils.

4.3.5. Carbonate Fractions

Carbonate fractions are composed of metal elements that are adsorbed on mineral salts that are adsorbed to the carbonates [37]. 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.

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

5. Conclusions

The study established that the concentration of bioavailable Cu and Co in soils increased at the end of the experiment in all treatments where contaminated water was used for irrigating the crops. The concentration of bioavailable Cu and Co on clay loam was 1.7 times higher than on sandy loam soil for both elements.

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. It was established that the concentrations of available Cu and Co in clay soil increased and levelled off at 38 and 21 mg/kg soil, respectively. The concentrations of available Cu and Co in sandy soil levelled off at 16.7 and 15.3 mg/kg soil, respectively. The increases in Cu were largely in the exchangeable and organic fractions for both soils. Co increased in the exchangeable and decreased in the water-soluble fractions for both soils. No apparent patterns of change were observed in the other fractions.

The general trend showed that, for Cu, the element on sandy loam soil was largely in the organic, residual, exchangeable, water-soluble, and carbonate fractions, whereas on clay loam soil the element was largely in organic, exchangeable, residual, carbonate, and water-soluble fractions. Cobalt was largely observed in the exchangeable, water-soluble, and carbonate fraction, with no particular trend observed on either clay loam or sandy loam soil.

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