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Influence of inorganic fertilizers and cattle manure on copper solubility and uptake by *Phaseolus vulgaris*

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ABSTRACT

Long term use of Cu-based fungicides has considerably polluted soils under coffee plantations in Kilimanjaro, Tanzania. High concentrations of copper in soils may lead to enhanced uptake by plants and contaminate the food web. This study explores the effects of inorganic fertilizers, cattle manure and their interaction effects on Cu availability to bean (*Phaseolus vulgaris*) grown on Cu contaminated soils with the purpose of assessing risks of contamination of the food web associated with growing bean plants on such soils. A pot experiment was carried out in a glass house using long-term Cu contaminated soils collected from Moshi Rural and Mwanga districts in Kilimanjaro, Tanzania. Air dried cattle manure was applied at two rates and applied both with and without inorganic fertilization. Cattle manure significantly increased soil pH, EC and water extractable organic carbon (WEOC) in both soil types. Fifteen percent of cattle manure application rate in Moshi soils decreased the Ethylenediaminetetraacetic acid (EDTA)-extractable Cu. The highest concentration of Cu in bean shoots was observed in the soils treated with inorganic fertilizers but without cattle manure in Moshi soils. Cattle manure in Moshi soils reduced the concentrations of Cu in bean. Bean shoots did not take up excessive quantities of Cu and therefore risks of growing beans on the soils studied appear to be low.

Key words: Bioavailability, pH, contamination, food web, soil

INTRODUCTION

Copper contamination in soils as a result of use of copper based fungicides has been reported worldwide. For example, 398 mg Cu kg^{-1} soil has been reported in vineyard soils in France (Chaignon *et al*., 2003). 3215 mg Cu kg^{-1} soil has been reported in vineyard soils in Brazil. In Australia, 247 mg Cu kg^{-1} soil was reported (Pietrzak and McPhail, 2004) whereas in Kenya 136 mg Cu kg^{-1} soil has been encountered in coffee fields (Lepp *et al*., 1984). In Kilimanjaro, Tanzania, Cu concentrations of as high as 2670 mg Cu kg^{-1} soil has been reported in coffee fields (Mzimba, 2001). One of the potential dangers of Cu contamination in the soils is that its bioavailable forms may excessively be taken up by plants, which may thus lead to food safety hazards. In China, 119 mg Cu kg^{-1} dry weight in Chinese cabbage were reported (Xiong and Wang, 2005). Adeyeye *et al*. (2006) reported 642 mg kg^{-1} Cu in cocoa seeds and 286 mg kg^{-1} in banana leaves in Nigeria. In Tanzania, Loland and Singh (2004) reported 991, 21 and 842 mg Cu kg^{-1} in coffee leaves, maize leaves and bean leaves, respectively.

In most traditional systems, soil is the main source of essential nutrients for crops. Under extensive systems of crop production, where nutrient mining and nutrient exports are low, the deterioration of soil fertility is not fast. Under coffee production systems, especially by small scale farmers, organic matter from coffee leaves and epicarps are returned to the soil. Banana leaves are used as mulching materials to conserve water and control weeds (Loland and Singh, 2004). Upon degradation, these organic residues release nutrients that are taken up by the growing crops. Thus, the system is sustained and most farmers could continue growing coffee for a long time without a need for inorganic fertilisers.

Despite the elevated levels of Cu in the soils of coffee farms in Kilimanjaro region, farmers

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who stopped the cultivation of coffee use the contaminated soils intensively for growing crops such as beans, maize and vegetables. These are shallow rooted crops whose roots extract water and dissolved nutrients from layers of Cu contaminated soils thereby posing risks of contaminating the food web. With increased intensity of farming in coffee growing areas, the soils face an accelerated decline of chemical fertility. Accordingly, the use of inorganic fertilizers becomes inevitable. Farmers in Kilimanjaro have started to use inorganic fertilizers in Cu contaminated farms. The addition of inorganic fertilisers, especially those containing exchangeable bases like Ca, Mg, and K, may change the EC and ionic strength of the soil solution and thus affect ion exchange reactions in the soil (El Bayaa *et al*., 2009). As reported by El Bayaa *et al*. (2009), an increase of K^+, Na^+ and NH_4^+ concentrations in soils increases the competition between Cu and the ions on the adsorbent surfaces, and thereby Cu solubility. KH_2PO_4 decreases Cu mobility due to the formation of insoluble Cu-phosphates (Liu *et al*., 2007). Li *et al*. (2007) did not find any significant differences in Cu concentrations between inorganic fertilizers and organic manure by maize and wheat. Apart from the inorganic fertilisers, organic compounds formed during the decomposition of soil organic matter can enhance Cu mobility (Hernandez-Soriano and Jimenez-Lopez, 2012) or can immobilise Cu (Sauvé *et al*., 1997). Mineralization of plant residues and the subsequent release of K^+, Na^+ and NH_4^+ can also increase Cu solubility (El-Bayaa *et al*., 2009).

Soil pH has a strong influence on metal solubility (McBride *et al*., 1997). Therefore activities that lower soil pH can cause metal toxicities, even if no additional metals have been introduced to the system (Robinson *et al*., 1995). However, little relationship between soil pH and Cu concentration in the soil solution has been reported (Sauvé *et al*., 1997). In Cu contaminated soils, more than 98% of the Cu in the soil solution was bound to organic complexes, regardless of pH (Sauvé *et al*., 1997) and this complexation reaction immobilizes Cu (McBride *et al*., 1997). Other factors which have an influence on metal bioavailability include the total metal load, clay content, presence of hydrous oxide, and redox conditions (El Kashouty and El Sabbagh, 2011). Therefore, the total metal contents provides the maximum metal content in the soil but the joint effects of soil properties have a great significance in deciding how much of Cu in soil pool will be bioavailable.

The effects of basic cations like K^+, Na^+, Mg^{2+} and Ca^{2+} on the bioavailabilty of Cu in agricultural fields can hardly be found in the literature. Most research on the influence of inorganic fertilizers on Cu bioavailability explored the effects of one or two cations on Cu bioavailability mainly for plant nutrition studies, for example Agbenin (2003); Mottaghian*et al*. (2008). Information on the influence of inorganic fertilizers and the interaction between the fertilizers and organic manure on Cu bioavailability is scant especially in long term Cu contaminated soils. A number of studies that explored the effects of organic fertilizers on Cu bioavailability used organic materials that have high concentrations of Cu, for example Mottaghian*et al*. (2008), Warman*et al*. (2009) and Walker *et al*. (2004). In order to study the effects of organic manure on Cu bioavailability, it is important to use organic materials containing low levels of Cu, like the one used in the present study. If rational judgments are to be made about how to make use of such potentially toxic soils, an understanding is required of plant responses, plant sensitivities and tolerances, and the substrate factors affecting plant responses to Cu toxicity (Reichman, 2002).

The objective of this study was to examine the effects of two levels of cow manure, and inorganic fertilizers and their interaction on Cu bioavailabilty to bean crop. Furthermore, the effects of cow manure, inorganic fertilizers and their interaction on soil pH, (Electrical conductivity (EC), WEOC, $CaCl_2$-extractable Cu and EDTA-extractable Cu were explored. The possible mechanisms involved in the uptake of Cu by bean shoots have been discussed.

MATERIALS AND METHODS

Soil Sampling and processing

Soils were collected from copper contaminated coffee farms in Mwanga and Moshi Rural districts in Kilimanjaro region, Tanzania, where Cu-based fungicides have been used for a long time. Moshi soils were collected from ManushiNdoo village from a farmer that has been applying Cu-based fungicides for 63 years. The farmer not only stopped the cultivation of coffee, but also the use of Cu-based fungicides. The farm is currently used for the production of banana and beans. In Mwanga, soils were collected from Raa village. The farmer in this village is still producing coffee, and used Cu-based fungicides for about 50 years up to the time of soil sampling. In addition to coffee production, the farmer is using the same piece of land for the production of bananas. Soils collected from Mwanga have been classified as Humic-Umbric Acrisols while those collected from Moshi Rural have been classified as EutricNitisols (Mlingano Agricultural Research Institute, 2006) according to FAO/UNESCO (1974) system of soil classification.

In the farms, surface soil samples (0 - 20 cm) soil depth was excavated from eight points using a shovel. The excavated soils in each farm were mixed together and homogenised to make composite samples. About 60 kg of dry soil was collected. The soils were used for the uptake studies carried out at the Tanzania Coffee Research Institute. The 20 cm depth was opted because studies (Loland and Singh, 2004) show that high concentrations of Cu in Cu contaminated farms is mainly found between 0 and 20 cm soil depth. Furthermore, most of the roots of the test crop in this study, *Phaseolusvulgaris* are mainly found between 0 and 20 cm soil depth.

At the Tanzania Coffee Research Institute, the soils were spread on a plastic sheet in a well-ventilated area for 12 days to allow drying. The soils were then ground and passed through a 2 mm sieve. The sieved soil samples were used for determination of selected soil properties presented in Table 1 and for the Cu uptake experiment. At the end of the uptake experiment 100 g soil samples were collected for further laboratory analysis using a spatula. These soil samples were taken to the Laboratory of Analytical Chemistry and Applied Ecochemistry, Ghent University for further analyses.

Selected chemical properties of soil samples

The selected chemical properties of the soils used in the study are presented in Table 1. Both soils are clay in texture, determined using a pipette method (Procedures for Soil Analysis, 2002) and have medium levels of organic carbon and CEC. Moshi soils are highly Cu contaminated while Mwanga soils are moderately contaminated. Each soil was air dried, homogenised and sieved through a 2 mm sieve and used to grow beans in a glass house. The glass house experiments were undertaken at the Tanzania Coffee Research Institute (TaCRI), based in Hai District, Kilimanjaro, Tanzania.

Table 1: Physical and chemical properties of soils used in the study

Soil type	Exchangeable bases and cation exchange capacity (cmol$_c$kg^{-1})					Total Cu (mg kg^{-1})	Available P (mg kg^{-1})	OC (%)	Texture (%)		
	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	CEC				Clay	Silt	Sand
Moshi	20	4.3	4.3	0.8	42	984	18	4.5	63.2	18.8	18
Mwanga	11	0.5	2.5	1	30	200	7	3.8	45.2	28.8	26

Source: Laboratory analysis of the soils carriedout at Tanzania Coffee Research Institute, Moshi.

Experimental design and treatments

Glass house experiments were carried out at the Tanzania Coffee Research institute. A factorial experiment was set-up involving the two soil types. Cattle manure was added at two levels, 7.5% and 15 % (w/w), on a dry weight basis and one rate of inorganic fertilizers. Treatments without addition of organic matter and/or inorganic fertilizers applications were as well included in the experiment. The experimental layout was a randomized design with three replicates (Figure 1). Each treatment involved 500 g of air dried soil, passed through a 2 mm sieve and then placed in 1 L plastic containers. The soils were mixed and homogenized with different rates of cattle manure then mixed with 25% (w/v) of distilled water and incubated for 78 days. The containers were covered by plastic bags to prevent the evaporation of water. Inorganic fertilizers were added and the soils incubated for another seven days before sowing the bean seeds. Two bean seeds per pot were sown. After five weeks of plant growth, the plants reached a flowering stage and the shoots were harvested by cutting the stems at 2 cm above the soil level. The shoots were then washed with distilled water, dried, weighed and ground using a laboratory grinding mill. The experimental treatments are as presented below:

Treatment 1: Mwanga soil + 0 inorganic fertilizers + 0% cattle manure
Treatment 2: Mwanga soil + 0 inorganic fertilizers + 7.5% w/w cattle manure
Treatment 3: Mwanga soil + 0 inorganic fertilizers + 15% w/w cattle manure
Treatment 4: Mwanga soil + inorganic fertilizers + 0% cattle manure
Treatment 5: Mwanga soil + inorganic fertilisers +7.5% w/w cattle manure
Treatment 6: Mwanga soil + inorganic fertilizers + 15% w/w cattle manure
Treatment 7: Moshi soil + 0 inorganic fertilizers + 0 cattle manure
Treatment 8: Moshi soil + 0 inorganic fertilizers + 7.5% w/w cattle manure
Treatment 9: Moshi soil + 0 inorganic fertilizers + 15% w/w cattle manure
Treatment 10: Moshi soil + inorganic fertilizers + 0% w/w cattle manure
Treatment 11: Moshi soil + inorganic fertilizers +7.5% w/w cattle manure
Treatment 12: Moshi soil + inorganic fertilizers + 15% w/ cattle manure

T11 R3	T4 R3	T10 R3	T9 R3	T1 R1	T7 R3
T3 R2	T6 R3	T10 R1	T6 R1	T9 R1	T11 R1
T3 R3	T8 R1	T1 R3	T8 R2	T6 R2	T4 R2
T1 R2	T3 R1	T5 R1	T12 R1	T2 R3	T12 R2
T7 R2	T8 R3	T12 R3	T2 R2	T10 R2	T5 R3
T11 R2	T5 R2	T9 R2	T4 R1	T7 R1	T2 R1

Figure 1: Experimental layout

Key: T = treatment; R = replicates

Cattle manure and inorganic fertilizers

The cattle manure was collected from a cattle auction market located at Weruweru along Moshi – Arusha Road in Moshi Rural, Kilimanjaro region. The cattle manure was characterised by pH of 8.6 and an EC of 9.6 mS cm^{-1}. The aqua regia- and $CaCl_2$-extractable Cu concentrations were 11 and 0.5 mg Cu kg^{-1} dry weight, respectively.

The inorganic fertilizers which were used in the study were obtained from YARA Tanzania limited and included (1) nitrogen, phosphorus and potassium (NPK) 15:15:15 at the rate of 0.8 g kg^{-1} soil, (2) calcium nitrate ($Ca(NO_3)_2$) (15.5% N and 19% Ca) each at 0.8 g kg^{-1} soil., (3) diammonium phosphate (DAP) 18:46:0 at the rate of 3.2 g kg^{-1} soil and (4) potassium nitrate (KNO_3) (13%N, 36%K) at the rate of 1.6 g kg^{-1} soil. These rates are equivalent to 50 kg ha^{-1}, 200 kg ha^{-1}, 50 kg ha^{-1} and 100 kg ha^{-1} $Ca(NO_3)_2$, DAP, NPK and KNO_3, respectively. In total, 58 kg N ha^{-1}, 97.5 kg P ha^{-1}, 43.5 kg K ha^{-1} and 9.5 kg Ca ha^{-1} were applied. These are the recommended rates for bean crop in Moshi Rural district (Mvungi, personal communication). These fertilizer materials were combined together to supply the macro nutrients, Ca, K, N and P which are essential nutrients.

Chemical analysis of soil samples

Determination of soil pH, EC, % OC, CEC and soil texture

Laboratory analysis of plant and soil samples used in the uptake experiment was carried out at the Laboratory of Analytical Chemistry and Applied Eco-Chemistry at Ghent University, Belgium. Soil pH, Electrical conductivity, organic carbon, cation exchange capacity and particle size distribution were determined using the standard methods as described below.

For soil pH and EC determination, soils and water were mixed at a 1:5 soil: water ratio. The solution was stirred and left for 16 hours (Van Ranst *et al*., 1999). After the equilibration, soil pH (pH-H_2O) was measured using a glass electrode (Orion pH meter Model 520A, Orion, Boston, MA, USA). Electrical conductivity (EC) was determined using a conductivity meter (WTW LF 537 electrode, (Wissenschafltich-TechnischenWerkstäten, Welheim, Germany). Organic carbon percentage (OC) was determined by the Walkley and Black method (Walkley and Black, 1934). Cation exchange capacity (CEC) was determined using the ammonium acetate (pH 7.0) saturation method (Chapman, 1965). Soil texture was determined by Bouyoucos hydrometer method (Procedures for Soil Analysis, 2002).

Determination of Copper fractions and water extractable organic carbon in soils

Copper contents in the treatments were determined using three different extraction methods. A 0.01 M $CaCl_2$ extraction method was carried out using a method described by Houba *et al*. (2000). In this method, soil and water samples were mixed at 1: 5 soil: $CaCl_2$ ratio. The mixture was shaken for two hours and filtered. This extraction method gives an indication of exchangeable and easily available Cu fractions in soils.

Copper was also extracted using Ammonium acetate (NH_4OAc)-0.02M Ethylenediaminetetraacetic acid (EDTA) (pH 4.65) method. The extraction was done at 1:5 soil: NH_4OAc-0.02M EDTA solution ratio. The mixture was shaken for 30 minutes and filtered as described in Lakanen and Erviö, (1971). This extraction method mimics complexing behaviour of root exudates which is exhibited by EDTA and acetic acid. Furthermore, NH_4^+ desorbs the exchangeable metal fractions. The pH of the extracting solution simulates the rhizosphere acidity (Meers *et al*., 2007).

Total Cu content was determined using aqua-regia at 1:10 soil:aquaregia ratio. After the soils were mixed with the aqua-regia, the mixture was heated for two hours and then filtered as described in Van Ranst*et al*. (1999). This fraction of copper gives an indication of the total copper load in soils which is an indication of a contamination level in the soils.

The Concentrations of Cu in all the three extraction methods were determined by an inductively coupled plasma optical emission spectrometer (ICP-OES; Varian Vista-MPX simultaneous, Varian, Palo Alto, CA, USA).

Water extractable organic carbon (WEOC) was determined as follows: Five grams of soil samples were mixed with 25 g of water in a bottle and shaken in a mechanical shaker for 24 hours then filtered using Chromafil filters, RC-45/25, of 0.45µm pore size. The solution was analyzed using the TOC analyzer (TOC-VCPH/CPN for TOC control V ver 2, Shimadzu Corporation, Kyoto, Japan).

Analysis of plant samples

Harvested plant samples were air dried for seven days then oven dried at 60 ^oC before they were ground and passed through a 1 mm sieve. 0.25 to 0.5 g of bean shoot samples were mixed with 20 mL of concentrated HNO_3 and heated at 150 ^oC for two hours. One mL of H_2O_2 was added before heating and after each 30 minutes of heating for a total of 4 mL. The solutions were then filtered in 25 mL volumetric flasks and adjusted to 25 mL using 2 M HNO_3. The concentration of Cu was determined by ICP-OES.

Quality control

Standard solutions which were prepared from standardised metal stock solutions (Merck, Darmstadt, Germany) were used for calibration. The detection limit was defined and calculated according to Hubaux and Vos (1970) (ISO 11843-2:2000) using four standards measured three times. To ascertain the accuracy of the ICP OES and the analytical procedures used, a standard reference material, rye grass referenced BCR 281, was analyzed in parallel with the plant samples. A recovery of 92% was obtained.

Data Analysis

ANOVA was carried out to detect the significance of factors on the concentrations of Cu in bean shoots, the dry matter production, soil pH, EC, WEOC and changes in $CaCl_2$- or EDTA- extractable Cu. Where the treatments showed significant differences based on F test, Tukey's method (p=0.05) was used to evaluate the significance of difference among the treatments.

RESULTS

Variations of pH and EC on soils treated with inorganic fertilizers

The chemical properties of the soils after they were treated with inorganic fertilizers and/ or cattle manure are presented in Table 2. Soil pH was increased by cattle manure in both Mwanga and Moshi soils. Manure application is expected to increase soil pH in acidic or neutral soils. Inorganic fertilizers had no significant effects on soil pH. The increase in soil pH matched with the increase in $CaCl_2$-extractable Cu in both soils (Table 2). A decrease in EDTA-extractable Cu corresponded with an increase in soil pH in Moshi soils. Cattle manure significantly increased soil EC in both soils, whereas inorganic fertilizers did not have any significant influence on the measured soil properties. An increase of soil EC is expected if EC of the organic materials added is higher than the EC of the soil.

Variations of concentrations of $CaCl_2$-and EDTA- extractable Cu on soils treated with cow manure and inorganic fertilizers

Cattle manure significantly increased the $CaCl_2$-extractable Cu in both Moshi and Mwanga soils. Cattle manure significantly decreased the EDTA-extractable Cu for Moshi soils at high rates while for Mwanga soils, no significant effect was observed at both rates (Table 3). There were no significant interaction effects of cattle manure and inorganic fertilizers on $CaCl_2$-extractable Cu in both soils.

Table 2: Soil properties and extractable Cu

Treatment	pH	EC ($\mu S\ cm^{-1}$)	$CaCl_2$ extractable Cu ($mg\ kg^{-1}$)	EDTA extractable Cu ($mg\ kg^{-1}$)
0% CM + INOR	6.4^{b}	384^{d}	0.9^{b}	686^{a}
7.5% CM + INOR	6.5^{ab}	1171^{c}	1.1^{ab}	662^{ab}
15% CM + INOR	6.7^{a}	1745^{a}	1.3^{a}	624^{bc}
Control	6.3^{b}	367^{d}	0.9^{b}	698^{a}
7.5% CM + 0 INOR	6.5^{ab}	1112^{c}	1.1^{ab}	686^{a}
15% CM + 0 INOR	6.8^{a}	1479^{b}	1.2^{ab}	652^{ac}
0% CM + INOR	6.2^{c}	327^{c}	0.1^{c}	100^{a}
7.5% CM + INOR	6.8^{b}	1133^{b}	0.3^{b}	96^{a}
15% CM + INOR	7.2^{a}	1716^{a}	0.5^{a}	90^{a}
Control	6.2^{c}	349^{c}	0.1^{c}	97^{a}
7.5% CM + 0 INOR	6.8^{b}	1109^{b}	0.3^{b}	86^{a}
15% CM + 0 INOR	7.2^{a}	1954^{a}	0.5^{a}	82^{a}

Key: CM = cattle manure; INOR = Inorganic fertilizers; 0 INOR = No inorganic fertilizer applied. Figures followed by the same letters in the same column within the same soil type are not significantly different statistically according to Tukey's method ($p=0.05$).

Variations of WEOC with cow manure treatment

In general, the different rates of organic amendments, as expected, increased significantly the WEOC in cattle manure treatments (Table 3). The effect of cattle manure on WEOC was significant at the 15% cattle manure application rate for Moshi soils whereas for Mwanga soils the significant WEOC increase was observed at both 7.5% and 15% application rates. In treatments that were not treated with cattle manure, WEOC did not differ significantly between the two soil types. However, the WEOC in Mwanga soils at both 7.5% and 15% cattle manure application rates was significantly higher than WEOC in Moshi soils (Table 3).

Variations of concentrations of Cu in bean shoots with cattle manure and inorganic fertilizers applications

Inorganic fertilizers applied to Moshi soil significantly increased Cu concentrations in bean shoots. In the present study, the highest concentration of Cu was observed in the treatment that received inorganic fertilizers without organic amendments (Figure 2). Cu concentration in bean shoots in inorganic fertilizers + cattle manure at 7.5 % application rate in Moshi soil was significantly higher than in the 7.5% cattle manure treatment that was not treated with inorganic fertilizers.

Table 3: Influence of inorganic fertilizers and cattle manure on WEOC

Treatment	**Moshi soil**	**Mwanga soil**
	WEOC ($mg\ kg^{-1}$)	WEOC ($mg\ kg^{-1}$)
0% OM + INOR	182^{a}	147^{a}
7.5% OM + INOR	285^{a*}	487^{b*}
15% OM + INOR	438^{b*}	762^{c*}
Control	175^{a}	188^{a}
7.5% OM + 0 INOR	268^{a*}	435^{b*}
15% OM + 0 INOR	398^{b*}	732^{c*}

Figures followed by the same letters in the same column are not significantly different statistically according to Tukey's method (p=0.05). Figures of the same parameter in the same row followed by asteric are statistically significant different (p=0.05)

In Moshi soils, cattle manure significantly decreased Cu concentrations in bean shoots at both 7.5% and 15% compared with the control treatment. However, between 7.5% and 15% cattle manure application rates, no significant effect was observed.

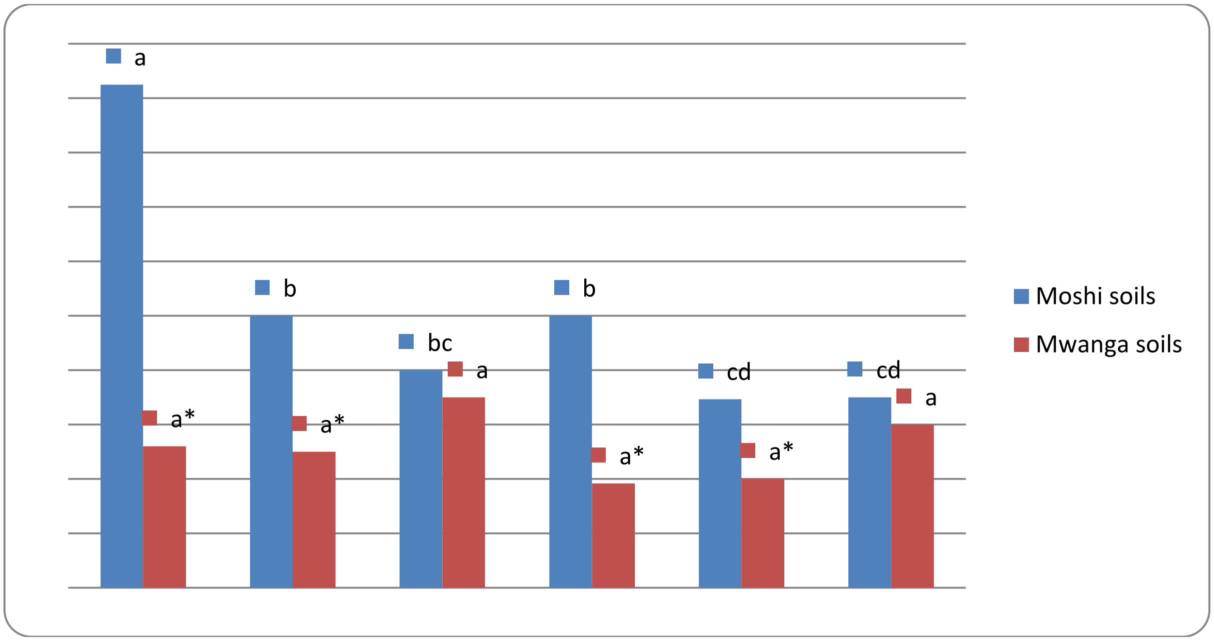

Figure 2: Influence of inorganic fertilizers and cattle manure on biomass production and concentration of Cu in bean plant

Figures followed by the same letters in the respective soil type are not significantly different statistically according to Tukey's method (p=0.05). Figures of the same treatment between the soil type with an asteric are significantly different according to Tukey's method (p=0.05).

Key: 0 OM = No organic matter applied; IN = inorganic fertilizer; 0 IN = No inorganic fertilizer applied.

For Mwanga soils, the main and interaction effects of inorganic fertilizers and cattle manure did not significantly affect Cu concentrations in bean shoots. The dry weight of bean shoots was not affected by the application of cattle manure and or inorganic fertilizers treatments in both soil types (Table 4).

Comparison of Cu concentrations in bean shoots between Moshi and Mwanga soils

Copper concentrations in Moshi soils at 0% and 7.5 % cattle manure was significantly higher than the concentrations of Cu in the corresponding treatments in Mwanga soils (Figure 2). However, at 15% cattle manure rate, the concentrations of Cu in bean shoots between Moshi and Mwanga soils did not differ significantly. In the treatments involving cattle manure without inorganic fertilizers, Cu concentration in bean shoots did not differ significantly between Moshi and Mwanga soils.

Table 4: Influence of inorganic fertilizers and cattle manure on biomass production

Treatment	Moshi soils	Mwanga soils
	Dry weight (g)	Dry weight (g)
0% OM + INOR	2.41[a]	1.91[a]
7.5% OM + INOR	1.88[a]	2.36[a]
15% OM + INOR	1.26[a]	2.36[a]
Control	1.49[a]	1.95[a]
7.5% OM + 0 INOR	1.76[a]	2.21[a]
15% OM + 0 INOR	2.03[a]	2.03[a]

Figures followed by the same letters in the same column are not significantly different statistically according to Tukey's method (p=0.05).

DISCUSSION

Variations of Copper concentrations on soils treated with cow manure

Metals within the soil solution are the only soil fraction directly available for uptake by the plants (Fageria *et al*., 1991). Hence, factors which affect the concentration and speciation of metals in the soil solution will affect the bioavailability of metals. Organic matter added to soils increase WEOC, which indeed may also increase DOC in the soil solution. Increased DOC in the soil may increase Cu mobility as Cu-organic complexes (Hernandez-Soriano and Jimenez-Lopez, 2012). This may lead to an increase of $CaCl_2$-extractable Cu as observed in the present study, as $CaCl_2$-extractable Cu is mostly related to the concentrations of Cu in the soil solution. The $CaCl_2$-extractable Cu can also be increased by cations like K^+, Na^+, NH_4^+ and Ca^{2+} released from cattle manure, which will compete with Cu for the adsorption sites (El-Bayaa *et al*., 2009). It is only when metals are present in bioavailable forms at excessive quantities that they have the potential to become toxic to plants (Reichman, 2002). The fraction of any metal occurring in the soil solution is usually small compared to the total metal quantities in the soil (Sauvé *et al*., 1997). In the present study, the $CaCl_2$-extractable Cu was only 0.1 to 0.13% and 0.05% to 0.25% of the total Cu contents in Moshi soils and in Mwanga soils, respectively. These values of the proportion of $CaCl_2$ extractable Cu as compared to the total Cu quantities in soils of the present study are in agreement with those reported by Narwal and Singh (1998) of only < 0.5%.

Copper forms insoluble complexes with organic matter (Sauvé *et al*., 1997). This is the main cause of low Cu solubility especially in soils containing high levels of organic matter such as these in the present study. In general, Cu solubility decreases with an increase of soil pH. The increase in $CaCl_2$-extractable Cu with increased soil pH, which is in contrast with the general rule, shows that in the present study, soil pH alone had a limited influence on $CaCl_2$-extractable Cu. It is probable that the cattle manure played a dominant role in Cu solubility. As reported by Sanders *et al*. (2006), the solubility of Cu was more affected by Cu-organic matter complexation than by soil pH. The contribution of cattle manure on $CaCl_2$-extractable Cu was 18% for 7.5% cow manure amended treatments compared with the control (Table 2).

Addition of organic matter to soils adds more solid OM (Komarek *et al*., 2010) and may also increase soil pH (Li *et al*., 2009) leading to less Cu mobility. This hypothesis explains the decrease in EDTA-extractable Cu in Moshi soils observed in the present study. The decrease in Cu concentrations in bean shoots in Moshi soils with increased pH is in line with the fact that Cu bioavailability decreases with an increase of soil pH (McBride *et al*., 1997).

Root exudates can change the chemistry of the soils in the root zone and solubilise organic matter which in turn complex and mobilize Cu and change the availability status of Cu

(Dessureault-Rompé*et al*., 2008). The insignificant decrease in EDTA extractable Cu with cattle manure application rates in Mwanga soils which is in contrast with the observation in Moshi soils can be attributed to lower Cu concentrations in Mwanga soils than in Moshi soils. EDTA- extraction method is good for predicting plant metal deficiencies, but it may not correlate very well with metal toxicity in plants (Merry *et al*., 1986).

Variations of Cu concentrations on bean shoots treated with inorganic fertilizers

Cu toxicity signs in the bean plants may start to show when the concentrations of Cu in the bean shoots exceed 20 mg Cu kg^{-1} dry weight (Shainberg *et al*., 2001). No toxicity symptoms such as leaf chlorosis and reduced shoot growth were observed in any of the treatments. Thus, plant growth did not seem to be affected by the high concentration of Cu in the soils. Accordingly, the concentrations of Cu remained within the normal concentrations of 4 and 20 mg kg^{-1} needed for most crops (Jones, 1972). The results of the present study demonstrate that bean crop does not take up excessive quantities of Cu, or the joint effects of soil properties deterred Cu uptake by the bean crop. Therefore, the risks related to the enhanced Cu uptake appear to be low. Apart from Cu immobilization by soil constituents like organic matter and immobilization under high CEC in soils, plants may convert Cu in the soils into metallic nanoparticles near the roots and reduce Cu toxicity to plants (Manceau *et al*., 2008). Plants may influence Cu availability in the rhizosphere by changing rhizosphere pH (Neumann and Römheld, 2002). Another report shows that Cu concentrations in tomato remained in the adequate range and were independent of single soil properties and soil Cu content (Chaignon *et al*., 2003). Furthermore, the workers did not find any significant difference in biomass production in tomato shoots grown on highly Cu contaminated soils and those grown on uncontaminated soils, which is in line with the results of the present study.

Inorganic fertilizers in Moshi soils enhanced Cu uptake by beans. The highest concentrations being observed in a treatment that did not receive cattle manure. This elucidates that depletion of organic matter contents in soils may enhance the uptake of Cu by bean crop. Senkondo *et al*. (2015) carried out a study that utilised the same Cu contaminated soils but artificially enriched with $CuSO_4$ and consequently leading to higher total Cu load. They found that bean shoots even from Cu enriched treatments had maximum Cu concentrations of 14 mg kg^{-1}. This shows that the presence of inorganic ions in the soil solution may be more important in enhancing Cu uptake by bean than the total Cu load. The effects of inorganic fertilizers on enhancing Cu uptake by bean shoots is deterred by high quantities of organic matter in Moshi soil treatments, demonstrating the Cu immobilization effects of cattle manure even in the presence of ions that can compete with Cu for the sorption surfaces. Mottaghian *et al*. (2008) reported significantly lower concentrations of Cu in soy bean in NPK treatment than in NPK + compost treated soils showing that the interaction effects of compost and NPK enhanced Cu concentrations in soy bean. The compost material used by the authors had elevated levels of Cu which may have contributed to the increased concentration of Cu. In the present study, the cattle manure which was used had a very low concentration of aqua regia and $CaCl_2$ extractable-Cu of (11 and 0.5 mg kg^{-1}respectively). It is of interest to carry out more studies on the effects of inorganic fertilizers in Cu contaminated soils without cattle manure for several seasons to investigate the uptake of Cu in Cu contaminated Moshi soils. Further research will be required to investigate the cause for the differences in response in Cu uptake as a result of inorganic fertilization between Moshi and Mwanga soils.

The 7.5 and the 15% dry cow manure application rates are on the extreme high. These high rates were selected because previous work (Senkondo *et al*., 2015) revealed that low application rates of cattle manure did not have a significant effect on Cu solubility. Because 7.5% cow manure drastically reduced Cu concentrations in bean shoots in Moshi soils (Figure 1), lower application rates can be researched for.

Variations of Cu concentrations in bean plants on soils treated with cattle manure

Cattle manure application to soils can immobilize Cu (Komarek *et al*., 2010) and deter Cu bioavailability. This may be a reason for the decrease in Cu concentrations with increased cattle manure application in Moshi soils. The decrease in Cu concentrations in bean shoots with increased cattle manure can be explained by the decrease in EDTA-extractable Cu with increased cattle manure rates. Consistent with the findings of the present study for Moshi soils, Li *et al*. (2009) found that pig manure and peat significantly decreased Cu concentrations in rice plant. A decrease in the uptake of Cu as a result of cow manure in soils has been reported for *Chenopodium album L*. by Walker *et al*. (2004).

The lack of a significant effect of cattle manure on the concentrations of Cu in bean leaves which were grown in Mwanga soils is consistent with the insignificant decrease in EDTA-extractable Cu in Mwanga soils in cattle manure treated treatments (Table 2). The phenomenon encountered in Mwanga soils has been reported by other workers, for example in winter squash (Warman *et al*., 2009) and tomato (Chaignon *et al*., 2003). An increase in Cu concentrations in plants has been reported in studies that used organic amendments that contained elevated levels of Cu, for example Warman *et al*. (2009) and Walker *et al*. (2004).

Apart from the joint effects of the soil properties, another possible governing factor that may have affected the concentrations of Cu in the bean shoots in the present study is that bean plants may have a mechanism of restricting excess Cu uptake and or its translocation to the above ground biomass (Li *et al*., 2009) as a mechanism of controlling toxicity to the plants. This hypothesis is supported by the observation that Moshi soils had about 5 times higher total Cu, 5 – 6 higher EDTA-extractable Cu and 2 – 9 higher $CaCl_2$-extractable Cu than Mwanga soils; yet Cu concentrations in bean shoots were only 1 – 3 fold higher in Moshi soils than was the case in Mwanga soils and remained within the normal ranges for plant Cu concentrations. Under toxic conditions, plants have mechanisms to regulate complexation of Cu within the xylem sap and hence minimize potential damage caused by high concentrations of free Cu ions (Welch, 1995). Under conditions of elevated metal supply, generally the majority of metals are restricted to the plant roots (Brown and Wilkins, 1985).

To combat high external metal concentrations there are two main categories of strategies; these are restriction of uptake or transport and internal tolerance mechanisms. The former strategy includes preventing or lessening toxic metals from entering the plants by precipitating or by complexing metals in the root environment (Reichman, 2002). The precipitation can be achieved by increasing pH of the rhizosphere or by excreting anions like phosphate (Taylor, 1991). Other mechanism include cellular exclusion where a large fraction of metals which are in plant roots are kept in the apoplastic free space and complexation at the cell wall-plasma membrane interface (Iwasaki *et al*., 1990).

In the present study, Cu concentration in shoots remained within the normal Cu concentrations of healthy plants. There was no difference in dry weights among the treatments. This shows that the mechanisms that may have been involved in keeping Cu at normal concentrations in bean shoots in the present study may include soil factors, precipitation by exudates from the plants in the rhizosphere or cellular exclusion. The experimental set up of the present study did not allow to differentiate between the cellular exclusion and precipitation by root exudates. A mechanism of compartmentation within the shoot cells may not have been involved because shoot analyses did not show elevated levels of Cu. It is of interest to carry out Cu bioavailability studies for other crops that are grown in these Cu contaminated fields. Since inorganic fertilizers enhanced Cu bioavailability in treatments with low levels of Cu, it is imperative to carry out further experiments to explore the effects of further depletion of organic matter in soils in copper contaminated coffee fields.

CONCLUSIONS

Inorganic fertilizers enhanced Cu availability to bean shoots in Moshi soil but not in Mwanga soil probably due to differences in the total Cu load. These effects were masked when cattle manure was also applied. Cattle manure increased pH, EC and $CaCl_2$-extractable Cu in both soils. The present study demonstrates that bean crop does not take up excessive quantities of Cu, and therefore, the risks related to the enhanced Cu uptake appear to be low. Soil factors like organic matter content, high CEC and pH, precipitation by exudates from the plants in the rhizosphere or cellular exclusion may be mechanisms involved in keeping Cu concentrations in bean shoots at normal levels. Bioavailability studies for other crops that are grown in Cu contaminated fields should be carried out.

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