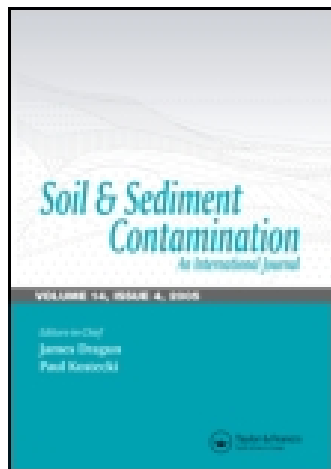


On: 09 January 2015, At: 10:02

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Soil and Sediment Contamination: An International Journal

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/bssc20>

Copper Bioavailability to Beans (*Phaseolus vulgaris*) in Long-Term Cu-Contaminated Soils, Uncontaminated Soils, and Recently Cu-Spiked Soils

Y. H. Senkondo^a, E. Semu^b & F. M. G. Tack^a

^a Department of Applied Analytical and Physical Chemistry, Gent University, Gent, Belgium

^b Department of Soil Science, Sokoine University of Agriculture, Morogoro, Tanzania

Accepted author version posted online: 20 May 2014. Published online: 07 Jan 2015.



CrossMark

[Click for updates](#)

To cite this article: Y. H. Senkondo, E. Semu & F. M. G. Tack (2015) Copper Bioavailability to Beans (*Phaseolus vulgaris*) in Long-Term Cu-Contaminated Soils, Uncontaminated Soils, and Recently Cu-Spiked Soils, *Soil and Sediment Contamination: An International Journal*, 24:2, 116-128, DOI: [10.1080/15320383.2014.920763](https://doi.org/10.1080/15320383.2014.920763)

To link to this article: <http://dx.doi.org/10.1080/15320383.2014.920763>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms &

Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

Copper Bioavailability to Beans (*Phaseolus vulgaris*) in Long-Term Cu-Contaminated Soils, Uncontaminated Soils, and Recently Cu-Spiked Soils

Y. H. SENKONDO,¹ E. SEMU,²
AND F. M. G. TACK¹

¹Department of Applied Analytical and Physical Chemistry, Gent University,
Gent, Belgium

²Department of Soil Science, Sokoine University of Agriculture, Morogoro,
Tanzania

Copper solubility and its bioavailability to Phaseolus vulgaris in long-term copper-contaminated soils, uncontaminated soils, and copper-spiked soils were studied. The role of plant factors, total copper load in soils, and/or the aging effect on the uptake of copper was explored so as to assess health risks through contamination of the food chain associated with growing the crop on such soils. Contaminated soils and clean soils were collected from coffee-growing fields in Kilimanjaro and Arusha, Tanzania. Two bean seeds were sown per pot, replicated three times, and arranged in a randomized design in a glass house. Copper spiking significantly increased extractable copper, as expected. For all of the treatments except for the Mwanga and Arumeru control soils, the addition of CuSO₄ did not significantly increase the humic-acid-bound copper, but it significantly increased the fulvic-acid-bound copper ($p = 0.05$). Moshi soils had significantly higher concentrations of copper in the bean shoots than was the case with other treatments ($p = 0.05$). For the respective soil types, there was no significant difference in the concentrations of copper in bean leaves between spiked and unspiked treatments ($p = 0.05$). Bean shoots did not accumulate copper beyond the normal concentrations.

Keywords Solubility, uptake, pH, fulvic acid, humic acid

Introduction

The continual use of copper-based fungicides in coffee fields to control fungal diseases has caused accumulations of copper in coffee-growing soils (Loland and Singh, 2004). The applied fungicides accumulate in soils through drops of solution containing the fungicides that fall on the soil during fungicide applications. Copper-contaminated leaves that drop on the ground as they atrophy or as pruned materials undergo mineralization, thereby releasing copper (dos Santos *et al.*, 2009). The accumulation of copper in the soils poses potential hazards to the environment and, consequently, the accumulation may be harmful to human beings (Lamb *et al.*, 2009) through contamination of the food chain (Loland and Singh,

Address correspondence to Y.H. Senkondo, Department of Applied Analytical and Physical Chemistry, Gent University, Coupure Links, B 653, 9000 Gent, Belgium. Email: hassan.senkondo@ugent.be or kangero70@yahoo.ca

2004). When the excess copper is ingested, it accumulates in the liver, brain, skin, pancreas, and myocardium (El Bayaa *et al.*, 2009).

Soil properties play a vital role in mobility and bioavailability of heavy metals such as copper (Gandois *et al.*, 2010; Sims, 1986; Sauvé *et al.*, 2000). When copper is introduced in the soils, it undergoes changes that may immobilize and render it insoluble, thereby making it unavailable for plant uptake (Brallier *et al.*, 1996).

The added copper in the soil may complex with organic matter, it (copper) may be bound to carbonates, or it may be adsorbed in the soil mineral colloids. This may lead to a decreased concentration of water-soluble copper with time, and make the copper less bioavailable (Ma *et al.*, 2006). Studies show that when copper is added onto the soil, most of it will be in the exchangeable and water-soluble form and hence more bioavailable; however, as time passes the copper becomes converted to other fractions which are less bioavailable (Jalali and Khanlari, 2008; Hogg *et al.*, 1993; McLaren and Ritchie, 1993). Other reactions that may take place after the copper has been added to the soil include complexation, surface adsorption, exchange reactions, chelation, and precipitation on the surfaces of the soil particles or diffusion into the mesopores and macropores of soils, converting the copper from available to less soluble forms (Cavallaro and McBride, 1978). Apart from environmental factors, soil components and properties, the degree of immobilization of copper is also determined by the total copper load in the soils (Jalali and Khanlari, 2008).

The amount of copper adsorbed by soil as a result of the aging effect is modified by soil pH and the quantity of organic matter in the soil. Lu *et al.* (2005), in their investigation of the aging effect on the distribution of metals to different fractions, found that, under acidic conditions, the rate of conversion of the exchangeable fraction of copper to other fractions was very low as compared with neutral or alkaline soils, and high organic matter resulted into high organic-matter-bound copper. Generally, the bioavailability of heavy metals in the soils decreases with an increase in the residence time (Zhang *et al.*, 2006; Ma *et al.*, 2006). Therefore, the mobility of copper in long-term copper-contaminated sites, as a result of continual additions of copper, may be completely different from the behavior which will be exhibited when a one-time heavy dose is applied to the soil and the mobility and bioavailability measured after a short incubation time (Lock and Janssen, 2003).

One of the soil constituents that can influence solubility behavior of metals in the soils is soil organic matter through the formation of soluble complexes which are formed upon organic matter degradation (Hernandez-Soriano and Lopez-Jimenez, 2012; Almås *et al.*, 1999) or through the formation of insoluble complexes (Narwal and Singh, 1998; Tandy *et al.*, 2009). According to Hernandez-Soriano and Lopez-Jimenez (2012), the binding of heavy metals to organic matter depends on the metal's affinity for different functional groups present in the organic matter and on the soil properties.

Humic acids and fulvic acids form a vital ingredient of the soil organic matter; the binding properties of the two organic acids may affect the fate of copper in the soil. Copper complexation by organic matter, in the form of humic acid and fulvic acid, is an effective mechanism of copper retention in the soils (Bradl, 2004). Fulvic acid in the soils may enhance copper solubility because it is soluble in water, while humic acid may form insoluble complexes, although the humic acid may also form soluble complexes, depending on the degree of dissociation of the humic molecules (Gondar *et al.*, 2006). The humic substances account for up to 70–80% of the organic matter and can also control the capacity of mineral matter in the soil to adsorb metals (Arias *et al.*, 2002). At pH values above 6, organic matter decreased plant absorption of Cu because of its ability to form chelates with humic acid and its strong adsorption to organic matter (Clemente *et al.*, 2003), as well as lower proton-metal ion competition (Gondar *et al.*, 2006).

In coffee-growing areas where the soils have long been contaminated by copper-based fungicides and where the addition of organic fertilizers has been a common practice, as is the case in Hai, Moshi, Mwanga and Arumeru Districts, it is imperative to study copper associations with these two important organic acids, humic acid and fulvic acid, because they (the acids) affect mobility of copper in the soils.

Our previous, unpublished studies reveal that, despite high concentrations of copper in the copper-contaminated soils, *Phaseolus vulgaris* did not take up excess quantities of copper. It was hypothesized that the aging effect or the crop characteristics were responsible for the phenomenon. Therefore, it is important to explore the factors that contributed to this phenomenon so as to include such information in the environmental risk assessment. Most studies on the influence of aging on copper solubility were undertaken in test tubes where adsorption isotherms of the soils were studied, or the soils were incubated for a few weeks to determine the distribution of copper species as affected by incubation time. Information on the comparisons between copper uptake from soils which have been contaminated by copper for a long time, uncontaminated soils, and recently spiked soils is hardly found in the literature.

To account for the role of aging effect on copper bioavailability, this study compared copper solubility in the soils and its bioavailability to bean shoots among long-term copper-contaminated soils, uncontaminated soils, and recently spiked soils. The study discusses the uptake of copper in relation to the total copper load and the soluble fractions of copper in the soils. The influence of spiking soils with CuSO_4 on copper association with humic or fulvic acids among different soil types have been explored in the present study.

Materials and Methods

Soil Sampling

Soils were collected from Hai, Moshi Rural, and Mwanga Districts in the Kilimanjaro region and Arumeru District in the Arusha region. The soils collected from Mwanga, Hai, and Moshi Rural Districts had been receiving copper fungicides for as long as 63 years in some soils. Two control soils were included in the study, one from Mwanga and the other from Arumeru. Kilimanjaro region is located in the northern part of Tanzania, between latitudes $3^{\circ}39'$ and $4^{\circ}37'$ south, and longitudes $36^{\circ}52'$ and $38^{\circ}25'$ east. Arumeru District is located between latitudes $3^{\circ}30'$ to $3^{\circ}30'18''$ south and longitudes between $36^{\circ}30'$ to $37^{\circ}30'$ east. The glass house experiments were undertaken at the Tanzania Coffee Research Institute (TaCRI), based in Hai District, Kilimanjaro region, Tanzania.

Mlingano Agricultural Research Institute, Tanzania (2006), compiled major soil types of Tanzania and their properties. The soils collected from Mwanga have been classified as Humic-Umbric Acrisols. The soils are strongly weathered, and characterized by low-activity clay, which developed from the weathering of acid rock materials on hilly or undulating topography. Moshi Rural and Hai soils have been classified as Eutric Nitisols. The soils are deep, well-drained with clayey subsurface horizons that have polyhedral, blocky structure elements with shiny ped surfaces. They have developed from parent materials that are finely textured with weathering products of intermediate to basic parent rock, and rejuvenated with admixtures of volcanic ash. The clay assemblage of this type of soil is rich in iron and has very little water-dispersible clay. The areas are hilly in topography. The soils collected from Arumeru District are Mollic Andosols. They are black, porous soils of volcanic materials resulting from the accumulation of stable organo-mineral complexes and short-range order minerals.

Experimental Treatments

About 500 g of soils (contaminated or control) were treated with CuSO_4 , mixed with 25% (w/v) water, covered with polythene bags, and equilibrated for 30 days. After 30 days, the copper-spiked soils were leached with 500 mL distilled water to remove the copper that was anticipated to be in the soil solution. The soils were left for three days to drain, after which two bean seeds were sown per pot and arranged in a glass house in a randomized design with three replicates. In the anticipation of germination failures as a result of copper toxicity, some bean seeds were separately planted in separate pots containing corresponding unspiked soils. On the spiked soils, the seeds did not germinate as anticipated and therefore the bean seedlings which were separately planted on the non-spiked soils were transplanted to the respective spiked treatments. After five weeks—that is, at flowering—the crop was harvested and dried. Soil samples were taken at harvesting for laboratory analysis.

The experimental treatments, replicated three times, are presented below:

1. Mwanga soil: Long-term contaminated soils;
2. Mwanga soil: Long-term contaminated soils + 200 mg/kg Cu from CuSO_4 ;
3. Moshi soil: Long-term contaminated soils;
4. Moshi soil: Long-term contaminated soils + 200 mg/kg Cu from CuSO_4 ;
5. Hai soil: Long-term contaminated soils;
6. Hai soil: Long-term contaminated soils + 200 mg/kg Cu from CuSO_4 ;
7. Mwanga control soil: Uncontaminated soils;
8. Mwanga control soil: Treated with 400 mg/kg Cu from CuSO_4 ;
9. Arumeru control soil: Uncontaminated soils;
10. Arumeru control soil: Treated with 800 mg/kg Cu from CuSO_4 .

Chemical Analysis of Soil Samples

Laboratory analysis was carried out at the Laboratory of Analytical Chemistry and Applied Ecochemistry, Ghent University. Soil pH (water) was measured using a glass electrode pH meter (Orion Model 520A, Orion, Boston, MA, USA) at a soil: water ratio of 1:5. Electrical conductivity (EC) was determined using a Microprocessor conductivity meter, WTW LF 537 electrode (Wissenschaftlich-Technischen Werkstätten, Weilheim, Germany) at the 1:5 soil: water ratio. Copper was extracted using two different extractants. These were 0.01 M CaCl_2 extraction at 1:5 soil: CaCl_2 ratio (Houba *et al.*, 1999) and ammonium acetate-EDTA (pH 4.65) at 1:5 soil: EDTA ratio (Lakanen and Ervio, 1971). CaCl_2 extractable copper represents soluble, easily leached copper fraction (Sauvé *et al.*, 1997); the advantage of this extractant is that its ionic strength is generally similar to that of the soil solution. Ammonium acetate-EDTA was selected because complexation between EDTA and acetic acid mimics complexing behavior by root exudates and NH_4^+ desorbs the exchangeable metal fractions, whereas the pH simulates rhizosphere acidity (Meers *et al.*, 2007). The copper was determined by inductively coupled plasma optical emission spectrometer (ICP-OES; Varian Vista-MPX simultaneous Varian, Palo Alto, CA, USA).

Humic Acid and Fulvic-Acid-Bound Copper

The association between copper with fulvic acid (FA) and humic acid (HA) was carried out as described by Mohamed *et al.* (2010). Five g of the soil samples were mixed with 50 mL of 0.1M NaOH and shaken for 24 hours in a mechanical shaker. The solution was centrifuged at 3800 rpm for 30 minutes. The 15 mL of the supernatant was taken and

acidified with HNO_3 ($\text{pH} = 1.2$) and centrifuged at 3800 for 30 minutes. The supernatant was decanted and digested with HNO_3 and the concentration of copper determined by ICP OES. This solution contains FA-bound copper. Another 15 mL of the 0.1M NaOH-treated solution was taken and digested with HNO_3 at 150°C for two hours and copper determined by ICP OES. This solution contains FA- and HA-bound copper. The HA-bound copper was obtained by difference.

Analysis of Plant Samples

About 0.3–0.5 g of bean shoot samples were mixed with 20 mL of HNO_3 acid and heated at 150°C for two hours. One (1) mL of H_2O_2 was added before heating and after 30 minutes of heating. In total, 4 mL of H_2O_2 were added to each sample. After cooling, the solutions were filtered in 25 mL volumetric flasks and made to the volume using 2 M HNO_3 . The concentration of copper was determined by the ICP OES.

Quality Control

The ICP OES used copper standards, Merck at four concentrations, and calibration controlled with independent standards. To determine the detection limit, the Hubaux and Vos method (1970) (ISO 11843-2:2000) model was used. Four standards were measured three times and, based on the standard deviations, the detection limit was calculated. 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 and 92% recovery was attained. Additionally, a calibration curve was recalibrated after every 20 samples. Moreover, a standard solution was included in every analysis.

Data Analysis

Analysis of variance (ANOVA) was carried out to examine any difference ($p = 0.01$) in the concentrations of copper in the bean crop from the different treatments using S PLUS 8.2 software (Insightful Inc., USA). ANOVA was also carried out to examine the effects of the treatments on soil pH, EC and CaCl_2 , EDTA, or aqua regia-extractable copper. Tukey's method ($p = 0.05$) was used to compare the means between the treatments.

Results

Variations in Soil pH and Soil EC

Physicochemical properties of soils used in the study are presented in Table 1. The pH and EC of the soils sampled at harvesting of the bean crop are presented in Table 2. Arumeru soil was in neutral range while all other soils were in acidic range, Hai soils being the most acidic. The pH of some different soil types varied significantly. The Arumeru soils had significantly higher pH than all of the other soil types, whereas Hai soils had significantly lower pH than all of the other soils. The differences in soil pH are a manifestation of different soil-forming factors, the nature of the parent materials, and human activities which exist between the different sampled farms. The pH values of Moshi soils, Mwangi and Mwangi control soils did not vary significantly.

The EC values showed that all of the soils were non-saline. The soil EC varied significantly among the different soil types ($p < 0.01$). Hai-spiked treatment had significantly

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

Soil type	pH	EC ($\mu\text{S}/\text{cm}$)	Exchangeable bases and CEC (meq/100g)						Available P (bray 1) (mg/kg)	Texture		
			Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	CEC	%OC		%Clay	%Silt	%Sand
Moshi	5.9	120.5	20	4.3	4.3	0.8	42	4.5	18	63.2	18.8	18
Hai	5.8	145.6	19	2	3.9	1.0	40	8.8	3	71.2	20.8	8
Mwanga	5.8	128.6	11	0.5	2.5	1.0	30	3.8	7	45.2	28.8	26
Arumeru control	6.9	167.1	28	0.0	8.5	1.0	39	2.8	25	53.2	32.8	14
Mwanga control	6.3	5.1	11	1	1.0	0.8	19	2.5	1	47.2	34.8	18

Table 2
pH and EC values of soils used in the study at harvesting

Treatment/Parameter	pH	EC ($\mu\text{S}/\text{cm}$)
Mwanga - spiked	6.20 ^b	112 ^{gh}
Mwanga	6.23 ^b	88 ^{gd}
Moshi - spiked	6.40 ^b	108 ^{ge}
Moshi	6.53 ^b	76 ^g
Arumeru control - spiked	7.00 ^a	235 ^{bc}
Arumeru control	7.20 ^a	146 ^{cg}
Hai - spiked	6.10 ^b	397 ^a
Hai	5.76 ^c	183 ^{cdef}
Mwanga control- spiked	6.13 ^b	113 ^{gh}
Mwanga control	6.4 ^b	68 ^g

The same letters in the same column are not significantly different according to Tukey's method of means comparison ($p = 0.05$).

higher EC than all of the other treatments. The addition of CuSO_4 significantly increased the EC for Hai soils, whereas the addition of CuSO_4 had no significant effect on soil EC for all of the other soil types.

Extractable Copper in Different Soil Types

Table 3 shows that extractable copper varied among the different soil types. For the contaminated, non-spiked treatments, Moshi soils had significantly the highest CaCl_2 -, EDTA-, and aqua-regia-extractable copper of all the other non-spiked treatments. The CaCl_2 -extractable copper for Mwanga-contaminated, non-spiked and Hai-contaminated, non-spiked soils were not significantly different, but the latter had significantly higher EDTA- and aqua-regia-extractable copper than the former. CaCl_2 -extractable copper in control soils was

Table 3
Copper extractability of soils used in the study

Treatment/Parameter	CaCl_2 (mg/kg)	EDTA (mg/kg)	AR (mg/kg)
Mwanga spiked	0.4 ^d	166 ^e	443 ^d
Mwanga	0.1 ^e	79 ^f	200 ^e
Moshi spiked	1.4 ^a	720 ^a	1444 ^a
Moshi	1.0 ^{cb}	608 ^b	984 ^b
Arumeru control - spiked	0.8 ^{cb}	94 ^f	898 ^b
Arumeru control	nd	12 ^g	46 ^f
Hai spiked	0.4 ^d	325 ^c	910 ^b
Hai	0.4 ^{ed}	300 ^c	678 ^c
Mwanga control- spiked	1.1 ^{ab}	237 ^d	414 ^e
Mwanga control	nd	22 ^g	49 ^f

The same letters in the same column are not significantly different according to Tukey's method of means comparison ($p = 0.05$).

Table 4
Humic- and fulvic-acid-bound copper of soils used in the study

Treatment/Parameter	HA-Cu (mg/kg)	FA-Cu (mg/kg)
Mwanga spiked	54 ^c	52 ^e
Mwanga	42 ^{ce}	22 ^f
Moshi spiked	122 ^b	208 ^a
Moshi	113 ^b	166 ^b
Arumeru control - spiked	173 ^a	104 ^{cd}
Arumeru control	15 ^e	2 ^f
Hai spiked	108 ^b	123 ^e
Hai	117 ^b	79 ^{de}
Mwanga control- spiked	46 ^{cd}	57 ^e
Mwanga control	17 ^e	4 ^f

The same letters in the same column are not significantly different according to Tukey's method of means comparison ($p = 0.05$).

below the detection limit of the ICP-OES used (0.005 mg/L or 0.0025 mg Cu/kg soil). The addition of CuSO_4 increased significantly ($p = 0.05$) the CaCl_2 - and EDTA-extractable copper for all soil types except for the Hai soils. As expected, the total copper contents of the soils, as measured by aqua-regia-extractable copper for all of the spiked treatments, were significantly higher than the treatments that were not spiked with CuSO_4 .

Binding of Copper to Humic and Fulvic Acids

Table 4 illustrates that the different treatments showed significant differences in capacities of the soil types to bind copper by fulvic acid or humic acid ($p < 0.01$). For all the treatments, except for the Arumeru and Mwanga control soils, the addition of CuSO_4 did not change significantly the HA-bound copper, but it significantly ($p = 0.05$) increased the FA-bound copper. Table 4 further depicts that control soils had lower HA- as well as FA-bound copper than the contaminated and spiked soils. It is interesting to note that the HA-bound copper was not statistically ($p = 0.05$) different between spiked and non-spiked soils for the respective contaminated soils (Table 4).

Influence of Different Soil Types and CuSO_4 on the Concentrations of Copper in Bean Shoots and Bean Dry Weights

Table 5 shows the concentrations of copper in bean shoots. The bean plants grown on Moshi soils had significantly ($p = 0.05$) higher concentrations of copper than the beans grown on all of the other soil types. The concentrations of copper among bean shoots grown on Hai, Mwanga, and Arumeru soils did not differ significantly. Furthermore, the addition of CuSO_4 to different soils did not significantly ($p = 0.05$) affect the concentrations of copper in bean shoots in all the respective soil types. Table 5 shows further that there was a significant ($p = 0.05$) difference in the bean dry matter among the different treatments. For Mwanga soils, the copper-spiked treatment produced significantly ($p = 0.05$) lower dry matter than the Mwanga-copper-unspiked treatment, implying possible toxicity. For other treatments, the bean dry matter between copper-spiked and copper-unspiked treatments did not vary

Table 5
Bean shoots dry weight and the concentrations of copper in bean shoots

Treatment/Parameter	Cu-bean (mg/kg)	Dry wt (g)
Mwanga spiked	6 ^b	1.04 ^b
Mwanga	6 ^b	1.84 ^a
Moshi spiked	14 ^a	1.23 ^{ab}
Moshi	14 ^a	1.41 ^{ab}
Arumeru control - spiked	5 ^b	1.26 ^{ab}
Arumeru control	4 ^b	1.00 ^b
Hai spiked	6 ^b	1.35 ^{ab}
Hai	7 ^b	1.34 ^{ab}
Mwanga control- spiked	5 ^b	0.87 ^b
Mwanga control	5 ^b	0.93 ^b

The same letters in the same column are not significantly different according to Tukey's method of means comparison ($p = 0.05$).

significantly. Bean seeds did not germinate on spiked soils but the transplanted seedlings continued growing with no differences on the biomass yields, except for the Mwanga soils.

Discussion

Soil pH and CaCl₂-Extractable Copper

Because soil pH differed significantly between the treatments, it would be expected that the copper solubility as measured by CaCl₂ extractability would be lower for high pH treatments and higher for low pH treatments. However, this was not the case. The converse phenomenon observed can be attributed to the differences in the contamination history among the soil types and the different spiking rates. The results in the present study have revealed that spiking introduces soluble fractions of copper to most of the soils studied (Table 3). This phenomenon signifies that the total copper load in the soils may be more important in controlling copper solubility in soils than soil pH. As Sauvé *et al.* (2000) reported, Cu solubility in the soils was a result of pH, the total metal burden, and organic matter contents. Lack of significant increase in CaCl₂-extractable copper in Hai soils between spiked and non-spiked treatments shows that Hai soils had high capacity to adsorb and retain the added copper. It can also be inferred that the conversion rate of copper from available forms to less available forms in Hai soils is higher than is the case in other soils; this is probably due to high organic matter content with the former as opposed to the latter soils (Table 1).

Copper Binding to Humic Acid and Fulvic Acid

The differences in the capacities of soils to bind copper to fulvic acid and humic acid can be attributed to the quantities and types of soil organic matter that may exist in different soil types and the total copper load. Humic substances that can be extracted from soils range in formula weight from as low as several hundred to over 300,000 (Stevenson, 1994 as cited in Certini, 2001). The differences in molecular weight for the humic materials in soils may affect copper binding (Inaba and Takanaka, 2005). Different plant materials have

different quantities of organic carbon (Clemente *et al.*, 2007) which, upon fractionation, may release different quantities of fulvic acid and humic acid. Differences in the quantities of released humic or fulvic acids can explain the differences in copper binding to these two important organic acids encountered in the present study. Observations in the present study (Tables 3 and 4) reveal further that, in most unspiked soils, more copper is bound to humic acid than to fulvic acid, which implies that for most unspiked soils the mobility and toxicity of copper are more controlled by humic acids than by fulvic acids. The observation that fulvic acid in spiked soils bound more copper than in unspiked soils shows that fulvic acid may be very important in binding recently added copper in the soils if the soils are in acidic pH ranges. This means that fulvic acid is an important determinant of copper mobility and toxicity in spiked soils. As Gondar *et al.* (2006) argue, copper binding to humic substances is influenced by a number of factors, such as the total amount of different functional groups and chemical structure. Lack of significant difference in humic-acid-bound copper between spiked and non-spiked soils in most soils shows the possibility that the binding sites in humic acid in the soils might have already been saturated with metals.

Influence of Different Soil Types and CuSO₄ on the Concentrations of Copper in Bean Shoots and Bean Dry Weights

Despite an increase in the CaCl₂- and EDTA-extractable copper as a result of the added CuSO₄, an increase in the extractable copper in the soils was not reflected in the concentrations of copper in bean shoots. This shows that even the recently added copper was not taken up by bean shoots, signifying that the aging effect had no influence on copper uptake by the bean shoots, which is contrary to what was hypothesized by Elzinga *et al.* (2006). The increased Cu solubility in spiked soils is a result of the low time of contact between the added copper and the soil mass (Zhou *et al.*, 2008). For example, Mwangi control-spiked soils had significantly higher CaCl₂-extractable copper than was the case with Moshi unspiked soils, despite significantly lower total copper contents in Mwangi soils. On the other hand, Mwangi control-spiked soils had significantly higher quantities of CaCl₂-extractable copper than was the case with Moshi unspiked soils, but the concentrations of copper in bean shoots in Moshi soils was significantly higher than the concentrations of copper in bean shoots in Mwangi control-spiked soils. It is therefore evident that the aging effect played a vital role in the solubility of copper in the soils but it had a limited role on copper uptake by bean shoots. It can therefore be inferred that relying on soluble quantities of copper in the soils, especially those generated by spiking soils, may exaggerate the risk of increased solubility posed by a particular metal in the soils, especially if uptake studies have not been carried out. The joint effects of soil properties (Brun *et al.*, 2001; Gandois *et al.*, 2010) may have played a more important role in deterring the uptake of copper than the aging effect or the total copper load. This hypothesis is supported by the fact that, in Moshi soils, despite the fact that the spiked treatment had significantly higher CaCl₂- and EDTA-extractable copper (Table 3) than the Moshi unspiked treatment, the concentration of copper in bean shoots was the same for both treatments (see Table 5). Furthermore, contrary to what was hypothesized by Brun *et al.* (1998) and Déportes *et al.* (1995) that copper load enhances copper bioavailability, the differences in the total copper load among different treatments in the present study did not affect copper concentrations in bean shoots (Table 5). The failure in seed germination in the spiked soils elucidates that copper toxicity is more critical at seed germination, but once the seeds escape copper toxicity at germination, the bean seedlings can tolerate higher concentrations of copper.

Apart from the joint effects of the soil properties, another possible governing factor that may have deterred the excessive uptake of copper by the bean shoots in the present study is that bean plants may have a mechanism of restricting excess copper uptake and/or its translocation to the above ground biomass (Miyazawa *et al.*, 2002) as a mechanism of controlling toxicity to the plants. This hypothesis is supported by the results obtained in Moshi treatments, which indicated that despite the differences in copper concentrations between Moshi spiked and Moshi unspiked treatments, the concentrations of copper in bean shoots in the two treatments were the same. The mechanism of restricted upward movement of copper by plants is not well understood (Pitch and Scholtz, 1996). In the present study, copper concentrations in bean roots were not measured due to difficulties in recovering root hair biomass. This happened due to the fact that the plants were grown on soils and it was difficult to recover substantial quantity of the roots. It is imperative to carry out a study that will make it possible to recover bean roots so as to measure the concentrations of copper in the roots so as to find out whether bean plants do not take excess copper to the root system or take copper to the root system but it hinders its translocation to the shoots.

The highest mean concentrations of Cu in the bean shoots encountered in the present study (Table 5) lie within the adequate copper concentrations of between 5 to 25 mg/kg for most plant species (Aasen, 1997, as cited in Loland and Singh, 2004). Jones (1972, as cited in Paramasivam *et al.*, 2009) reports that the concentrations of less than 4 mg/kg are considered deficient and copper concentrations of more than 20 mg/kg are considered toxic. Therefore, bean plants in the present study did not take up copper in excess quantities.

In conclusion, soluble fractions of copper and total copper load are not good indicators of copper bioavailability to the bean shoots and, therefore, spiking can exaggerate the risks of contamination of the food chain. The aging effect had no influence on copper uptake by bean shoots. Plant factors and the joint effects of soil properties played a major role in controlling copper uptake by bean shoots. Fulvic acid was a major factor in binding the recently added copper rather than humic acid, and therefore it (fulvic acid) may govern the mobility and toxicity of the recently added copper. Humic acid was a major factor controlling the binding of copper in unspiked soils rather than fulvic acid, and therefore it (humic acid) may dictate the mobility and toxicity of copper in unspiked soils.

References

- Almås, A., Singh, B. R., and Salbu, B. 1999. Mobility of cadmium-109 and zinc-65 in soil influenced by equilibrium time, temperature and organic matter. *J. Environ. Qual.* **28**, 1742–1750.
- Arias, M., Barral, M. T., and Mejuto, J. C. 2002. Enhancement of copper and cadmium adsorption on kaolin by the presence of humic acids. *Chemosphere*, **48**, 1081–1088.
- Bradl, H. B. 2004. Adsorption of heavy metal ions on soils and soils constituents. *J. Colloid. Interf. Sci.* **277**, 1–18.
- Brallier, S., Harrison, R. B., Henry, C. L., and Dongsen, X. 1996. Liming effects on availability of Cd, Cu, Ni, and Zn in a soil amended with sewage sludge 16 years previously. *Water Air Soil Pollut.* **86**, 195–206.
- Brun, L. A., Maillet, J., Richarte, J., Herrmann, P., and Remy, J. C. 1998. Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard soils. *Environ. Pollut.* **102**, 151–161.
- Brun, L. A., Maillet, J., Hinsinger, P., and Pépin, M. 2001. Evaluation of copper availability to plants in copper-contaminated vineyard soils. *Environ. Pollut.* **111**, 293–302.

- Cavallaro, N. and McBride, M. B. 1978. Copper and cadmium adsorption characteristics of selected acid and calcareous soils. *Soil Sci. Soc. Am. J.* **42**, 550–556.
- Certini, G. 2001. Nitrogen in humic and fulvic acid fractions from a volcanic soil under pine: Recovery efficiency of two extractants. *J. Plant Nutr. Soil Sci.* **164**, 267–269.
- Clemente, R., Walker, D. J., Roig, A., and Bernal, M. P. 2003. Heavy metal bioavailability in a soil affected by mineral sulphides contamination following the mine spillage at Aznalcóllar (Spain). *Biodegradation*, **14**, 199–205.
- Clemente, R., Paredes, C., and Bernal, M. P. 2007. A field experiment investigating the effects of olive husk and cow manure on heavy metal availability in a contaminated calcareous soil from Murcia (Spain). *Agric. Ecosys. Environ.* **118**, 319–326.
- Déportes, I., Benoit-Guyed, J., and Zmirou, D. 1995. Hazard to man and the environment posed by the use of urban waste compost: A review. *Sci. Tot. Environ.* **172**, 197–222.
- dos Santos, J. S., dos Santos, M. L. P., Conti, M. M., dos Santos, S. N., and de Oliveira, E. 2009. Evaluation of some metals in Brazilian coffees cultivated during the process of conversion from conventional to organic agriculture. *Food Chem.* **115**, 1405–1410.
- El-Bayaa, A. A., Badawy, N. A., and Alkhalic, E. A. 2009. Effect of ionic strength on the adsorption of copper and chromium ions by vermiculite pure mineral. *J. Hazard. Mater.* **170**, 1204–1209.
- Elzinga, E. J., Rouff, A. A., and Reeder, R. J. 2006. The long-term fate of Cu^{2+} , Zn^{2+} , and Pb^{2+} adsorption complexes at the calcite surface: An X-ray absorption spectroscopy study. *Geochim. Cosmochim. Acta*, **70**, 2715–2725.
- Gandois, L., Probst, A., and Dumat, C. 2010. Modelling trace metal extractability and solubility in French forest soils by using soil properties. *Eur. J. Soil Sci.* **61**, 271–286.
- Gondar, D., Iglesias, A., López, R., Fiol, S., Antelo, J. M., and Arce, F. 2006. Copper binding by peat fulvic and humic acids extracted from two horizons of an ombrotrophic peat bog. *Chemosphere*, **63**, 82–88.
- Hernandez-Soriano, M. C. and Jimenez-Lopez, J. C. 2012. Effects of soil water content and organic matter addition on the speciation and bioavailability of heavy metals. *Sci. Tot. Environ.* **423**, 55–61.
- Hogg, D. S., McLaren, R. G., and Swift, R. S. 1993. Desorption of copper from some New Zealand soils. *Soil Sci. Soc. Am. J.* **57**, 361–366.
- Houba, V. J. G., Temminghoff, E. J. M., Gaikhorst, G. A., and van Vark, W. 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. *Communications in Soil Science and Plant Analysis*. **31**, 1299–1396.
- Hubaux, A. and Vos, G. 1970. Decision and detection limits for calibration curves. *Anal. Chem.* **42**, 849–855.
- Inaba, S. and Takaneke, C. 2005. Effects of dissolved organic matter on toxicity and bioavailability of copper for lettuce sprouts. *Environ. Int.* **31**, 603–608.
- Jalali, M. and Khanlari, Z. V. 2008. Effect of aging process on the fractionation of heavy metals in some calcareous soils of Iran. *Geoderma*, **143**, 26–40.
- Lakanen, E. and Ervio, R. 1971. A comparison of eight extractants for the determination of plant available micronutrients in soils. *Acta Agri. Fenn.* **123**, 223–232.
- Lamb, D. T., Ming, H., Megharaj, M., and Naidu, R. 2009. Heavy metal (Cu, Zn, Cd, and Pb) partitioning and bioaccessibility in uncontaminated and long-term contaminated soils. *J. Hazard. Mater.* **171**, 1150–1158.
- Lock, K. and Janssen, C. R. 2003. Influence of aging on metal availability in soils. *Rev. Environ. Contam. Toxicol.* **178**, 1–21.
- Loland, J. O. and Singh, B. R. 2004. Extractability and plant uptake of copper in contaminated coffee orchard soils as affected by different amendments. *Acta Agr. Scand. B-S P.* **54**, 121–128.
- Lu, A., Zhang, S., and Shan, X. 2005. Time effect on the fractionation of heavy metals in soils. *Geoderma*, **125**, 225–234.
- Ma, Y., Lombi, E., Oliver, I. W., Nolan, A. L., and McLaughlin, M. J. 2006. Long-term aging of copper added to soils. *Environ. Sci. Technol.* **40**, 6310–6317.

- McLaren, R. G. and Ritchie, G. S. P. 1993. The long term fate of copper fertilizer applied to a lateritic sandy soil in Western Australia. *Aust. J. Soil Res.* **31**, 36–50.
- Meers, E., Samson, R., Tack, F. M. G., Ruttens, A., Vandegheuchte, M., Vangronsveld, J., and Verloo, M. G. 2007. Phytoavailability assessment of heavy metals in soils by single extractions and accumulation by *Phaseolus vulgaris*. *Environ. Exp. Bot.* **60**, 385–396.
- Miyazawa, M., Giminez, S. M. N., Yabe, M. J. S., Oliveira, E. L., and Kamogawa, M. Y. 2002. Adsorption and toxicity of copper and zinc in bean plants cultivated in soil treated with chicken manure. *Water Air Soil Pollut.* **138**, 211–222.
- Mlingano Agricultural Research Institute. 2006. *Soils of Tanzania and their Potential for Agriculture Development*, Report, Ministry of Agriculture, Food Security and Co-operatives, Dar Es Salaam, Tanzania.
- Mohamed, I., Ahamadou, B., Li, M., Gong, C., Cai, P., Liang, W., and Huang, Q. 2010. Fractionation of copper and cadmium and their binding with soil organic matter in a contaminated soil amended with organic materials. *J. Soils Sediments*, **10**, 973–982.
- Narwal, R. P. and Singh, B. R. 1998. Effect of organic materials on partitioning, extractability and plant uptake of metals in an alum shale soil. *Water Air Soil Pollut.* **103**, 405–421.
- Paramasivam, S., Richards, K. A., Alva, A. K., Richards, A. M., Sajwan, K. S., Jayaraman, K., Heanacho, A., and Afolabi, J. 2009. Evaluation of poultry litter amendment to agricultural soils: Leaching losses and partitioning of trace elements in collard greens. *Water Air Soil Pollut.* **202**, 229–243.
- Pitch, A. and Scholtz, G. 1996. Translocation of copper and other micronutrients in tomato plants (*Lycopersicon esculentum* Mill): Nicotianamine-simulated copper transport in the xylem. *J. Experimen. Bot.* **47**, 41–47.
- Sauvé, S., McBride, M. B., Norvell, W. A., and Hendershot, W. H., 1997. Copper solubility and speciation of in situ contaminated soils: Effects of copper level, pH and organic matter. *Water Air Soil Pollut.* **100**, 133–149.
- Sauvé, S., Hendershot, W., and Allen, H. E. 2000. Solid-solution partitioning of metals in contaminated soils: Dependence on pH, total metal burden and organic matter. *Environ. Sci. and Technol.* **34**, 1125–1131.
- Sims, J. T. 1986. Soil pH effects on the distribution and plant availability of manganese, copper and zinc. *Soil Sci. Soc. Am. J.* **50**, 367–373.
- Tandy, S., Healey, J. R., Nason, M. A., Williamson, J. C., and Jones, D. L. 2009. Remediation of metal polluted mine soil with compost: Co-composting versus incorporation. *Environ. Pollut.* **157**, 690–697.
- Zhang, J. H., Li, Q. Y., and Yan, D. 2004. Centrifuge modelling of copper ion migration in unsaturated silty clay. *Chinese Journal of Geotechnical Engineering.* **26**, 792–796.
- Zhou, S., Xu, M., Ma, Y., Chen, S., and Wei, D. 2008. Aging mechanism of copper added to bentonite. *Geoderma* **147**, 86–92.