Pigeon Pea Biochar as a Soil Amendment to Repress Copper Mobility in Soil and Its Uptake by Spinach

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A pot crop experiment was conducted to study the effect of biochar on Cu mobility in a soil-plant system. Pigeon pea biochar was prepared by slow pyrolysis at 300 °C. The experiment had three levels of Cu (0, 250, and 500 mg Cu kg^{-1} soil) and three levels of biochar (0, 2.5, and 5 g kg^{-1} soil), using spinach as the test crop. The dry matter yield of edible spinach leaf decreased by 16.7% and 27.9% at 250 and 500 mg Cu kg^{-1} soil concentration, respectively. The soil organic carbon (SOC) increased by 27.08% and 45.83% at 2.5 and 5 g kg^{-1} soil application of biochar, respectively. Cu mobility in soil was significantly reduced as a result of biochar application, as evident from the reduction in DTPA extractable Cu in soil, the transfer coefficient value (soil to plant), and the Cu concentration in the leaf and root. The increases in SOC and pH in the biochar amended soil affect copper dynamics because they control adsorption and precipitation on solid phase. Cu has higher affinity towards SOC and makes stable complexes, thereby decreasing the Cu mobility in soil. Adsorption and precipitation of heavy metals to solid phases and also increasing the negatively charged functional group due to increase in soil pH resulted in reduction of Cu mobility in soil.

Keywords: Pigeon pea; Biochar; Copper; Spinach; Transfer coefficient

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INTRODUCTION

In recent decades, attention has been focused on the heavy metal pollution in soil; metals may persist in soil for a longer period than previously thought, and they are relatively non-biodegradable (Kumar *et al.* 2007). In general, soil contamination from heavy metals is prominent in areas adjacent to industrial and urban centers. The sources of heavy metals in the soil are from natural (geogenic process) and anthropogenic activities. The most common heavy metals that occur in soil are lead, chromium, cobalt, nickel, copper, zinc, arsenic, cadmium, selenium, silver, and mercury. Like other heavy metals, gradual increases in soil Cu levels can create serious ecological problems. The Cu level in the environment is raised mainly because of emissions from major industries, including copper mining, metallurgy, electroplating, explosive, pesticides, fertilizers, petroleum-refinery, paints, and dyes. Elevated levels of Cu in the environment have generated attention of environmentalist because they pose a serious threat to soil micro-organisms, fauna, and human health (Shrivastava 2009).

Heavy metals are often referred to as trace or toxic elements because of their toxic effects on living things (Jarup 2003; Silva *et al.* 2005). The level of toxicity and the bioavailability to the plant species depends on several factors, *i.e.*, chemical form (species) and the amount that is presented to the plant in the environment, as well as certain external factors like soil pH, oxidation-reduction potential, presence of other cations and anions in the system, clay, and organic matter content in soil (Leiros *et al.* 1999; Loska and Wiechula 2000; Wyszkowska and Wyszkowski 2003). Because of the persistent and non-biodegradable nature of metals in soil, remediation of heavy metal polluted soil is a greater task (Gao 1986). Integrating the remediation options and the ecosystem services, such as carbon sequestration through the addition of carbon-rich material, are the most attractive land management options for the contaminated sites.

During the last few decades, research has been focused on inorganic contaminant stabilization in soil, particularly heavy metal containment by the processes of adsorption, binding, or co-precipitation through amendment additions (Kumpiene *et al.* 2008). Of the several amendments used for *in-situ* stabilization of contaminants, organic materials, such as biosolids, manures, and composts, have proved successful in reducing the mobility of contaminants in multi-metal polluted soils (Clemente and Bernal 2006). More recently, carbon-rich biochar (carbonaceous residue of incomplete burning of carbon-rich biomass) has been recognized as an important soil amendment because of its potential benefits for carbon sequestration in soil (Lehmann 2007) and for heavy metal remediation purposes (complexes with metal ions) (Liang *et al.* 2006). However, there is a paucity of data about the impact of biochar on Cu mobility in soil and its uptake in crop plants. Hence, the present investigation was intended to study the effect of biochar on Cu mobility in soil and its uptake by spinach crops that were grown in a Cu-treated soil.

EXPERIMENTAL

Materials

Soil collection and physico-chemical analysis

A greenhouse experiment was conducted to assess the impact of biochar application on spinach yield and Cu mobility in a soil-plant system. The bulk experimental soil for the study was collected from the surface soil (0-15 cm layer) of an agricultural field in the village of Nipaniya Jatkhedi, Bhopal. The soil was processed, sieved through a 2 mm sieve, and analyzed for its physico-chemical properties, following standard methods (Chaudhury *et al.* 2005). The experimental soil was sandy loam with a pH of 7.9 and an electrical conductivity (EC) of 0.12 dS/m. The soil was low in soil organic carbon (SOC), available nitrogen (N), available phosphorus (P), available potassium (K), and available sulphur (S) content, indicative of a poor nutrient status. The diethylene triamine penta-acetic acid (DTPA) extractable heavy metals content was 9.30, 0.03, 0.34, 0.09, and 0.60 mg/kg for Cu, Cd, Pb, Cr and Ni, respectively (Table 1).

Biochar preparation and chemical characterization

Biochar was prepared at the Central Institute of Agricultural Engineering (CIAE), Bhopal, Madhya Pradesh, India, using pigeon pea stalk as a feedstock material. After sun drying, the pigeon pea stem was cut into pieces (10-20 cm), and pyrolyzed at 300 °C for 2 h, followed by quenching and subsequent drying in an oven at 105 °C. The dried biochar was crushed in a 24 blade variable-speed rotor mill (Pulversittee 14, Fritsch Laboratory Instruments, Germany) and sieved to obtain a uniform 53 to 75 µm particle size. The representative samples of ground biochar were analyzed for plant nutrient composition and heavy metals content (Table 2).

Table 1. Physico- Chemical Properties of Experimental Soil

pH (soil : water, 1:2)	7.90
EC (soil: water, 1:2) (dS/m)	0.12
Sand (%)	73.16
Silt (%)	09.30
Clay (%)	17.54
Textural class	Sandy loam
SOC (%)	0.49
AN (kg/ha)	167.00
AP (kg/ha)	9.34
AK (kg/ha)	138.9
AS (kg/ha)	7.1
Total heavy metal content	
Cu (ppm)	61.30 (*9.30)
Cd (ppm)	00.20 (0.03)
Pb (ppm)	20.87 (0.34)
Cr (ppm)	24.10 (0.09)
Ni (ppm)	60.83 (0.60)

EC: Electrical Conductivity; SOC: Soil Organic Carbon; AN: Available Nitrogen; AP: Available Phosphorus; AK: Available Potassium; AS: Available Sulphur; *DTPA extractable heavy metals content

Table 2. Chemical Composition of Pigeon Pea Biochar

pH	8.45
EC (dS/m)	0.50
TOC (%)	56.25
TN (%)	1.07
TP (%)	00.07
TK (%)	00.62
TS (%)	00.12
Total heavy metal content	
Cu (ppm)	18.78
Cd (ppm)	00.31
Pb (ppm)	00.80
Cr (ppm)	12.45
Ni (ppm)	01.20
Zn (ppm)	22.52

EC: Electrical Conductivity; TOC: Total Organic Carbon; TN: Total Nitrogen; TP: Total Phosphorus; TK: Total Potassium; TS: Total Sulphur

Methods

Experimental design and procedure

A greenhouse experiment was conducted with sandy loam soil and spinach as the test crop. The processed soil (5 kg) was poured into wide mouth glazed pots with a 7 kg capacity.

The experiment measured three levels of applied Cu content (0, 250, and 500 mg Cd/kg soil) and three levels of biochar applied (0, 2.5, and 5.0 g/kg soil) in a completely randomized design. The required amount of Cu was added in solution form ($CuSO_4.5H_2O$) to the powdered biochar, and then they were mixed thoroughly according to the experimental treatments. The potted soil mixed with biochar and heavy metal (Cu) was allowed to equilibrate for two week under field capacity conditions prior to the sowing of the spinach seeds.

The recommended uniform dose of N (0.03 g N/kg soil), P (0.018 g P_2O_5/kg soil), and K (0.018 g K_2O/kg soil) were supplied in the form of urea, di-ammonium phosphate, and potassium chloride, respectively, in order to ensure adequate supply of the nutrients. The entire dose of P and K, and a one half dose of N was applied basally before sowing, while the remainder of N was top-dressed 20 days after sowing. The seeds of spinach (variety *Selection-1*) were treated with Bavistin® DF (BASF, New Zealand) at 2 g/kg seed in order to avoid fungal infection. Ten healthy seeds of spinach were sown by making holes at 2 to 3 cm depth equidistantly in the soil, which were subsequently covered. After germination, the entire seedling was allowed to grow for 10 days. Then, the plants were finally thinned out leaving five healthy spinach plants per pot.

Harvesting, processing, and analysis of plant and soil samples

Sixty days after sowing, the above-ground portion (leaf) and below-ground portion (root) of the spinach plants were harvested separately, washed with distilled water, and air-dried. Roots were washed thoroughly with tap water to remove adhering soil particles, followed by washing with dilute acid (HCl) and distilled water in sequence. The air dried leaf and root samples were then oven dried at 65 °C until a constant weight was obtained.

Oven dried leaves and roots were ground in a Wiley mill and passed through a 2 mm sieve. Homogenized tissue samples were digested in a di-acid mixture containing HNO_3 and $HClO_4$ (9:4 v/v) on a hot plate at 150 to 175 ºC for approximately 2 h until a clear liquid was obtained.

Soil samples were collected separately from each pot after the crops were harvested. The post-harvest soil samples were analyzed for pH and EC, which were measured in a soil to water solution of 1:2, whereas the SOC was estimated by following the wet oxidation method (Walkley and Black 1934). Soil samples were digested in a di-acid mixture (HNO_3/$HClO_4$ at 9:4) for total Cu content, and the plant available Cu in the soil was extracted using a DTPA extractant (Lindsay and Norvell 1978). The concentrations of Cu in the digested samples (plant and soil) and DTPA extractant were determined using an inductively coupled plasma-optical emission spectrophotometer (Optima DV 2100, PerkinElmer, USA).

Statistical analysis

The analysis of variance technique was carried out for each pairing in the experiment as applicable to factorial completely randomized design (Gomez and Gomez 1984). To determine the significant differences between the means, the least significant differences (LSD) were estimated at a 5% probability level and Duncan's multiple range test was used for comparing the means.

RESULTS AND DISCUSSION

Dry Matter Yield of Spinach Crop as Influenced by Pigeon Pea Biochar and Copper Levels

Soil Cu levels significantly influenced the dry matter (DM) yield of the spinach leaf and root (Fig. 1). The main observable effect of Cu on spinach was a decrease in the DM yield when the soil Cu level was increased. This was likely a result of the deleterious effect of Cu on several of the plant's physiological processes (Stiborova 1988). The percent decrease in DM yield from the control level in spinach leaves was 16.7% and 27.9% at the 250 and 500 mg of Cu/kg soil levels, respectively. Similarly, the DM yield of the spinach root was reduced from the control level by 20.6% and 34.9% at the 250 and 500 mg of Cu/kg soil levels, respectively. However, the application of biochar increased the DM yield of the spinach crop at their corresponding levels of Cu. The results were highly consistent with the finding of Xu *et al.* (2005); the grain yield and total biomass of the rice crop significantly decreased with an increase in the soil Cu level above 200 mg Cu/kg soil.

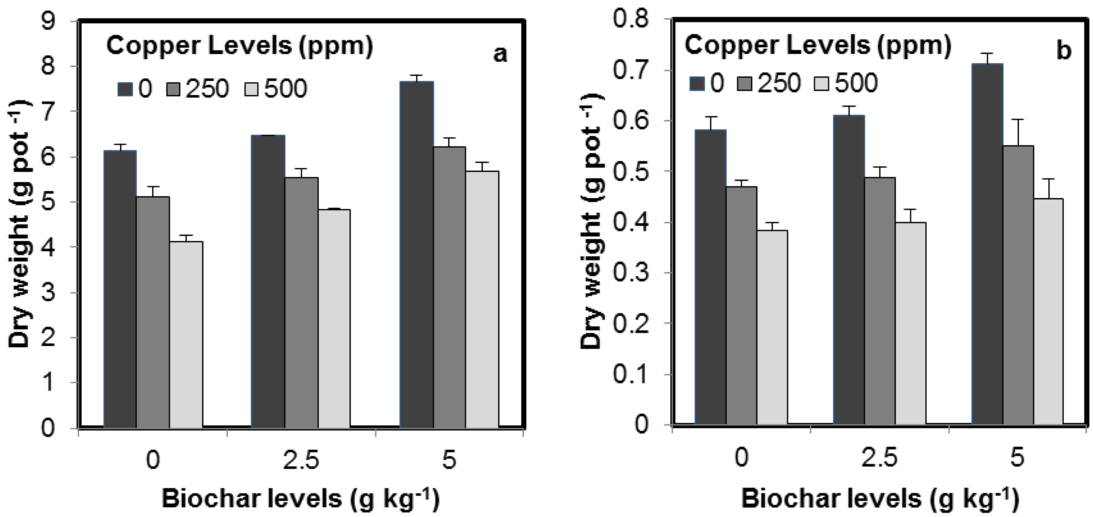

Fig. 1. The effect of pigeon pea biochar and copper on the dry matter yield of a) spinach leaves and b) spinach root. Each bar represents the mean ± SEM (n=3)

One of the most interesting and potentially important properties of biochar is its effect on the crop yield. Several studies also found that the crop yield can be improved using biochar (Blackwell *et al.* 2009). Results worldwide have indicated improvements in crop yield from 30 to 100% in soybeans, maize, carrots, and beans that was attributed to the use of biochar (Yamato *et al.* 2006; Rondon *et al.* 2007; Kimetu *et al.* 2008). Results from our potted crop experiment showed that the spinach leaf and root DM yields were significantly influenced by the application of biochar. The application of biochar at 2.5 and 5 g/kg soil increased the DM yield of spinach leaf by 9.8% and 27.4%, respectively, and the root DM yield by 4.2% and 18.8%, respectively. The maximum DM yield of the spinach leaf and root (7.65 and 0.71 g/pot, respectively) was recorded at the 5 g/kg soil biochar application, in the absence of Cu (0 mg Cu/kg soil), which was found to be statistically significant (Fig. 1). This increase in the spinach DM yield of the leaf and root may be because of an improvement in the nutrient availability, favorable chemical and biological environment, and/or better physical properties in the biochar amended soil (Blackwell *et al.* 2009).

Biochar's Effect on Cu Content and Uptake in the Leaf and Root

Heavy metal content and its uptake by vegetable crops raised on contaminated soils greatly differed by individual plant species. In addition, the concentration in edible plant tissues increased with increasing levels of heavy metal contamination (Intawongse and Dean 2006). The results from our experiment also indicated that increasing levels of Cu addition to soil significantly increased the concentration of Cu in the plant parts (leaf and root) and its uptake by the spinach crop. In general, Cu content was greater in the roots than the leaf, at their corresponding levels of soil Cu and biochar application. The mean leaf and root Cu content ranged from 10.13 to 211.7 mg/kg and 31.6 to 276.9 mg/kg, respectively (Table 3). Similarly, the mean uptake of Cu by the spinach leaf and root ranged from 84.9 to 921.8 µg/pot and 20.9 to 106.7 µg/pot, respectively. The highest content and uptake of Cu in the leaf and root was observed at 500 mg Cu/kg soil level in the absence of the biochar application. The results were in agreement with the findings from Yang *et al.* (2002); the concentration of the Cu in plant parts increased with an increase in the soil Cu level or nutrient solution concentration. Yang *et al.* (2002) reported that the shoot and the root concentrations of vegetable crops increased with an increasing soil Cu level; however, the Cu concentration was greater in the root than the shoot.

Table 3. Effect of Pigeon Pea Biochar on Copper Content and Uptake by Spinach in a Copper Contaminated Soil

Biochar (g/kg)	Copper (mg/kg)	Copper Concentration (mg/kg)		Copper Uptake (µg/pot)	
		Leaf	Root	Leaf	Root
0	0	14.7 ± 0.07	44.6 ± 0.05	101.5 ± 1.2	25.6 ± 0.04
2.5	0	12.03 ± 0.05	34.1 ± 0.08	88.7 ± 1.2	20.9 ± 0.06
5	0	10.13 ± 0.06	31.6 ± 0.03	84.9 ± 1.4	22.2 ± 0.03
0	250	109.4 ± 1.5	181.8 ± 1.4	609.7 ± 11.7	85.3 ± 1.4
2.5	250	89.7 ± 1.1	154.6 ± 1.2	528.1 ± 12.3	75.2 ± 1.5
5	250	77.1 ± 1.5	124.3 ± 1.5	499.9 ± 11.8	68.6 ± 1.2
0	500	211.7 ± 1.5	276.9 ± 1.6	921.8 ± 15.5	106.7 ± 1.6
2.5	500	174.4 ± 1.3	235.4 ± 1.8	836.5 ± 14.2	93.6 ± 1.1
5	500	151.5 ± 1.1	202.4 ± 1.4	842.3 ± 13.5	89.8 ± 1.2
LSD ($P < 0.05$)	Biochar	8.12	15.8	61.7	9.8
	Copper	7.95	13.5	55.2	7.4
	Biochar x Copper	14.56	25.6	127.6	16.9

In contrast, the application of biochar in heavy metal (Cu) treated soil significantly ($P<0.05$) reduced the Cu content and its uptake by the spinach crop. The Cu content in the spinach leaf were considerably reduced by 17.9% and 28.9%, while its uptake was also reduced by 11.0% and 12.6% at 2.5 and 5 g/kg soil of biochar from the control, respectively (Table 3). It was clear from the result that the heavy metal mobility in soil as a measure of DTPA extractable Cu was reduced and its subsequent uptake by the spinach leaf and root were significantly ($P<0.05$) decreased. This may have been because of a definite increase in the cation exchange capacity (CEC), pH, and/or other soil properties which may have improved the overall sorption capacity of the soils as a result of the biochar application. Namgay *et al.* (2010) also showed that the biochar application decreased the concentration of heavy metal in maize shoots, which was attributed to the formation of stable metal-organic complexes.

Changes in Soil pH, EC, and Organic Carbon as Influenced by Biochar

The results from the potted crop experiments with spinach have shown that some of the important properties of soil were clearly influenced (altered), resulting from the application of biochar. The soil pH, EC, and organic carbon content were significantly ($P<0.05$) increased in the post-harvest soil samples because of the biochar application, as compared to control samples. The soil pH increased from 7.85 to 8.21 and was statistically significant ($P<0.05$) at both levels (2.5 g/kg and 5 g/kg) of biochar application. An increase in pH by 0.2 and 0.36 was observed at the 2.5 g/kg and 5 g/kg soil application of biochar levels, respectively (Table 4). On the other hand, soil Cu levels significantly ($P<0.05$) reduced the soil pH value from 8.13 to 7.96. The soil EC level of the post-harvest samples increased with an increase in the levels of soil Cu and biochar application.

Table 4. Effect of Pigeon Pea Biochar on pH, EC, and Soil Organic Carbon Content of Copper Contaminated Soil

Biochar (g/kg)	Copper (mg/kg)	Soil Properties		
		pH	EC (ds/m)	OC (%)
0	0	8.01 ± 0.03	0.14 ± 0.03	0.46 ± 0.02
2.5	0	8.11 ± 0.02	0.23 ± 0.03	0.60 ± 0.04
5	0	8.26 ± 0.04	0.25 ± 0.02	0.73 ± 0.02
0	250	7.80 ± 0.02	0.23 ± 0.07	0.48 ± 0.06
2.5	250	8.04 ± 0.04	0.24 ± 0.03	0.62 ± 0.04
5	250	8.20 ± 0.03	0.26 ± 0.03	0.67 ± 0.03
0	500	7.74 ± 0.05	0.31 ± 0.02	0.48 ± 0.05
2.5	500	7.99 ± 0.03	0.35 ± 0.02	0.60 ± 0.05
5	500	8.16 ± 0.05	0.37 ± 0.03	0.70 ± 0.03
LSD (P < 0.05)	Biochar	0.10	0.03	0.04
	Copper	0.07	0.02	0.05
	Biochar x Copper	0.15	0.04	0.07

Results further showed that the soil amended with biochar at different levels significantly ($P<0.05$) increased the SOC. The percent increase in organic carbon content at 2.5 and 5 g/kg soil application of biochar was 27.1% and 45.8%, respectively, from the control level (Table 4). The highest value for organic carbon content (0.73%) was observed in the treatment where biochar was applied at 5 g/kg soil in the absence of Cu, and the lowest value (0.46%) was observed in the control. The Cu in the soil might have adsorbed and precipitated as Cu solid due to an increase in soil pH and EC (Sun *et al.* 2006). Copper transport (mobility) has been significantly retarded by the presence of organic amendments (biochar) as Cu has stronger affinity towards organic carbon and makes stable complexes (Sposito 1989) and thus reduces its bioavailability.

DTPA-Extractable Copper and Transfer Coefficient for Copper from the Soil-Plant System

The DTPA-extractable Cu in the post-harvest soil sample ranged from 5.35 to 222.75 mg/kg soil. Increasing the level of Cu in soil significantly (P<0.05) increased the amount of DTPA extractable Cu. The highest value (222.75 mg/kg) was observed in the treatment where Cu was applied to the soil at the highest level (500 mg/kg), in the absence of biochar. However, a significant (P<0.05) reduction in plant bioavailable fraction (DTPA extractable Cu) was observed with the increasing levels of the biochar application (Fig. 2). The application of biochar at 5 and 10 g/kg decreased the extractability of Cu by 24.3% and 39.0%, respectively. It was clearly evident from the results that the biochar application significantly (P<0.05) reduced the Cu availability in the soil. Namgay *et al.* (2010) also showed that the addition of biochar to the soil would increase the cation exchange capacity (CEC), pH, and other properties, and decrease the heavy metal mobility. Hence, the addition of biochar improved the overall sorption capacity of soils, and therefore it may be capable of reducing the heavy metal mobility in the soil (DTPA extractable Cu).

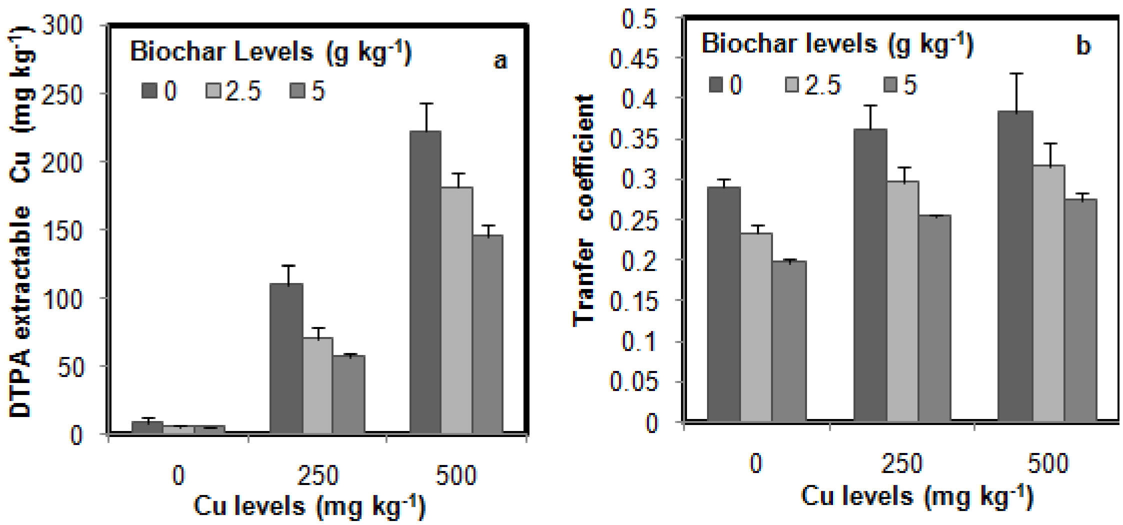

Fig. 2. The effect of pigeon pea biochar and copper on the DTPA extractable Cu (a) and transfer coefficient value (b) from the soil-plant system. Each bar represents the mean ± SEM (n=3)

The transfer coefficient value for Cu was calculated as the ratio of the concentration of metal in the plant versus the total concentration of metal in the soil. The highest value for the transfer coefficient indicates the greatest mobility of the trace metal from the soil to the plant. Increasing levels of soil Cu resulted in an increase in the transfer coefficient values, whereas the application of biochar, as a soil amendment in the Cu contaminated soil, reduced its value significant (P<0.05). The transfer coefficient for Cu was 0.38 in the absence of biochar, which was reduced to 0.24 at 5 g biochar/kg soil application (Fig. 2). This indicated that the biochar application reduced the Cu uptake by the above-ground biomass of spinach. Such a reduced uptake of Cu by the plant may also be caused by a lower extractability (by DTPA) in the soil because of the biochar application. Various studies have shown that the mobility of metals in soils and their uptake by plants can be reduced by addition of biochar (Namgay *et al.* 2010; Fellet *et al.* 2011; Park *et al.* 2011). The application of biochar significantly reduces the soil solution concentration of heavy metals (DTPA extractable Cu) by making stable complexes with heavy metals such as organic matter-trace metal complex. Biochar, a highly condensed aromatic structure with a large specific surface area (Downie *et al.* 2009) and high cation exchange capacity (CEC) (Liang *et al.* 2006; Glaser and Birk 2012) addition to soils provides negatively charged surfaces for ion exchange which are responsible for the ability to sorb heavy metals in soil (Lehmann *et al.* 2005; Liang *et al.* 2006). Another mechanism for the immobilization of heavy metals might be due to an increase in soil pH leading to an increase in the sorption capacity of functional groups of variable charges or precipitation of mineral phases (Cao *et al.* 2009). All these mechanisms in total might have resulted in reduction of Cu mobility, Cu concentration in the leaf and root as well as transfer co-efficient values. Therefore, it was apparent from the results that the biochar application reduced the Cu mobility in soil and subsequently its transfer in the soil-plant system.

CONCLUSIONS

1. Application of biochar in the heavy metal contaminated site provides better option for managing contaminated soil for sustaining crop production and soil health.
2. The study has shown that pigeon pea biochar addition (5 g/kg soil) increased the dry matter yield of spinach by 27% and decreased the DTPA extractable copper and transfer coefficient values.
3. Soil pH and organic carbon increased in the biochar amended soil was substantial enough to reduce Cu mobility in soil through organic matter-trace metal complex and or adsorption process.
4. To conclude, the study demonstrated that pigeon pea biochar has inherent potential to increase the spinach yield and reduce the copper bioavailability to a toxic level for spinach crop growth in a contaminated sandy soil.

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