INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-4, Issue-3, July-Sept-2014

ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 4th April-2014 *Revised: 24th May-2014* *Accepted: 26th May-2014*

Research article

BIOCHAR EFFECTS ON COPPER AVAILABILITY AND UPTAKE BY SUNFLOWER IN A COPPER CONTAMINATED CALCAREOUS SOIL

Mina Sharifat Salmani[a*], Farhad Khorsandi[b], Jafar Yasrebi[c] and Najafali Karimian[c]

[a]Department of Soil Science, Science and Research Branch, Islamic Azad University, Fars, Iran
[b]Department of Soil Science, Darab Branch, Islamic Azad University, Darab, Iran
[c]Department of Soil Science, Shiraz University, Shiraz, Iran
[*]Corresponding author: mina.sharifat@yahoo.com

ABSTRACT: Biochar, a carbon rich soil amendment, could be beneficial in decreasing copper toxicity in plants. An open air, pot experiment was carried out to study the effects of biochar on soil available copper and sunflower yield and Cu uptake under copper toxicity stress. The experimental design was complete randomized block with three levels of copper fertilizer (0, 50 and 200 mg/kg), four levels of citrus wood biochar (0%, 1%, 2% and 4% by weight) and three replications. The results showed that application of biochar at medium and high Cu toxicity levels can significantly reduce available Cu level in calcareous soil, thereby, significantly reducing Cu concentration in sunflower aerial parts to normal levels. Cu uptake by sunflower also decreased significantly by biochar application to soil. Considering the results of this experiment, it is recommended to use at least 1 % and at most 2% citrus wood biochar under medium to high Cu toxicity conditions.
Keywords: Biochar, Copper toxicity, Copper uptake, *Helianthus annuus*, Calcareous soil

INTRODUCTION

Many agricultural and non-agricultural lands are contaminated with heavy metals such as nickel, copper and zinc. The main reason for this phenomenon is waste and sewage disposal in these lands by industrial and urban communities as well as abundant use of chemicals in agriculture, especially pesticides and herbicides. Highly contaminated soils containing copper and other heavy metals are toxic to soil microorganisms, as well as plants and animals feeding on them. Reduced yields and plant growth, structural changes in soil microorganisms and nematodes have been observed in heavy metal contaminated soils. Heavy metal contamination of agricultural lands is a critical environmental issue in a large area of the country where urban or industrial wastewater is used to irrigate crops, horticultural products and vegetables. Urban and industrial wastewater is a major source of heavy metals and may contaminate agricultural soils, and consequently food chain of the community. Moreover, over-use of copper rich fungicides and pesticides is another important source of agricultural lands contamination.

Copper is an essential micronutrient necessary for appropriate growth of crop plants. The copper deficiency level for plants has been reported as 1-5 mg/kg dry mass and the copper toxicity level in plants has said to be 20-30 mg/kg dry mass [1]. The amount of copper in soils varies from 5 to 60 mg/kg although less than 2 mg/kg and more than 60 mg/kg have been seen frequently in soils [2]. The critical level of copper in calcareous soils has been reported to be 1 mg/kg [1]. Although Cu is a micronutrient essential at low concentrations for normal and healthy plant growth, its presence at high concentrations induces severe phytotoxicity and retards plant growth [3]. Biochar is a solid, carbon rich material made by chemical-thermal conversion of biomass in an environment with shortage or absence of oxygen [4]. Research has shown that adding biochar to the soil increases the soil fertility and agricultural productivity significantly [5-8]. It has been reported that biochar can reduce the bioavailability of heavy metals, such as copper, in soils [9-11]. Calcareous soils of Iran contains high amounts of lime and their pH levels are high; thus, it is very probable to suffer from copper deficiency. This can be a limiting factor for optimal growth of crops. The presence of biochar in calcareous soil may increase the availability of micronutrients, specifically the copper.

On the other hand, as biochar have special physical and chemical properties, especially in soils containing high amounts of copper, it may reduce the toxic effects of copper on the plant. Buss et al. [11] carried out an experiment to study the effects of biochar on reducing copper toxicity for *Chenopodium quinoa* in a sandy soil. Their results showed that the plant will uptake less amounts of copper in the presence of biochar.
There is very limited information on the effects of biochar on copper availability and uptake by plants in copper contaminated soils. Therefore, the main objective of this study is to investigate the effects of biochar on copper availability and copper uptake by sunflower in a calcareous soil.

MATERIALS AND METHODS

This research was carried out during summer of 2013 in Shiraz, Iran. The experimental design of this open-air, pot experiment was randomized complete block design with three replications. Treatments consisted of three copper fertilizer levels from hydrated copper sulfate source (0, 50 and 200 mg/kg soil) and four levels of biochar (0%, 1%, 2% and 4% by weight). The plant under study was Sunflower (*Helianthus annuus).* The 50 and 200 mg Cu/kg soil are considered as medium and highly toxic levels of Cu to sunflower plants. The soil used in this research was silt loam and in regards to salinity and copper content had no restriction for growing sunflower. Some physical and chemical properties of the soil are shown in Table 1. The biochar used was citrus wood charcoal which was ground into powder. Some physical and chemical properties of biochar are shown in Table 2. In order to measure biochar pH and EC, 2 grams biochar powder and 40 ml distilled water were shaken for an hour in a shaker. The solution was then filtered by using filter paper, and pH and EC were measured.
The specific amounts of copper and biochar were added to 4 kg soil in each pot. Eight sunflower seeds were planted at the depth of 2 cm from the soil surface. The pots were watered with tap water. The first watering was done after the end of planting the seeds and the pots were given 800 ml water, and after 3 weeks of plant growth, the pots were irrigated with 1000 ml of tap water. All pots were watered equally.
A week after sprouting, the plants were thinned to 2 plants per pot. Harvesting of sunflower plants was done 50 days after planting. The aerial parts of the plants were detached from the soil surface, and fresh weights were recorded. The plant samples were then washed with distilled water and dried and then put into paper bags. They were dried for 72 hours in 68^{0}C and their dry weight was measured. Oven dried plant samples were powdered. Then, 1 g of powdered oven dried sample was completely burnt in the oven at 550 ºC to determine concentration of copper in plant tissue by dry ashing method [12]. About 1 kg of soil from each pot was also taken to measure soil available copper in the laboratory. DTPA extractable copper content of soil was also measured [12]. Copper uptake by sunflower was determined by multiplying the plant’s dry weigh in the pot by copper concentration of the plant.
The data were statistically analyzed by SAS statistical software. The comparison between treatments were performed by Duncan's multiple range test (DMRT) at 5% probability level.

RESULTS AND DISCUSSION

Soil Available Copper

The results showed that as added copper to soil increased, the mean available copper level in soil increased significantly as compared to the control (Table 3). As biochar level increased, without considering the copper level, the amount of mean copper available in soil was reduced significantly, and the lowest reduction was observed at 2% biochar level (Table 3). There was no significant differences between biochar levels in control (0 mg Cu/kg soil), but as biochar level increased, an increasing trend was also observed in soil available copper (Table 3). In 50 mg Cu/kg soil treatment, the available copper in soil significantly decreased in biochar treatments as compared to control treatment, but there was no significant difference between 1% and 4% biochar treatments. The available Cu at 2% biochar level, was significantly higher than 1 and 4% levels (Table 3). At 200 mg Cu/kg soil treatment, the available copper also showed a significant reduction in all biochar treatments as compared to control (Table 3). The available Cu at 2% biochar level was significantly higher than 1 and 4% biochar levels.

Copper Concentration In Sunflower

The mean copper concentration in sunflower aerial parts increased significantly with increasing levels of copper fertilizer addition to soil (Table 3). However, as biochar level in soil increased, mean copper concentration in the shoot significantly decreased in all biochar treatments as compared to the control. The highest reduction in mean copper concentration of plant was observed at 4% biochar treatment (Table 3).

Copper concentration of the plant decreased significantly in control (0 mg Cu/kg soil) by increasing levels of biochar (Table 3). In 50 mg Cu/kg soil treatment, no significant difference was observed between 1% biochar treatment and control, but as biochar increased to the levels of 2% and 4%, copper concentration of the plant significantly decreased (Table 3). When biochar level increased in 200 mg Cu/kg soil treatment, plant Cu concentration significantly decreased as compared to control in all biochar levels. In both 50 and 200 mg Cu/kg soil treatments, the most significant reduction was observed in 4% biochar treatment (Table 3). Similar findings were reported by Uchimiya et al. [9], Park et al. [10] and Buss et al. [11].
The copper toxicity level in crops is between 20-30 mg Cu/kg dry mass [1]. In this study, at 200 mg Cu/kg soil treatment, without using biochar, plant Cu concentration was 21.16 mg Cu/kg dry mass (Table 3), which is in Cu toxicity range. All treatments of biochar significantly reduced this value to normal Cu level in plants (Table 3).

Table 1. Some physical and chemical properties of the soil used in the experiment

Soil properties	Unit	Amount
Sand	%	40.3
Silt	%	50.3
Clay	%	9.4
Soil texture	--	Silt loam
pH	--	7.65
EC_e	dS/m	0.63
Lime content	%	32
Organic carbon	%	1.44
DTPA available copper	mg/kg	0.68

Table 2. Some properties of the citrus wood biochar used in the experiment

Biochar properties	Unit	Amount
Ash	%	15.3
Moisture	%	4.25
CEC	cmol/kg	12.84
Copper (Cu)	mg/kg	0.27
pH (1:20)	--	11.95
EC (1:20)	dS/m	5.47
Organic carbon	%	77.84

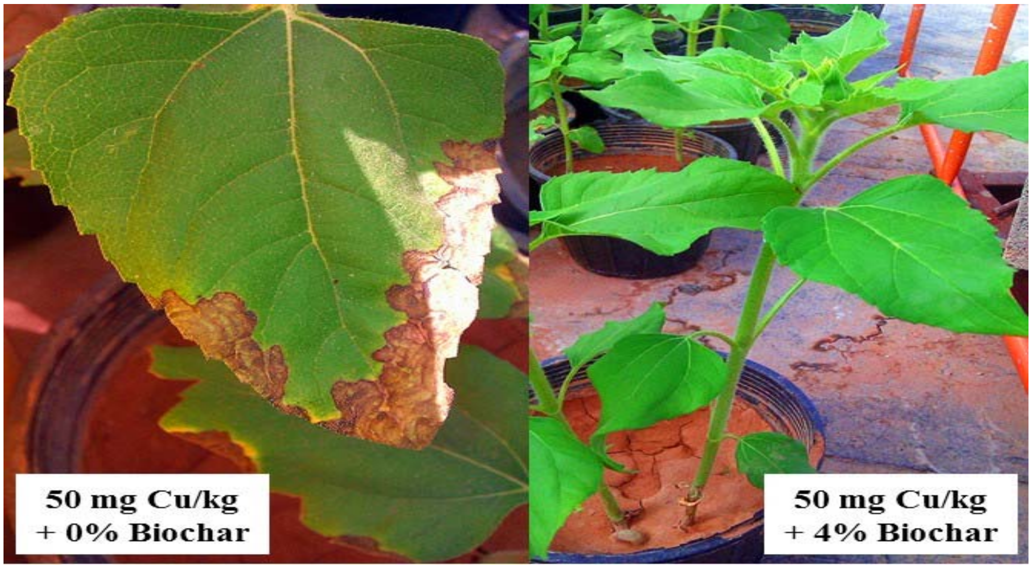

Figure 1. Effect of biochar application on copper toxicity in sunflower plant at 50 mg Cu/kg soil treatment (medium Cu toxicity)

Table 3. Effects of biochar and copper fertilization treatments on selected properties measured in the experiment

Biochar level (%)	Cu treatments (mg/kg soil)			Mean
	0	50	200	
Soil available Cu (mg/kg soil)*				
0	0.60 h	33.60 e	95.87 a	43.36 A
1	0.62 h	20.60 g	86.67 c	35.96 C
2	0.67 h	29.0 f	92.33 b	40.47 B
4	1.04 h	20.47 g	80.47 d	33.99 D
Mean	0.73 C	25.92 B	88.84 A	
Cu concentration in sunflower plant (mg/kg plant dry weight)*				
0	10.20 f	12.67 d	21.16 a	14.68 A
1	8.10 g	12.50 d	15.83 b	12.14 B
2	3.67 i	11.33 e	14.66 c	9.89 C
4	6.80 h	8.73 g	11.0 fe	8.84 D
Mean	7.19 C	11.31 B	15.67 A	
Sunflower dry weight (g/pot)*				
0	17.14 a	12.83 bc	8.91 dc	13.0 A
1	13.25 b	14.05 ab	9.28 dc	12.19 A
2	17.23 a	10.93 bc	12.22 bc	13.5 A
4	10.81 bc	6.79 de	4.91 e	7.5 B
Mean	14.6 A	11.1 B	8.8 C	
Cu uptake by sunflower (mg/pot)*				
0	0.17 a	0.16 ab	0.19 a	0.18 A
1	0.11 bdc	0.18 a	0.15 ab	0.14 AB
2	0.06 dc	0.12 abc	0.18 a	0.12 B
4	0.07 dc	0.06 dc	0.06 d	0.063 B
Mean	0.10 B	0.13 AB	0.14 A	

*For each property, numbers with at least a letter in common are not statistically different than each other according to Duncan's Multiple Range test at 5% probability level.

Figure 2. Effect of biochar application on copper toxicity in sunflower plant at 200 mg Cu/kg soil treatment (high Cu toxicity)

Sunflower Dry Weight

Biochar effects on fresh and dry weights of sunflower under copper toxicity stress of this experiment has been discussed in detail by Sharifat et al [13]. When copper levels in soil increased, the mean dry weight of sunflower plants decreased significantly (Table 3). The reason was copper toxicity due to the increasing use of copper fertilizer and its negative effects on sunflower growth. The increase of biochar levels in soil resulted in a significant decrease of mean dry weight only in 4% treatment as compared to the control. The other biochar levels did not have any significant differences with control (Table 3).

At 0 mg Cu/kg soil treatment, the plant's dry weight in 1% and 4% biochar treatments decreased significantly in comparison to the control but they had no significant difference with each other (Table 3). In the 2% biochar treatment the plant's dry weight showed no significant difference with the control. In 50 and 200 mg Cu/kg treatment levels, the plant's dry weight in 1% and 2% biochar treatments had no significant difference with the control but in 4% biochar treatment the plant's dry weight decreased significantly in comparison to the control. The highest sunflower dry weight was observed in 200 mg Cu/kg soil treatment in 2% biochar level, but it had no significant difference with the control (Table 3).

Copper Uptake by Sunflower

The mean copper uptake by sunflower plant increased significantly with increasing levels of copper fertilizer addition to soil (Table 3). As biochar content of soil increased, the mean Cu uptake by plant decreased significantly in 2 and 4% levels as compared to the control (Table 3). Biochar was reported to decrease copper uptake by *Chenopodium quinoa* plant in a sandy soil [11], which is consistent with the results of this experiment.

Copper uptake by plant decreased significantly in control (0 mg Cu/kg soil) by increasing levels of biochar, but there was no significant differences in Cu uptake between biochar levels (Table 3). In 50 mg Cu/kg soil treatment, Cu uptake was significantly reduced only at 4% biochar level as compared to the control. Cu uptake at 1 and 2% biochar levels were not significantly different than control (Table 3). Similar results were also observed in 200 mg Cu/kg soil treatment.

It seems that biochar by adsorption of available Cu cations, reduced Cu availability to the plant, and consequently, its uptake by sunflower. Therefore, Cu toxicity effects on sunflower plants were reduced. Figures 1 and 2 clearly illustrate toxicity symptoms in sunflower, and the reduction of Cu toxicity in the plant through biochar application. Toxicity symptoms can be easily observed in 50 mg Cu/kg soil treatment without the application of biochar treatment, while by application of 4% biochar, Cu toxicity symptoms were eliminated (Figure 1). Intense Cu toxicity symptoms can be clearly observed in sunflower in of 200 mg Cu/ kg soil treatment application of biochar, while Cu toxicity effects significantly decreases as a result of 4% biochar (Figure 2).

CONCLUSION

The application of biochar at medium and high Cu toxicity conditions can significantly reduce the available Cu level in calcareous soil. As a result, Cu concentration in sunflower's aerial parts significantly decreased to the nontoxic Cu concentration level in plant. Cu uptake by sunflower also decreased through increasing biochar application to the soil.

In general, application of biochar under Cu deficiency condition in soil did not affect the availability of Cu in soil, but reduced Cu concentration in plant, Cu uptake by plant and therefore, dry matter production. Thus, biochar application is not recommended under such conditions. The use of 1% and 2% biochar reduced Cu availability and uptake, and thereby prevented significant reduction of plant dry weight at medium and high Cu toxicity levels. Considering the results of this experiment, it is recommended to use at least 1 % and at most 2% citrus wood biochar under medium to high Cu toxicity conditions.

REFERENCES

[1] Marschner, H. 1985. Mineral Nutrition of Higher Plants. 2nd edition, Academic Press, London, UK.

[2] Stevenson, F.J. 1986. Cycles of Soil. John Wiley and Sons, New York, USA.

[3] Alushllari, M., Cicicil, N., Deda, A. 2014. The bioaccumulation factor of essential metals in maize. Sci. Agri., 1(2), pp. 76-79.

[4] International Biochar Initiative (IBI). 2012. Standardized Product Definition and Product Testing Guidelines for Biochar That Is Used in Soil. Document Reference Code: IBI-STD-01, IBI.

[5] Lehmann, J., da Silva, J.P., Steiner, C., Nehls, T., Zech, W., Glaser, B. 2003. Nutrient availability and leaching in an archaeological anthrosol and a ferrosol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant Soil, 249, pp. 343-57.

[6] Rondon, M., Lehmann, J., Ramirez, J., Hurtado, M. 2007. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. Biol. Fert. Soil, 43, pp. 699-708.

[7] Chan, C.H., Lehmann, J., Engelhard, M. 2008. Natural oxidation of black carbon in soils: changes in molecular form and surface charge along a climosequence. Geochimica et Cosmochimica Acta, 72, pp. 1598-1610.

[8] Lehmann, J., Joseph, S. 2009. Biochar for Environmental Management: Science and Technology. Earthscan, Sterling, VA.

[9] Uchimiya, M., Lima, I.M., Klasson, K.T., Wartelle, L.H. 2010. Contaminant immobilization and nutrient release by biochar soil amendment: roles of natural organic matter. Chemosphere, 80, pp. 935–940.
[10] Park, J.H., Bolan, N.S., Chuasavathi, T. 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil, 348, pp. 439-451.
[11] Buss, W., Kammann, C., Koyro, H.W. 2012. Biochar reduces copper toxicity in *Chenopodium quinoa* Wild in a sandy soil. J. Environ. Qual., 41, pp. 1157-1165.
[12] Lindsay, W.L., Norvell, W.A. 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci. Soc. Am. J., 42, pp. 421-428.
[13] Sharifat, M., Khorsandi, F., Yasrebi, J., Karimian, N. 2014. Effects of biochar on sunflower yield and some soil properties in a copper contaminated calcareous soil. Adv. Environ. Bio, (In Press).