Carpathian Journal of Earth and Environmental Sciences, July 2017, Vol. 12, No. 2, p. 447 - 456

THE EFFECTS OF BIOCHAR APPLICATION ON CADMIUM UPTAKE OF TOBACCO

Halil ERDEM[1*], Ahmet KINAY[2], Elif GÜNAL[1], Hüseyin YABAN[1] & Yusuf TUTUŞ[3]

[1]*Gaziosmanpasa University, Faculty of Agriculture, Soil Science and Plant Nutrition Department, 60240 Tokat/Turkey*
[2]*Gaziosmanpasa University, Faculty of Agriculture, Department of Field Crops, Tokat/Turkey*
[3]*Sabanci University, Faculty of Engineering and Natural Sciences, Istanbul/Turkey*
**Corresponding author: halil.erdem@gop.edu.tr Fax: +90 356 2521480*

Abstract: Biochar could be used to improve soil physico chemical properties of Cd contaminated soils for safe crop production. A greenhouse experiemnt was conducted to investigate the effects of biochar application on growth and nutrient uptake of tobacco plants (Xhanti 2A tobacco genotype) in a Cd contaimaned soil. Mahleb cherry seed shell biochar was incorporated into a Cd-contaminated soil at four application rates (0, 1, 2, and 3 % (v/v)). The biochar application decreased the toxic amounts of Cd in tobacco shoots, whereas the Fe, Zn, Mn and Cu concentrations of shoots were also significantly reduced with the highest biochar treatment of 3.0%. These decreases were 19.5% for Fe, 51.0% for Zn, 28.9% for Mn and 8.51% for Cu. The decrease in Fe, Zn, Mn and Cu concentrations in tobacco shoots due to biochar application is a result of lowered availability of these metals in the soil with increasing biochar doses. However, the findings from biochar studies can not easily be trasferred by others due to differences in feedstocks used to produce biochars, environements in which biochars produced and the application rates of biochar.

Keywords: Mahalep, cadimium, tobacco, macronutirent, biochar, micronutirent, shoot dry matter

1. INTRODUCTION

The increase of cadmium (Cd) concentration in agricultural fields has been a serious concern due to the risk of transferring into the food chain (Peralta-Videa et al., 2009). Thus, soil amendments such as alkaline materials, limestone, and organic matter have been used to lower the mobility of Cd and reduce the Cd uptake by crops (Baker et al. 2011; Zhang et al., 2013). The results on application of organic and inorganic amanedments revealed that soil pH and soil organic carbon (SOC) content can be manipulated by application of amendments which decrease the mobility of Cd and eventually prevents transferring to the crops (Bradl, 2004; Zheng et al., 2013). Therefore, high cation exchange capacity of soil amendments is a prerequisite for the efficiency in reclaiming the excess Cd concentrations of soils. Safety for the environment and not having adverse effects on soil structure, soil fertility, or the ecosystem are also important additional features to looked for in an amendment. Biochar produced from pyroloysis of feedstocks at temperatures varying between 300 and 1000°C can be considered as an alternative additive, which significantly changes the physico-chemical and biological properties of soils (Lehmann et al., 2003; Chan et al., 2008; Ibrahim et al., 2013). Biochar is an effective sorbent for various hazardous inorganic and organic contaminants due to its high functional and sorptive characteristics (Vithanage et al., 2014; Cao et al., 2009).

The wooddy materials, crop residues, switch grass, organic wastes, sewage sludge, bagasse from the sugarcane industry, olive mill waste, animal manure, and paper sludge are widely used in biochar production (Sohi et al., 2009). Biochars produced from various feedstocks have different physico-chemical properties as a function of variability in the composition of feedstocks (Brewer, 2012). Biochars contains a significant amount of recalcitrant organic material, and aromatic groups are the major carbon components (Harvey et al., 2012).

High binding capacity of biochars increases the capacity of soil to hold plant nutrients and decreases leaching of chemicals from soil profile (Uzoma et al., 2011; Chan et al., 2008). Beesley et al. (2011) have also reported that biochar is an effective additive for heavy metal-contaminated soil to decrease the mobility of heavy metals and plant uptake depending on metal type and biochar properties. The decrease in Cd concentration of plants with the addition of biochar was attributed to the immobilization of bioavailable metals and also to the dilution effect due to increased plant biomass (Park et al., 2011) In a shorth term incubation study, biochar application significanlty immobilized the Cd, Pb, and Cu in soil (Park et al. 2011; Lucchini et al., 2014). Biochar application on a long term polluted rice growing soil immobilized the soluble Cd and reduced the Cd uptake of rice plants (Cui et al., 2011). Wagner & Kaupenjohann (2015) also reported significant reduction of Zn, Cd and Cu concentrations in plants with the addition of biochar to sewage field soils. The application of wheat chaff-derived biochar at an application rate of 5% significantly ($P< 0.05$) reduced the Cd concentration of J. subsecundus N.A. Wakef (Zhang et al., 2013).

The impact of a biochar on uptake of trace elements by plants may depend on types of biochar trace element and soil (Rizwan et al., 2015). Tobacco can accumulate relatively high Cd concentrations in the leaves, and thus considered as an efficient Cd accumulator (Lugon-Moulin et al, 2006) that boosts the harmful affect of tobacco when smaked. Therefore, the purpose of this study was to investigate the effects of biochar applications on Cd uptake of tobacco plants grown under high Cd concentrations.

2. MATERIAL AND METHODS

2.1. Material

Xhanti 2A tobacco genotype was used in individual pots at the experimental greenhouse located in the University of Gaziosmanpasa Tokat/Turkey. Xanthi 2A is a mid early genotype, average plant height is between 125 and 140 cm, and the number of leaves for each plant ranges from 28 to 30. The genotype is fine textured, very fragrant, and nicotine rate is approximately 1.6% with 15% sugar content (Peksuslu, 1998).

The soil used in the experiment had the following chemical and physical properties; texture was SiCl, $CaCO_3$ content was 15.8%, pH was 8.02, organic matter content was 1.14%. DTPA-extractable concentrations of Zn, Fe, Mn, Cu and Cd were 0.52, 2.11, 3.81, 1.37 and 0.005 mg kg^{-1}, respectively (Erdem et al., 2012). Soils were classified as Typic Fluvents (Durak & Yıldız, 1997).

2.2. Methods

2.2.1. Biochar production

The biochar applied in this study was produced by pyrolysis of mahleb cherry (*Prunus mahaleb L.*) seed shells. Biochars were produced by slow pyrolysis of feedstocks at 500°C in an ingeniously developed reactor. Fine biochars are more effective in reducing heavy metal concentration in shoots compared to coarse biochar due to the larger specific surface area of the finer biochar (Zheng et al., 2013). Thus, biochars were grinded to maximum size 2 mm before mixing to soil. Slow pyrolysis process was characterized by slow heating rates (a rate of approximately 10°C min^{-1}) and long residence times of biomass. The pyrolysis temperature was kept constant at 500°C and biochar was held in the unit until pyrolysis gas disappeared. After heating for almost 4 to 6 hours, the biochars were allowed to cool to room temperature.

2.2.2. Experiment

All experiments were conducted in greenhouse at the Faculty of Agriculture, Gaziosmanpasa University, in Turkey. Tobacco seedlings were transplanted in individual plastic pots with 2.5 kg soil (220 mm upper diameter, 180 mm lower diameter and 3-litre volume). Before potting, the soil was mixed homogenously with basal treatment of N 250 mg kg^{-1} soil as $Ca(NO_3)_2.4H_2O$, P 100 mg kg^{-1} soil as KH_2PO_4 and Fe 2.5 mg kg^{-1} soil as Fe-EDTA and Zn 2.5 mg kg^{-1} soil as $ZnSO_4.7H_2O$. Cadmium was applied at 0, 10 and 20 mg kg^{-1} soil doses in the form of $3(CdSO_4).8H_2O$ with three replicates. The soils were treated with four doses of mahalep biochar (0%, 1%, 2% and 3% biochar). Plants were harvested when the Cd toxicity symptoms were observed at 54 days of the growth. Harvested plants were dried at 70°C in an oven. Dry weights (DW) were recorded and expressed as g DW of plants.

2.2.3. Plant and Soil Analyses

The pH of mahalep biochar was measured with a pH meter in a suspension of using a biochar to water ratio of 1:5. Electrical conductivity (EC) of biochar was measured with a EC meter 1:5 extract of biochar to water (Zhang et al., 2013). The total C and N concentrations were measured by CHN analyzer

(Leco, USA). The total Cd concentration in biochar samples were measured by ICP-OES (Varian Vista). Harvested plants were ashed in a microwave oven by using 2 ml of 35% H_2O_2 and 5 ml of 65% HNO_3. Following the digestions, Cd, P, K, Fe, Zn, Cu and Mn were analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES; Varian Vista) (Bataglia et al., 1983). Total nitrogen was measured by Kjeldahl method (Bremner, 1965). Soil samples were collected from each of the pot, and analyzed for pH (Jackson, 1959) and DTPA-extractable Cd concentrations (Lindsay and Norvell, 1978).

2.2.4. Statistical Analysis

Standard analyses of variance techniques were used to assess the significance of treatment means. The data presented in the tables are mean values. Differences in the means of were compared using the Duncan`s test at 0.05 probability level.

3. RESULTS AND DISCUSSIONS

3.1. Characterics of Biochar

The mahalep biochar was strongly alkaline pH (8.86) and sliglhtly saline (EC: 1.01 dS m^{-1}) with a quite high C /N ratio (172). The total concentrations of Cd, Fe, Zn, P and K were 0.09 mg kg^{-1}, 26.39 mg kg^{-1}, 650 mg kg^{-1}, 904.36 mg kg^{-1} and 28440 mg kg^{-1}, respectively. Cation exchange capacity of biochar was 33.66 cmol kg^{-1} and specific surface area was 289.6 m^2 g^{-1} (Bayram, 2016) that would be expected to be suitable for immobilizing the cationic heavy metals in soil.

3.2. Soil DTPA-extractable Cd concentration and pH

The DTPA-extractable soil Cd concentratin was significantly influenced by the biochar additions (Table 1). The increased rate of biochar additions significantly ($P<0.05$) reduced the DTPA extractable Cd in soil which was similar to the results reported by Cui et al., (2012) in two year experiment of biochar effect on wheat Cd uptake. Cadmium concentration in soil at Cd-10 dose was 6.96 mg kg^{-1} before addition of biochar, and it was decreased to 6.08 mg kg^{-1} (12.6% decline), to 4.92% (29.3% decline) and to 4.46 mg kg^{-1} (35.9% decline) with 1.0%, 2.0% and 3.0% biochar additions, respectively. The DTPA extractable Cd concentration was 13.41 mg kg^{-1} in control for Cd-20 treatment, and soil Cd concentration was drastically reduced to 6.55 mg kg^{-1} which corresponds to 51.2% decline compared to the initial concentration with the 3% biochar addition, (Table 1). Lu et al., (2014) obtained the greatest decrease in extractable Cd in soil with 5% fine grained biochar treatment. The impact of biochar application on soil pH is presented in table 1. Biochar addition significantly ($P<0.05$) increased the soil pH. The highest mean value of pH was recorded in soils treated with 3.0% biochar.

The soil pH was increased by 0.27 unit (in Cd 20) at the highest application rate compared to control treatment. The Cd doses did not have any effect on soil pH and Cd dose*biochar interreaction had also not effective on soil pH. Zheng et al., (2013) indicated that an increase in soil pH may accelarete the metal adsorption and enhance the precipitation, thus reduces the transfer to the plants grown in contaminate soils. However, correlation analyses showed that soil pH had no significant relationship with any of the metal composion of tobacco dry matter (Table 3).

Table 1. Changes in DTPA extractable Cd concentrations and pH values of soil depending on biochar and Cd doses

Cd treatment (mg kg^{-1})	BC 0%	BC 1%	BC 2%	BC 3%
	DTPA- Extractable Cd in soil (mg kg^{-1})			
Cd 0	0.05 ± 0.00	0.03 ± 0.00	0.06 ± 0.02	0.05 ± 0.01
Cd 10	6.96 ± 0.12	6.08 ± 1.04	4.90 ± 0.62	4.46 ± 1.01
Cd 20	13.41 ± 0.47	11.57 ± 0.52	9.06 ± 0.10	6.55 ± 1.14
Cd Dose (Cd)	**	**	**	**
Biochar Dose (BC)	**	**	**	**
Cd*BC	**	**	**	**
Soil pH				
Cd 0	8.01 ± 0.15	8.06 ± 0.01	8.15 ± 0.03	8.22 ± 0.08
Cd 10	7.91 ± 0.17	8.03 ± 0.13	8.23 ± 0.01	8.18 ± 0.04
Cd 20	7.94 ± 0.14	8.09 ± 0.04	8.18 ± 0.06	8.21 ± 0.11
Cd Dose (Cd)	ns	ns	ns	ns
Biochar Dose (BC)	**	**	**	**
Cd*BC	ns	ns	ns	ns

* Significant at $P<0.05$ level ** Significant at $P<0.01$ level

3.3. Dry matter productions and Cd contents in shoots

The aboveground total biomass of tobacco was significantly influenced by different biochar and Cd additions but not with their interactions after 6 weeks of tobacco growth. In Cd-contaminated soil, the total biomass significantly varied with biochar additions compared to the Cd-contaminated soil without biochar. Application of mahalep biochar at 1.0% treatment in Cd-containinated soil increased the shoot dry matter weight (g plant^{-1}) of tobacco, however higher doses of biochar treatments (≥ 1.0%) lowered the shoot dry matter compared to the 1.0% biochar addition (Table 2). Despite the beneficial effects of increased Cd immobilization shown in lower Cd concentration with higher biochar doses, no significant increases in yield were observed at high biochar application rates due to high Cd decreased the phytoavailable soil nitrogen (Table 3).

Compared to the control, the 1.0% biochar treatment with Cd-20 dose significantly increased the shoot dry matter by 49.3%. Initial increase in dry matter yield may be due to the decreased availability of heavy metals (Cd) in the soil and increasing the availability of P added by the biochar. Increased doses of biochar applications (0.1, 5.0 and 10%) to a Cd enriched soil (24 mg Cd kg^{-1}) in a greenhouse experiment increased the dry matter of rape from 13 mg plant^{-1} (control, 0% biochar) to 15, 16 and 49 mg plant^{-1} with 1.0, 5.0 and 10.0% biochar applications, respectively (Houben et al., 2013). However, Jeffrey et al., (2011) indicated that experimental results for effects of biochars on crop yields are mostly dependent on the experimental design, setup, soil properties and conditions. In some of studies, researchers (Borchard et al., 2014; Rees et al., 2015) reported that biochar improves soil physic chemical properties and increases nutrient uptake but may have no significant effects on plant growth even at a rate of 40 t ha^{-1}. Although biochar applications and Cd treatments alone had significant effects on shoot dry matter of tobacco plants, impact of both factors did not yield significant changes of shoot dry matter yield (Table 2).

Table 2. The effect of Cd and biochar treatments on shoot dry matter production and shoot Cd content of tobacco plant.

Cd treatment (mg kg^{-1})	BC 0%	BC 1%	BC 2%	BC 3%
	Shoot Dry Matter (g plant^{-1})			
Cd 0	2.82 ± 0.23	3.08 ± 0.17	3.15 ± 0.45	2.60 ± 0.28
Cd 10	2.12 ± 0.54	2.38 ± 0.36	2.25 ± 0.35	1.84 ± 0.60
Cd 20	1.48 ± 0.64	2.21 ± 0.09	1.75 ± 0.52	1.32 ± 0.36
Cd Dose (Cd)	**	**	**	**
Biochar Dose (BC)	**	**	**	**
Cd*BC	ns	ns	ns	ns
	Cd in shoot (µg shoot^{-1})			
Cd 0	1.85 ± 0.30	1.31 ± 0.65	2.25 ± 1.23	1.76 ± 1.11
Cd 10	68.9 ± 0.85	61.1 ± 4.68	55.8 ± 7.29	49.1 ± 6.98
Cd 20	98.7 ± 9.65	74.5 ± 14.2	73.2 ± 8.43	51.7 ± 5.86
Cd Dose (Cd)	**	**	**	**
Biochar Dose (BC)	**	**	**	**
Cd*BC	**	**	**	**

* Significant at P<0.05 level ** Significant at P<0.01 level

Table 3. Correlations among variables tested in the experiment

		Soil		Plant								
	DM	Cd	pH	N	K	Mg	P	Cd	Fe	Zn	Mn	Cu
DM	1											
Cd-S	-0.66**	1										
pH-S	-0.07	-0.24	1									
N	0.849**	-0.67**	-0,19	1								
K	0.83**	-0.86**	0,20	0.73**	1							
Mg	0.75**	-0.56**	-0,22	0.82**	0.58**	1						
P	0.94**	-0.68**	-0,01	0.85**	0.85**	0.79**	1					
Cd	-0.68**	0.95**	-0,18	-0.70**	-0.89**	-0.57**	-0.70**	1				
Fe	0.86**	-0.64**	-0,03	0.83**	0.80**	0.73**	0.87**	-0.67**	1			
Zn	0.86**	-0.72**	-0,13	0.89**	0.79**	0.83**	0.84**	-0.74**	0.83**	1		
Mn	0.78**	-0.79**	0,00	0.81**	0.81**	0.85**	0.82**	-0.81**	0.80**	0.88**	1	
Cu	0.89**	-0.80**	0,06	0.86**	0.83**	0.82**	0.91**	-0.79**	0.79**	0.88**	0.86**	1

DM: Dry Matter S: Soil * Significant at P<0.05 level ** Significant at P<0.01 level

Shoot dry matter yield of tobacco significantly ($P<0.05$) decreased with increased Cd tratements and higher decreases ocurred at the highes application rate of Cd. Because, when plants uptake higher concentration of Cd, various physiological processes are directly or indirectly inhibited consequently result in retardation of growth and low plant biomass (Adrees et al., 2015). Thus, shoot dry matter of tobacco reduced from 3.08 g plant^{-1} to 2.12 and 1.48 g plant^{-1} with the Cd-10 and Cd-20 treatments (Table 2). Biochar addition slightly alleviated the negative effects of Cd induced toxicity by lowering the available Cd to plant roots though the highest dose of 3% biochar did not further increase the dry matter yield of tobacco shoots. Younis et al., (2015) also reported that biochar application increased the fenugreek biomass production in Cd-spiked soil. The increased shoot dry matter in Cd contaminated soil may also be related to the improved the root development which might be due higher water holding capacity, aeration and nutrient uptake other physico chemical properties of soils (Rees et al., 2015). Although, reports describing the effect of biochar on the biochemical and physiological activities of plants are limited (Rizwan et al., 2015), Younis et al. (2015) attributed to the increased photosynthetic pigments, photosynthetic rate and transpiration rate, and Fiaz et al., (2014) to the increased photosynthetic, Chl a, Chl b, and carotenoid, and accessory pigments', anthocyanin and lycopene, concentrations of plants.

The shoot Cd concentrations were significantly ($P<0.05$) reduced with increased biochar treatments as compared to unamended control (Table 2). The increase in soil pH with the higher doses of biochar applications may have promoted the Cd adsorption due to the increases in negative charges and reduced bioavailability of Cd. This resulted in the reduction in shoot Cd concentrations of tobacco plants. Mousavi et al., (2010) found similar results and concluded that pH increase with the biochar treatment caused to precipitation of Cd as $CdCO_3$. Lu et al., (2014) reported that the Cd concentration in shoots of *Sedum plumbizincicola* significantly ($P<0.05$) decreased with both bamboo and rice straw derived biochar applications by up to 49% and 20%, respectively. The highest reduction of Cd (upto 80%) in mustard shoots was observed with the 5.0% chicken biochar addition. The 80% decrease in Cd concentration with biochar treatments was connected to the immobilization of bioavailable metals and also to the dilution effect due to higher plant biomass production (Park et al., 2011). The Cd concentration of oat shoots grown in soil receiving sewage sludge in long term was lowered from 2.95 mg kg^{-1} to 0.99 mg kg^{-1} with the 5.0% biochar treatment (Wagner & Kaupenjohann, 2015).

The correlations among concentrations of elements determined in tobacco shoot reflect the behaviors of these components (Table 3). Positive correlation between two elements means that both are acting in soil in a similar way. In this perspective, Cd has negative correlations with all elements determined in tobacco shoots indicating that increased concentration of Cd decreases the concentration of macro and micro nutrient contents.

3.4. Macronutrients in shoots

The variation of N, K, Mg and P concentrations in tobacco shoots with different doses of biochar and Cd were presented in table 4. The biochar additions significantly ($P<0.05$) lowered the total nitrogen concentration of tobacco shoots (Table 4), with higher reduction at the high application rate (3%) compared to the control and lower applicating rates (1.0 and 2.0%). The shoot N concentration in Cd-0 treatment was decreased from 71.2 mg plant^{-1} to 60.9, 60.2 and 50.9 mg plant^{-1} with 1.0, 2.0 and 3.0% biochar additions, respectively. Low N uptake in the higher doses of biochar treatments is probably related to the high C:N ratio (172/1) of biochar used in the experiment. Zheng et al., (2013) also indicated that N in biochars produced at a temperature higher than 500^{o}C would not be bioavailable. Similar to the biochar treatment, increased Cd doses also lowered the total N contents of the tobacco shoots. Yildiz (2005) reported significant decrease in N concentrations for tomato and corn with increased Cd doses. This decline in N concentration might be related to the inhibation of nitrate reductase activity and decrease of nitrate adsorption and transfer from roots to the above ground mass of plants (Hernandez et al., 1996). Application of 1.0% biochar significantly lowered the negative effect of Cd and increased the shoot dry matter, however, higher doses (3% BC) did not further increase the nitrogen concentration of tobacco shoots, moreover decreased the shoot dry matter (Tables 2 and 4). Similarly, rice hull biochar application leaded to nitrogen deficiency in lettuce which was associated to the adsorption of available N on to the biochar. Further increase in biochar application rate resulted in growth decreased (Kim et al. 2015).

Potassium concentrations of shoots were significantly increased with biochar additions. The increase of K concentrations in shoots at control were 24.6%, 26.3% and 13.6% for 1.0, 2.0 and 3.0%

biochar treatments, respectively (Table 4). Puga et al., (2015) similarly observed an increaseof 104% and 234% for jack beans and *Mucuna aterrima*. The increases of K concentrations for Cd contaminaed soils were more prominent compared to the unamended control. Application of the K rich biochar enhanced the concentration of bioavailable K concentration in soil wihich resulted in increase shoot K concentrations of tobacco plants. In Cd-20 treatment, 1.0% biochar addition increased the shoot K concentration by 56.6% compared to the control (no biochar with Cd-20) (Table 4).

The biochar treatments significant increased the shoot P concentrations of tobacco up to 3% biochar application, and then decreased with higher biochar rate. The increased Cd doses significantly lowered the P concentrations of tobacco shoots. However, the interreaction effect of biochar and Cd doses had no effect on P concentration of tobacco shoots (Table 4).

Rees et al., (2015) reported decreased bioavailability of N, P, and Ca in Cd contaminated soils with biochar application. Similar to the findings of our study, Yang et al., (2015) also showed that plant available P in the soil increased with the biochar addition in a dose-dependent manner. Magnesium concentrations of tobacco shoots were significantly reduced with the biochar addition and the impact on Mg concentration was increased with the increased biochar rate (Table 4). Biochar binds some of essential plant nutrients on the surfaces and reduces their bioavailability (Beesley et al., 2011). The reduction of plant nutrients particularly with the 3.0% biochar addition is probably related to the increased binding of plant nutrients on biochar surfaces. The response of magnesium concentration to Cd is significantly pronounced (P<0.05) at increasing its application rate for all biochar doses (Table 4).

3.5. Micronutrients in shoots

The pH of the biochar (8.86) was higher than that of the soil (8.02) which indicates that biochar may increase immobilision of micronutirients. Biochar studies conducted in a more acidic soil conditions showed that biochar application caused a linear reduction of the available heavy metals (Puga

Table 4. Effect of Cd and biochar treatments on shoot N, K, P and Mg concents

Cd treatment (mg kg^{-1})	**BC 0%**	**BC 1%**	**BC 2%**	**BC 3%**
	N (mg shoot^{-1})			
Cd 0	71.2 ± 5.41	60.9 ± 5.53	60.2 ± 7.83	50.9 ± 0.38
Cd 10	46.2 ± 5.84	54.4 ± 2.13	41.7± 5.82	37.7 ± 10.1
Cd 20	30.7 ± 1.49	47.7 ± 3.72	34.3 ± 6.2	30.9 ± 7.88
Cd Dose (Cd)	**	**	**	**
Biochar Dose (BC)	**	**	**	**
Cd*BC	*	*	*	*
	K (mg shoot^{-1})			
Cd 0	111.5 ± 8.47	139.0 ± 5.60	140.9 ± 18.6	126.7 ± 11.9
Cd 10	66.4 ± 1.91	90.8 ± 3.41	92.3 ± 4.33	86.1 ± 8.91
Cd 20	51.3 ± 12.6	77.1 ± 4.78	65.1 ± 4.37	67.8 ± 10.4
Cd Dose (Cd)	**	**	**	**
Biochar Dose (BC)	**	**	**	**
Cd*BC	ns	ns	ns	ns
	P (mg shoot^{-1})			
Cd 0	6.13 ± 0.85	6.29 ± 0.18	6.36 ± 1.10	5.57 ± 0.45
Cd 10	4.10 ± 0.82	5.06 ± 0.76	4.52^{b} ± 0.47	4.15 ± 1.34
Cd 20	3.00 ± 1.10	4.70 ± 0.71	3.67 ± 1.06	2.91 ± 0.75
Cd Dose (Cd)	**	**	**	**
Biochar Dose (BC)	*	*	*	*
Cd*BC	ns	ns	Ns	Ns
	Mg (mg shoot^{-1})			
Cd 0	12.9 ± 1.17	9.13 ± 0.39	9.77 ± 1.53	8.04 ± 0.48
Cd 10	8.9 ± 1.52	8.09 ± 1.05	8.21 ± 0.19	7.23 ± 1.61
Cd 20	6.9 ± 2.14	7.31 ± 0.57	5.78 ± 0.11	5.70 ± 1.24
Cd Dose (Cd)	**	**	**	**
Biochar Dose (BC)	**	**	**	**
Cd*BC	ns	ns	ns	ns

et al., 2015). The results are consistent with regarding the reduction in the availability of micronutrients in contaminated soils with biochar application in a dose-dependent manner. In this greenhouse experient, biochar additions to an alkaline soil had the greatest impact on availability of Fe, Zn, Mn and Cu concentration with the highest application dose of 3.0%. Similar results for Pb and Zn were observed in uptake by Jack bean (*Canavalia ensiformis*) and *Mucuna aterrima* plants with application of 5% biochar of sugar cane straw (Puga et al., 2015). The higher decrease micronutients with the increase dose of biochar is probably due to retention of nutrients on the biochar surfaces. This decrease complied with 19.6% for Fe, 51.0% for Zn, 28.9% for Mn and 8.51% for Cu, at the highest dose of biochar additions in Cd-0 treatments (Table 5).

The variation of shoot Mn and Cu concentrations were not statistically significant, but the interreaction of biochar doses by Cd doses yielded significant changes for shoot Mn concentrations of tobacco. Fellet et al. (2014) indicated the presence of exchange sites on the surfaces of biochars that greatly affect the adsorption of elements and cause to the low bioavailability of nutrients. The results reported by Wagner & Kaupenjohann (2015) were accord the above statement. They found that application of 5% biochar in soil receiving sewage sludge in the longterm resulted in a significant reduction in Cd concentration from 2.95 to 0.99 mg kg^{-1}, and Zn concentration from 183 to 38 mg kg^{-1} Zn in the shoots of oats.

In case of Cd treated soils, 1.0% and 2.0% biochar applications significantly increased the shoot Fe, Zn and Mn and Cu concentrations. The Fe, Zn, Mn and Cu concentrations of Cd contaminated soil (Cd 20) showed an increase of 89.7%, 60.3%, 47.8% and 46.3% at the 1.0% biochar dose as compared to the control (Table 5). Similarly, biochar application decreased the Cu and Pb concentrations in shoots of ryegrass grown in a former Cu mine soil (Karami et al., 2011). The Cu concentrations of tobacco shoots significantly varied with the Cd doses, though biochar additions cleared off the impact of Cd doses on shoot Cu concentrations (Table 5). Park et al., (2015) found that adsorption capacities for some of trace elements by chicken bone biochar were in order of Cu>Cd≥Zn. The results indicating that Cu is the most retained cation and Zn can be easily exchanged by Cu.

Table 5. Effect of Cd and biochar treatments on shoot Fe, Zn, Mn and Cu contents

Cd treatment (mg kg^{-1})	**BC 0%**	**BC 1%**	**BC 2%**	**BC 3%**
	Fe (µg $shoot^{-1}$)			
Cd 0	185.4 ± 31.4	178.3 ± 8.78	202.0 ± 42.6	149.0 ± 12.1
Cd 10	122.7 ± 35.9	169.8 ± 21.7	152.2 ± 8.07	100.4 ± 23.1
Cd 20	72.4 ± 0.51	137.4 ± 22.7	115.2 ± 2.42	81.2 ± 8.41
Cd Dose (Cd)	**	**	**	**
Biochar Dose (BC)	**	**	**	**
Cd*BC	ns	ns	ns	ns
	Zn (µg $shoot^{-1}$)			
Cd 0	79.7 ± 4.10	71.0 ± 1.28	79.0 ± 4.01	39.0 ± 5.28
Cd 10	41.0 ± 9.23	43.9 ± 5.26	41.7 ± 6.07	32.0 ± 11,0
Cd 20	23.2 ± 8.27	37.2 ± 4.81	26.6 ± 1.49	20.2 ± 3.36
Cd Dose (Cd)	**	**	**	**
Biochar Dose (BC)	**	**	**	**
Cd*BC	**	**	**	**
	Mn (µg $shoot^{-1}$)			
Cd 0	99.5 ± 12.0	70.1 ± 2.65	89.5 ± 3.64	70.7 ± 3.58
Cd 10	51.3 ± 6.92	57.0 ± 3.60	59.3 ± 2.42	58.0 ± 2.94
Cd 20	36.6 ± 10.6	54.1 ± 4.06	38.8 ± 4.07	40.7 ± 9.95
Cd Dose (Cd)	**	**	**	**
Biochar Dose (BC)	ns	ns	ns	ns
Cd*BC	**	**	**	**
	Cu (µg $shoot^{-1}$)			
Cd 0	14.1 ± 0.60	15.8 ± 1.42	15.7 ± 1.75	12.9 ± 1.02
Cd 10	9.20 ± 4.12	9.90 ± 1.78	9.60 ± 1.31	9.80 ± 4.16
Cd 20	5.40 ± 1.78	7.90 ± 2.55	6.60 ± 2.03	6.20 ± 1.39
Cd Dose (Cd)	**	**	**	**
Biochar Dose (BC)	ns	ns	ns	ns
Cd*BC	ns	ns	ns	ns

4. CONCLUSIONS

The biochar application to the Cd contaminated soil was able to reduce available concentrations of Cd into the plant-soil system and increase the concentrations of P (except 3% biochar) in tobacco shoots. The decline in Cd phytoavailability can be attributed to the high pH and specific surface area of mahleb cherry seed shell biochar. Effect of biochar on shoot dry matter of tobacco plants varied with the biochar application rate and the highest does of 3.0% lowered the shoot yield despite the increasing the immobilization of Cd in soil. Biochar reduced the phytoavailability of N, Mg, Cd, Fe, Zn and Mn to tobacco plants as compared to unamended control.

REFERENCES

Adrees, M., Ali, S., Rizwan, M., Rehman, M.Z., Ibrahim, M., Abbas, F., Farid, M., Qayyum, M.K. & Irshad, M.K., 2015. Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. Ecotoxicol Environ Saf 199:186–197.

Baker, L. R., White, P. M., & Pierzynski, G. M., 2011. Changes in microbial properties after manure, lime, and bentonite application to a heavy metal-contaminated mine waste. Applied soil ecology, 48(1), 1-10.

Bataglia, O.C., Furlani, A.M.C., Teixeira, J.P.F., Furlani, P.R. & Gallo, J.R., 1983. Metodos de analise química de plantas. Instituto Agronomico, Campinas, p. 48.

Bayram, O. 2016. Determination of various physical and chemical characteristics of biochars produced from different agricultural wastes. MSc Thesis. University of Gaziosmanpasa, Tokat, Turkey.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., Harris, E., Robinson, B. & Sizmur, T., 2011. A review of biochars' potential role in the remediation, revegetation andrestoration of contaminated soils. Environ. Pollut. 159, 3269–3282.

Borchard, N., Siemens, J., Ladd, B., Möller, A. & Amelung, W., 2014. Application of biochars to sandy and silty soil failed to increase maize yield under common agricultural practice. Soil & Tillage Research 144:184–194.

Bradl, H. B., 2004. Adsorption of heavy metal ions on soils and soils constituents. Journal of Colloid and Interface Science, 277(1), 1-18.

Bremner, J. M., 1965. Total nitrogen. Methods of soil analysis. Part 2. Chemical and microbiological properties, (methodsofsoilanb), 1149-1178.

Brewer, C. E. 2012. Biochar characterization and engineering. PhD. Dissertation, University of Iowa. USA.

Cao, X.D., Ma, L.N., Gao, B. & Harris, W., 2009. Dairy-manure derived biochar effectively sorbs lead and atrazine. Environ. Sci. Technol. 43, 3285–3291.

Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A. & Joseph, S., 2008. Agronomic values of greenwaste biochar as a soil amendment. Aust. J. Soil Res. 45, 629–634.

Cui, L., Li, L., Zhang, A., Pan, G., Bao, D. & Chang, A., 2011. Biochar amendment greatly reduces rice Cd uptake in a contaminated paddy soil: a two-year field experiment. BioResources 6, 2605–2618.

Cui, L., Pan, G., Li, L., Yan, J., Zhang, A., Bian, R. & Chang, A., 2012. The reduction of wheat Cd uptake in contaminated soil via biochar amendment: a two-year field experiment. BioResources, 7(4), 5666-5676.

Durak, A. and Yıldız, H., 1997. Tokat Meyvecilik Üretme İstasyonu Topraklarının Detaylı Etüdü, Haritalanması ve Sınıflandırılması. Journal of Agricultural Faculty of Gaziosmanpasa University, pp 327-341.

Erdem, H, Kınay, A., Oztürk, M. & Tutus, Y., 2012. Effect of cadmium stress on growth and mineral composition of two tobacco cultivars. Journal of Food, Agriculture & Environment Vol.10 (1):965-969.

Fellet, G., Marmiroli, M. & Marchiol, L., 2014. Elements uptake by metal accumulator species grown on mine tailings amended with three types of biochar. Sci. Total Environ. 468-469, 598-608.

Fiaz, K., Danish, S., Younis, U., Malik, S.A, Raza Shah, M.H. & Niaz, S., 2014. Drought impact on Pb/Cd toxicity remediated by biochar in Brassica campestris. J Soil Sci Plant Nutr 14:845–854.

Harvey, O. R., Kuo, L. J., Zimmerman, A. R., Louchouarn, P., Amonette, J. E., & Herbert, B. E., 2012. An index-based approach to assessing recalcitrance and soil carbon sequestration potential of engineered black carbons (biochars). Environmental science & technology, 46(3), 1415-1421.

Hernandez, L.E., Carpena-Ruiz, R. & Garate, A., 1996. Alterations in the mineral nutrition of pea seedlings exposed to cadmium J. Plant Nutr. 19: 1581-1598.

Houben, D., Evrard, L. & Sonnet, P., 2013. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere, 92(11), 1450-1457.

Ibrahim, H.M., Al-Wabel, M.I., Usman, A.R. & Al-Omran, A., 2013. Effect of Conocarpus biochar application on the hydraulic properties of a sandy loam soil. Soil Sci. 178, 165–173.

Jackson, M.L., 1959. Soil chemical analysis. Englewood Cliffs, New Jersey.

Jeffery, S., Verheijen, F. G. A., Van Der Velde, M., & Bastos, A. C. 2011. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agriculture, ecosystems & environment, 144(1), 175-187.

Karami, N., Clemente, R., Moreno-Jiménez, E., Lepp, N.W. & Beesley, L., 2011. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. J Hazard Mater 191:41–48

Kim, H.S., Kim, K.R., Kim, H.J., Yoon J.H., Yang, J.E., Ok, Y.S., Owens, G. & Kim, K.H., 2015. Effect of biochar on heavy metal immobilization and uptake by lettuce (Lactuca sativa L.) in agricultural soil. Environ Earth Sci 74:1249–1259.

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 Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant Soil 249, 343.

Lindsay, W. L. & Norvell, W.A., 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Amer.J. 42:421-428.

Lu, K., Yang, X., Shen, J., Robinson, B., Huang, H., Liu, D., Bolan, N., Pei, J. & Wang, H., 2014. Effect of bamboo and rice straw biochars on the bioavailability of Cd, Cu, Pb and Zn to Sedum plumbizincicola. Agriculture, Ecosystems & Environment, 191, 124-132.

Lucchini, P., Quilliam, R. S., DeLuca, T. H., Vamerali, T. & Jones, D. L. 2014. Does biochar application alter heavy metal dynamics in agricultural soil?. Agriculture, Ecosystems & Environment, 184, 149-157.

Lugon-Moulin, N., Martin, F., Krauss, M. R., Ramey, P. B., & Rossi, L., 2006. Cadmium concentration in tobacco (Nicotiana tabacum L.) from different countries and its relationship with other elements. Chemosphere, 63(7), 1074-1086.

Mousavi, Z. H., Hosseinifar, A. & Jahed, V., 2010. Removal of Cu (II) from wastewater by waste tire rubber ash. J Serb Chem Soc 75:845 – 853.

Park, J.H., Choppala, G.K., Bolan, N.S., Chung, J.W. & Chuasavathi, T., 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil 348,439–451.

Park, J. H., Cho, J. S., Ok, Y. S., Kim, S. H., Kang, S. W., Choi, I. W., Heo, J.S., De Laune, D. & Seo, D. C. 2015. Competitive adsorption and selectivity sequence of heavy metals by chicken bone-derived biochar: Batch and column experiment. Journal of Environmental Science and Health, Part A, 50(11), 1194-1204.

Peksuslu, A. 1998. Morphological, Physiological and Biochemical Properties of Some Turkish Tobacco Varieties Grown under İzmir- Bornova Conditions. PhD. thesis, Univ. Ege, Bornova, İzmir, Turkey.

Peralta-Videa, J. R., Lopez, M. L., Narayan, M., Saupe, G., & Gardea-Torresdey, J., 2009. The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. The international journal of biochemistry & cell biology, 41(8), 1665-1677.

Puga, A. P., Abreu, C. A., Melo, L. C. A. & Beesley, L., 2015. Biochar application to a contaminated soil reduces the availability and plant uptake of zinc, lead and cadmium. Journal of environmental management, 159, 86-93.

Rees, F., Germain, C., Sterckeman, T. & Morel, J. L., 2015. Plant growth and metal uptake by a non-hyperaccumulating species (Lolium perenne) and a Cd-Zn hyperaccumulator (Noccaea caerulescens) in contaminated soils amended with biochar. Plant and Soil, 1-17.

Rizwan, M., Ali, S., Qayyum, M. F., Ibrahim, M., Zia-ur-Rehman, M., Abbas, T., & Ok, Y. S., 2015. Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: a critical review. Environmental science and pollution research international.

Sohi, S., Lopez-Capel, E., Krull, E. & Bol, R., 2009. Biochar, climatechange and soil: a review to guide future research, in CSIRO Land and Water Science Report series ISSN: 1834-6618.

Uzoma, K.C., Inoue, M., Andry, H., Fujimaki, H., Zahoor, Z. & Nishihara, E., 2011. Effect of cow manure biochar on maize productivity under sandy soil condition. Soil Use Manage. 27, 205–212.

Vithanage, M., Rajapaksha, A.U., Zhang, M., Thiele-Bruhn, S., Lee, S.S. & Ok, Y.S., 2014. Acid-activated biochar increased sulfamethazine retention in soils. Environ. Sci. Pollut. Res.. http://dx.doi.org/ 10.1007/s11356-014-3434-2.

Wagner, A. & Kaupenjohann, M., 2015. Biochar addition enhanced growth of Dactylis glomerata L. and immobilized Zn and Cd but mobilized Cu and Pb on a former sewage field soil. European Journal of Soil Science, 66(3), 505-515.

Yang, X., Liu, J., McGrouther, K., Huang, H., Lu, K., Guo, X. & Wang, H., 2015. Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil. Environ Sci Pollut Res. doi:10. 1007/s11356-015-4233-0.

Yildiz, N., 2005. Response of tomato and corn plants to increasing Cd levels in nutrient culture. Pak. J. Bot. 37 (3): 593-599.

Younis, U., Malik, S.A., Qayyum, M.F., Shah, M.H.R., Shahzad, A.N & Mahmood, S., 2015. Biochar affects growth and biochemical activities of fenugreek (Trigonella corniculata) in cadmium polluted soil. J App Bot Food Qual 88:29–33.

Zhang, Z., Solaiman, Z.M., Meney, K., Murphy, D.V. & Rengel, Z., 2013. Biochars immobilize soil cadmium, but do not improve growth of emergent wetland species Juncus subsecundus in cadmium-contaminated soil. J. Soils Sediments13, 140–151.

Zheng, R., Chen, Z., Cai, C., Wang, X., Huang, Y., Xiao, B., & Sun, G., 2013. Effect of biochars from rice husk, bran, and straw on heavy metal uptake by pot-grown wheat seedling in a historically contaminated soil. BioResources, 8(4), 5965-5982.

Received at: 25. 04. 2016
Revised at: 13. 01. 2017
Accepted for publication at: 10. 02. 2017
Published online at: 20. 02. 2017