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Chemical Stabilization of Some Heavy Metals in an Artificially Multi-Elements Contaminated Soil, Using Rice Husk Biochar and Coal Fly Ash

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ABSTRACT: A greenhouse experiment has been planned for this study to delineate the benefits of two types of rice husk biochars (namely B300 and B600 which are prepared at 300°C and 600°C, respectvely) and coal fly ash (CFA), as soil amendments, for decreasing the amount of some heavy metals (like Pb, Cd, Ni, Cr, and Cu) as well as mobility and phytoavailability in an artificially-calcareous multi-element-contaminated soil. The effect of soil amendment on heavy metals' availability has been evaluated via sequential extraction experiment and phytoavailability of the plant. According to the results, among the studied amendments, B600 has had the highest positive effect on both dry matter yield in corn and heavy metals' availability reduction in post-harvest soil samples (with the exception of Cr), compared to CFA and B300, due to the increasing specific surface area, CEC, and pH that promote heavy metals' sorption in the soil through surface complexation and ion exchange mechanisms. Evaluation of heavy metals' chemical forms in post-harvest soil samples indicates that addition of amendments has significantly decreased mobility factor of heavy metals (with the exception of Cr in CFA-amended soils). In general, application of three soil amendments to this polluted soil has considerable effect on the reduction of heavy metals' availability and phytoavailability. However, among the studied amendments, B600 and CFA have had the maximum and minimum effect on heavy metals' availability reduction, respectively.

Keywords: Amendments, Calcareous soil, Remediation, Mobility factor

INTRODUCTION

Soil pollution with Heavy Metals (HMs) has been known as one of the environmental challenges in recent decades (Grimm et al., 2008). HMs' leaching from contaminated soils into groundwater and their uptake by the plants can adversely affect food security and human health (Wongsasuluk et al., 2014). In some abandoned industrial areas and mining sites, soils are contaminated with a variety of HMs (multi-element-contaminated soils), and there is limited information about their remediation process.

In response to a growing need to address soil pollution, soil remediation practices in soils are necessary to treat polluted soils. Traditional methods of HMs remediation such as landfill techniques have become out of date due to its high expense, while others such as soil stabilization and phytoremediation are greatly potential for HMs' remediation. In situ chemical

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stabilization has been introduced as one of the cost-effective remediation techniques wherein soil amendments chemically decrease the hazardous potential of HMs through converting the contaminants into less mobile fractions by adsorption, complex formation, or (co)precipitation process (Saffari et al., 2016).

Low-cost and widely available amendments such as waste product from agricultural and industrial, which have low environmental impact, are being increasingly used for HM stabilization. In recent years, biochar has been used as an effective soil amendment to reduce HM availability. It is the solid product from pyrolysis of waste biomass product under oxygen-limited conditions. Depending on pyrolysis temperature, pyrolysis time, and the type of biomass (e.g., dairy manure and crop residues) biochars have different properties. Coal fly Ash (CFA) is another amendment, used to reduce HMs' mobility in the soil (Saffari et al., 2015; Kumpiene et al., 2008). Reduction of HMs' availability with CFA is due to the increase of pH and specific surface area that increase precipitation of insoluble phases and promoting metal sorption through surface complexation or cation exchange (Saffari et al., 2016; Kumpiene et al., 2007). The efficacy of soil amendment could be measured with various methods such as adsorption-desorption process (Saffari et al., 2016), sequential extraction methods (Saffari et al., 2015), Toxic Characteristic Leaching Procedure (TCLP), and HMs bioavailability for plant.

In arid and semi-arid conditions, due to limited water resources, municipal wastewater is being used as water sources for plant irrigation (Saffari and Saffari, 2013). Wastewater application has led to changes in some soil properties (Saffari and Saffari, 2013); therefore, wastewater can play an important role in fractionation and mobility of HMs.

Nonetheless, little attention has been paid to the role of soil amendments to improve HMs stabilization in multi-element-contaminated calcareous soils. In addition, application of amendments in wastewater-irrigated soils could be important for better understanding of amendments' performance. As such, the present study aims at investigating the effect of two types of rice husk biochars and coal fly ash as soil amendments on HMs uptake of grown corn, irrigated with wastewater and freshwater in an artificially calcareous multi-element-contaminated soil as well as its relation to changes in HMs fractionation under different amendments.

MATERIALS AND METHODS

A greenhouse experiment was performed, based on a completely randomized design with three replicates, using the pot culture of corn (Zea mays, AS-71) in artificially calcareous multi-element-contaminated soil. The treatments included three types of amendments, namely rice husk biochars prepared at 300°C (B300) and 600°C (B600) as well as coal fly ash (CFA), two rates of amendments (2% and 5% W/W), and two irrigation sources. Freshwater and wastewater. The study was carried out in a greenhouse at Shiraz University, Shiraz, Iran.

Soil sample was collected from the depth of 0–30 cm in a calcareous soil type (Fine, mixed, mesic, Fluventic Calcixerepts) from agricultural fields, located at the college of agriculture, Bajgah, Shiraz, Fars Province, Iran. The soil samples were air-dried and passed through a 2-mm sieve. Soil texture was analyzed, using hydrometer method (Bouyoucos, 1962, while pH was measured in saturated paste. Percentage of Calcium Carbonate Equivalent (CCE) was determined through acid neutralization (Loeppert and Suarez 1996) with Organic Matter (OM) content, determined via wet oxidation (Nelson & Sommers 1996) and Cation Exchange Capacity (CEC), measured by replacing exchangeable cations with sodium acetate (Sumner & Miller 1996). Plant-availablity of the heavy metals was extracted with diethylenetriaminepentaacetic acid (DTPA) and determined via atomic absorption spectrophotometer (Lindsay and Norvell, 1978). Total content of heavy metals was determined, using 4M HNO_3 (Sposito et al., 1982), and determined via atomic absorption spectrophotometer. Table 1 presents some soil chemical and physical properties. Three kinds of amendments, namely B300, B600, and CFA, were applied in this study. CFA was collected from Zarand coal washing factory of Kerman, Iran. It contained 46.47% of SIO_2, 27.32% of Al_2O_3, 0.9% of TiO_2, 6.73% of Fe_2O_3, 4.56% of CaO, 0.15% of BaO, 0.14% of SrO, 2.32% of MgO, 3.42% of K_2O, 0.82% of Na_2O, 4.6% of SO_3, 4.6% of P_2O_5, and 0.82% of Mn_3O_4, with a pH value of 9.1.

Table 1. Selected chemical and physical properties of the studied soil

Property	Value	Property	Value
pH	7.8	Total Cd (mg/kg)	0.65
CCE (%)	39.5	Total Cu (mg/kg)	45
Sand (%)	27	Soluble Cd in DTPA(mg/kg)	Trace
Clay (%)	35	Hexavalent Cr (mg/kg)	8.5
OM (%)	1.4	Soluble Cu in DTPA(mg/kg)	0.92
CEC (Cmol(+)/kg)	15.8	Soluble Mn in DTPA(mg/kg)	5.6
EC (dS/m)	0.65	Soluble Pb in DTPA(mg/kg)	Trace
Total Ni (mg/kg)	51	Soluble Ni in DTPA(mg/kg)	Trace
Total Cr (mg/kg)	76	Soluble Fe in DTPA(mg/kg)	4.1
Total Pb (mg/kg)	Trace		

Biochars were prepared at 300°C and 600°C from rice husk, covered in aluminum foil (to simulate limited oxygen accessibility for the period of wildfires) and placed in a preheated muffle furnace for 4h in order to produce them. The concentrations of carbon, hydrogen, and nitrogen in biochars samples were determined with CHN analyzer (varioMACRO CHNS) with the elemental content of C, H, and N in B300 sample being 51.57%, 2.11%, and 1.52%, respectively. As for its pH, EC, and CEC values, they were 6.2, 13.1 dS/m, and 420 mmol$_{(+)}$/kg, respectively. In contrast, the average elemental composition of B600 was 58.99% C and 1.55% H, with the percentage of N being trace. Also, its pH, EC, and CEC values were 8.7, 21.2 dS/m, and 580 mmol$_c$/kg, respectively. European Biochar Certificate (EBC, 2012) have introduced a variety of this subtance with a minimum of 50.0% for Carbon (C) and a maximum of 0.7 for H/C. Here, B300 and B600 contained 51.57 and 58.99% C, while their H/C ratio was 0.04 and 0.02, respectively. In addition, this study used Fourier Transform Infrared Spectroscopy (PerkinElmer FT-IR: Spectrum RXI) in order to identify functional groups of the produced biochars, the results of which had been previously reported by Saffari et al. (2015). To determine HMs bioavailability, soil samples were placed in plastic cups and Ni, Pb, Cu, Cr, and Cd were added at the rates of 150, 600, 200, 200, and 150 μg/g, respectively. The metal cations were applied in forms of $Ni(NO_3)_2$, $Pb(NO_3)_2.4H_2O$, $CuSO_4.5H_2O$, $K_2Cr_2O_7$, and $CdCl_2.5H_2O$. Afterwards, selected amendments including CFA, B300 and B600 were added to each polluted soil sample separately, at two rates of 2% and 5% W/W, with each soil sample getting mixed thoroughly. The soil samples were incubated for 14 days at 25°C and the moisture was kept almost at field capacity moisture by adding distilled water to a constant weight. After incubation, soil samples were transferred to pots and used for growing plants.

Six seeds of corn (Zea mays, AS-71) got planted in each pot and narrowed down to three uniform plants six days after planting. The irrigation treatments consisted of irrigation with freshwater (FW) and irrigation with wastewater (WW). The municipal wastewater, used in this experiment, was prepared from the wastewater station of Shiraz, containing 0.1 meq/l of nitrate, 0.2 meq/l of phosphorus, 0.003 ppm of Pb, 0.002 ppm of Cr, 0.0002 ppm of Cd, 0.002 ppm of Ni, and 0.03 ppm of Cu. Its EC and pH were 1.1 dS/m and 6.3, respectively. Irrigation was conducted every three days and the moisture was kept at field capacity. The plants were allowed to grow for two months under natural sunlight in the greenhouse. Aboveground parts of plants were harvested and rinsed with deionized water and dried at 65°C for 48 h, to be ashed in a muffle furnace for 2h at 550 ◦C. This resultant ash was dissolved in 2M hydrochloric acid (HCl) and filtered through a filter paper, then to get diluted to 50 ml with deionized water (Jones et al., 1991). Afterwards, they were analyzed by atomic absorption spectrophotometer (Shimadzu AA-670G). At the end of the experiment, soil samples of the respective pots were removed and taken for bioavailble form (extracted by DTPA) and chemical fractionation of selected heavy metals. Chemical fractionation of HMs in soil samples was determined, using Singh et al. (1988) procedure, which called for HMs to be separated into seven forms. Residual fraction (Res) was calculated by subtracting the sum of six fractions from total HMs. Table 2 gives the outline of this method.

Table 2. Outline of the sequential extraction procedure, used in this study

g soil:mL solution	Extracting solution	Shaking time (h)	Chemical form of HMs	Symbol
10:40	1 M $Mg(NO_3)_2$	2	Exchangeable	EX
10:40	1M NaOAc (pH=5 CH_3COOH)	5	Carbonate- bound	Car
10:20	0.7M NaOCl (pH=8.5)	0.5 in boiling water bath	Organically- bound*	Om
5:50	0.1M $NH_2OH.HCl$ (pH=2 HNO_3)	0.5 in boiling water bath	Mn-oxide- bound	Mn-OX
5:50	0.25M $NH_2OH.HCl$+ 0.25M HCl	0.5 at 50 °C in water bath	Amorphous Fe-oxide-bound	FeA-Ox
5:50	0.2M $(NH4)_2C_2O_4$ +0.2M $H_2C_2O_4$ +0.1 $MC_6H_8O_6$	0.5 in boiling water bath	Crystalline Fe- oxide-bound	FeC-Ox

*Two times extraction

HMs mobility was determined by a Mobility Factor (MF), calculated according to the following equation: (EX+Car/sum of fractions) ×100 (Saffari et al., 2015)

The Duncan's multiple-range test procedure and other statistical analyses were calculated by means of Microsoft Excel 2007, SAS V9.1.3, and SPSS V19.

RESULTS AND DISCUSSION

Results from analysis of variance (ANOVA) showed that both Fresh Matter Yield (FMY) and Dry Matter Yield (DMY) in corn shoot were significantly influenced by the amendments, themselves, amendment rates (with the exception of FMY), irrigation sources, and their interaction (Table 3). Namgay et al., (2010) applied three different rates of biochar (viz., 0, 5, and 15 g/kg), prepared from *Eucalyptus saligna* at 550°C, on polluted soil to report that addition of biochar did not have any significant effect on DMY of maize. They explained that low-level application of biochar and addition of basal fertilizers in all treatments to soils caused no significantly different DMY between treated soils and the control. It seems that in the present study, application of appropriate rate of biochar (i.e., 2% and 5%, equal to 20 and 50 g/kg) in the nonfertilized soil increased fertility through boosting both water-holding capacity and CEC. Similar results were obtained by Rondon et al. (2007), who observed an increase in DMY of maize at biochar application rates of 60 and 90 g/kg soil, which they attributed to increased CEC. Existence of HMs in the studied soils led to various toxicity symptoms such as chlorosis and necrosis. In addition, shoots of plants had noticeable and gradual stunted growth, though none of them got wasted. These symptoms were more obvious in soils treated with CFA and the control. Figure 1 illustrates the influence of amendment application on FMY and DMY. The highest and lowest FMY and DMY were obtained in soil samples, treated with B600 and CFA, respectively (Figure 1). The beneficial effects of various biochar application on decreasing HMs availability in contaminated soils have been reported by several experiments (Saffari et al., 2015, b: Saffari et al., 2016). The mean rate of DMY and FMY in treated soils with B300 and B600 increased by 47.99% and 102.9%; and 10.74 and 32.43%, compared to control treatment, respectively (Figure 1). Depending on the temperature, biochars had different properties. Production of biochar at high temperature often produced biochar with highly aromatic substances, recalcitrant to breakdown, with high adsorptivity for heavy metals and high surface area, compared to prepared biochars at low temperature (Ladygina and Rineau, 2013).

The characteristics of B300 and B600, previously reported by Saffari et al. (2015), showed that the functional groups such as carboxylic bonds and aromatic C=O ring stretching (likely –COOH) in B600 was higher than B300, the pH and CEC of which had been increased. In addition, higher amount of CEC was obtained in B600 than B300. As a result, it was expected that B600 could efficiently affect immobilization of HMs, consequently increasing the plant's DMY and FMY. Usman et al (2016) evaluated sorption process of date palm biochar (prepared at two pyrolysis temperatures of 300 °C and 700 °C) for Cd removal from aqueous solutions. Their results showed that, ion exchange with Ca and Mg, precipitation, or co-precipitation (rather than surface complexation with oxygen-containing functional groups) incorporated the main process for Cd removal via prepared biochar at high pyrolysis temperature. On the other hand, they showed that sorption of Cd on biochar, prepared at low pyrolysis temperature (with more pronounced oxygen-containing functional groups), might be controlled by ion exchange and surface complexation. Therefore, it seems that, existence of various functional groups could lead to heavy metals stabilization in soils through ion exchange with Ca and Mg, precipitation or co-precipitation for treated-B600, and ion exchange and surface complexation for treated-B300. Houben et al. (2013) studied the beneficial effects of biochar (prepared from plant's straw at 600C^{o} in three rates, viz. 1%, 5%, and 10%) and application of lime in contaminated soils the biomass production of rapeseed. Their results showed that the biomass of plants, harvested from the biochar-10% treatment, was 9.7 and 3.1 times higher than that of plants, grown on the biochar-5% and lime treatments, respectively. Application of CFA decreased the mean of DMY and FMY by 74.8% and 75.5%, compared to control treatment. Seaman et al. (2001) and Saffari et al. (2014) reported that CFA can increase the mobility of Cr by oxidizing Cr(III) to Cr(VI) in soil, likely the main reason of decreased FMY and DMY in soil samples, treated by CFA. Based on the results, application of the amendment at high rate treatment (5% W/W) could provide higher adsorption sites for HMs, increasing FMY and DMY, compared to low rate treatment (2% W/W); however, there was no significant difference, observed in FMY (Figure 2).

Table 3. Analysis of variance of FMY, DMY, HMs concentrations (C.) and uptake (U.) by plant, and DTPA–extractable (DTPA-) concentration of HMS in soils after plant harvesting, as affected by amendments, amendment rates, and irrigation sources.

	Source	Amendments (A)	Amendments Rates (B)	Irrigation Sources (C)	A×B	A×C	B×C	A×B×C	Error
	†D.F.	3	1	1	3	3	1	3	32
Mean Squares	FMY	300.037**	2.786^{ns}	44.867**	7.129*	5.645**	0.268**	0.139**	0.929
	DMY	14.843**	0.698**	4.014**	0.582**	0.149**	0.003**	0.021**	0.054
	C. Cu	71495.523**	5.267^{ns}	22376.740**	1453.017**	2888.624*	6.092**	40.042**	313.122
	C. Cd	12535.948**	0.867^{ns}	472.696**	46.985^{ns}	31.609**	10.407**	3.176*	6.089
	C. Ni	3543.004**	0.891^{ns}	95.937**	49.336**	44.054**	25.931**	11.777**	8.129
	C. Pb	103461.19**	72.207^{ns}	1668.615**	1803.549*	546.472*	460.462**	83.865*	184.812
	C. Cr	44267.239**	9.586^{ns}	8638.992**	119.681^{ns}	1989.302**	0.023**	1.768**	150.768
	U. Cu	84487.36**	104.46^{ns}	1897.70^{ns}	3028.99^{ns}	24314.54*	16.63*	532.34**	1991.53
	U. Cd	3615.37**	65.56^{ns}	1568.97**	136.72^{ns}	611.75*	16.85**	32.41**	56.18
	U. Ni	8266.52**	50.72^{ns}	2664.74**	28.02^{ns}	1129.02*	10.03^{ns}	20.65*	84.36
	U. Pb	67207.34**	16.25^{ns}	31882.23**	2070.61^{ns}	6894.76^{ns}	611.16**	93.28**	661.6
	U. Cr	137637.40**	2248.00^{ns}	108694.90**	5416.98^{ns}	24057.24*	12.02**	29.50**	2107.7
	DTPA-Cu	2874.0**	393.3**	35.6^{ns}	72.3*	78.3^{ns}	0.01**	27.8*	12.7
	DTPA-Cd	1682.7**	37.5*	1.7^{ns}	11.4*	73.9^{ns}	6.5**	2.3**	6.4
	DTPA-Ni	587.2**	61.8**	245.6**	8.4^{ns}	35.6*	68.0*	13.0**	5.7
	DTPA-Pb	5946.9**	828.7**	9723.1**	102.8**	459.6**	5.7**	56.9**	44.4
	DTPA-Cr	1194.7**	0.03^{ns}	18135.2**	35.9^{ns}	515.7**	113.5^{ns}	42.6**	45.1

†Degrees of freedom

*, **Significant at 5% and 1%, respectively.

ns: non-significant.

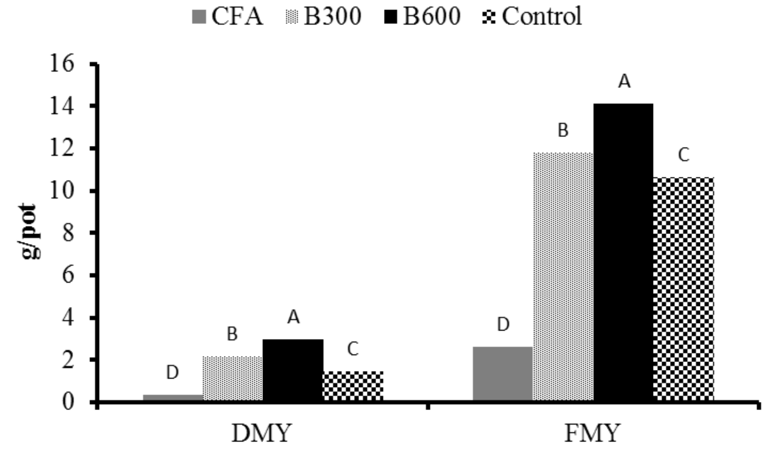

Fig. 1. Amendments' effect on DMY and FMY of corn shoot (g/pot). Different letters (in each separate cluster) indicate significant differences among the means of different treatments (p<0.05).

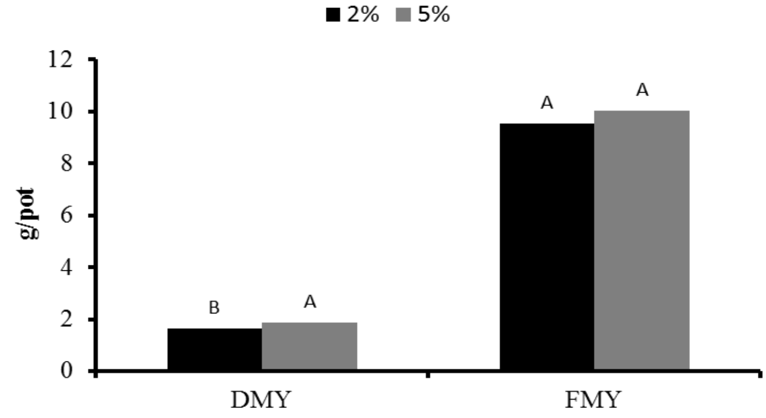

Fig. 2. Effect of amendment rates on DMY and FMY of corn shoot (g/pot). Different letters (in each separate cluster) indicate significant differences among the mean of different treatments (p<0.05).

The mean of DMY and FMY in treated soils at 5% rate rose by 12.8% and 4.8%, compared to 2% rate, respectively (Figure 2). Figure 3 demonstrates the impacts of irrigation sources on FMY and DMY. With application of wastewater, the mean rate of FMY and DMY plummeted, compared to freshwater treatment. This decreased FMY and DMY about by 17.95% and 28.43, respectively compared to WW-treatment soils.

Saffari and Saffari (2013) studied the impact of treated municipal wastewater on soil chemical properties, reporting that application of wastewater had slightly decreased soil pH (7.5). They explained that the slight pH change of the soils through increasing wastewater irrigation might be due to the higher inputs of sulfate loads of wastewater, release of exchangeable cations, oxidation of organic compounds, and nitrification of ammonium. It seems that, by reducing the soil pH, HMs' availability for plants had increased, consequently leading to a decrease in FMY and DMY, compared to the plants, irrigated with freshwater.

Table of ANOVA (Table 3) showed that the application of amendments and irrigation sources on HMs' concentration and total uptake in corn shoot was statistically significant; however, there was no considerable difference among applied amendments rates. Furthermore, the interactions among the treatments were significantin some cases and insignificant in the others. Figure 4 illustrates amendments' impact on HMs' concentrations via the corn shoots. The highest and lowest concentrations of Cu, Cd, Pb, and Ni in corn belonged to CFA and B600 treatments, respectively (Figure 4). The dilution effect of plant biomass (concentration of plant's HMs increased as DMY declined) was responsible for the highest concentrations of HMS in amended soils with CFA. On the other hand, the highest and lowest concentrations of Cr were observed in control and B300-treated soil, respectively.

Total uptake of HMs, with the exception of Cu, were higher in the plants, grown in control soils, than in the ones, grown in soils treated with amendments (Figure 5). Compared to the control soil, application of the amendments significantly decreased the total uptake of Cr, Ni, Pb, and Cd in corn shoot, with the lowest and highest total uptake of Cr, Ni, Pb, and Cd in corn belonging to CFA and control treatment, respectively. As for Cu, its highest and lowest total uptake in corn shoot were observed in B600 and CFA treatments, respectively.

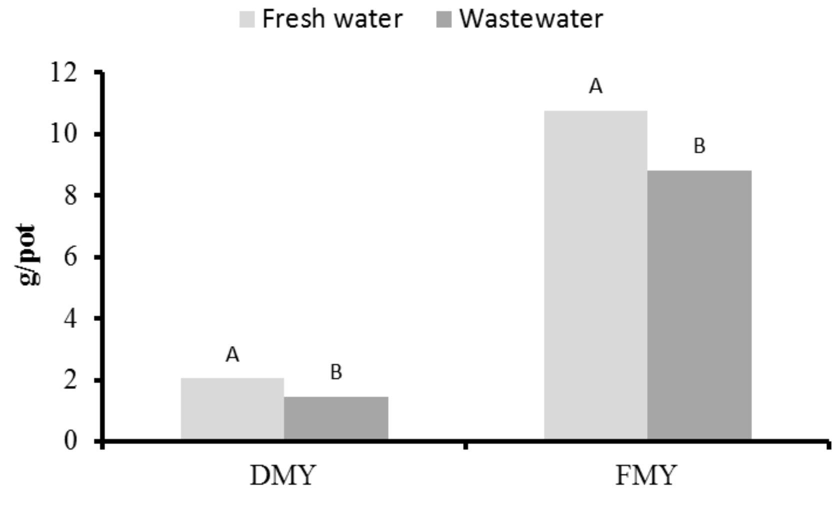

Fig. 3. Effect of irrigation sources on DMY and FMY of corn shoot (g/pot). Different letters (in each separate cluster) indicate significant differences among the means of different treatments ($p<0.05$)

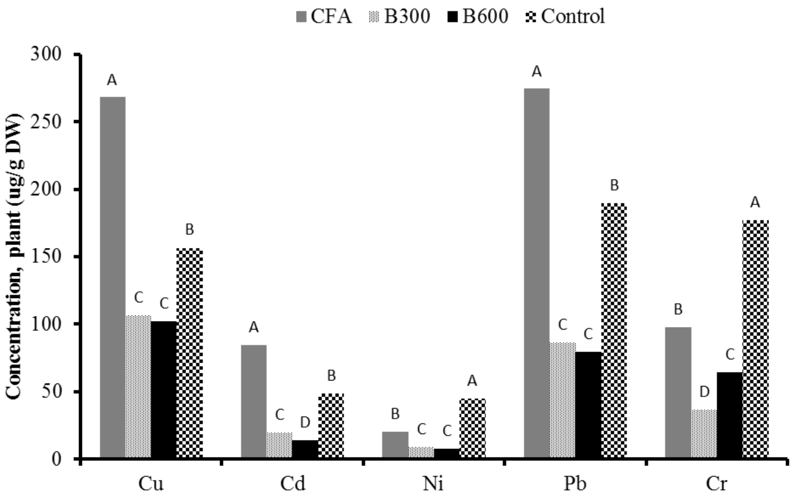

Fig. 4. Amendments' effect on HMs concentration via the corn shoots (ug/g DW). Different letters (in each separate group of bars) indicate significant differences among the means of different treatments (p<0.05).

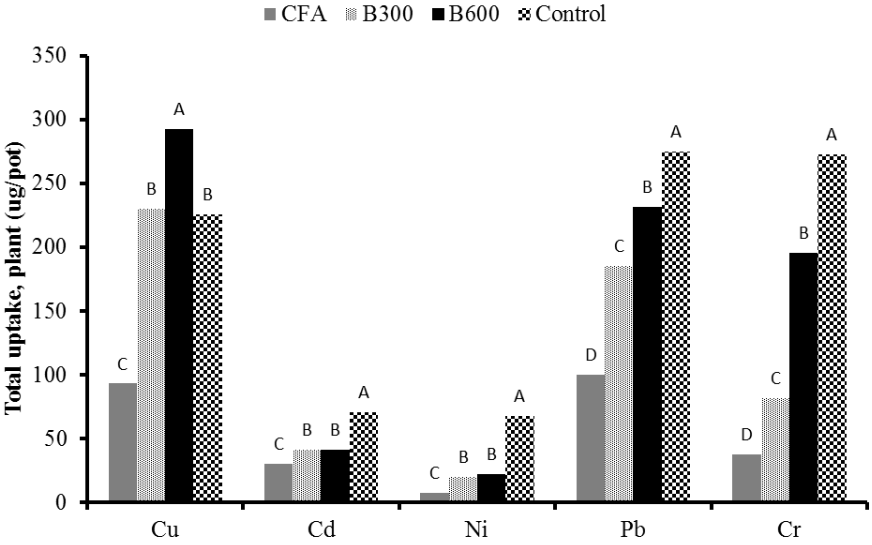

Fig. 5. Amendments' impact on HMs' uptake via corn shoots (ug/pot). Different letters (in each separate group of bars) indicate significant differences among the means of different treatments (p<0.05)

Figures 6 and 7 show the impact of amendments' rates on HMs concentration and total uptake in corn shoot. Results indicated that there was no significant difference in HMs concentration and total uptake in corn shoot from the soil, treated with different rates of amendments. Application of wastewater significantly increased Cu, Cd, and Pb concentrations, while decreasing Ni and Cr in the corn shoots (Figure 8). On the other hands, it significantly decreased HMs' total uptake in corn shoot (Figure 9). Chang et al. (2013) investigated chemical stabilization of cadmium in acidic soil, using different amendments (at the rates of 1%, 2%, and 4%), such as wood biochar (650°C), crushed oyster shell, blast furnace slag, and fluidized-bed crystallized calcium. Their results showed that thanks to by-product application, Cd concentration in the shoots stayed below 10.0 mg/kg, as compared to 24 mg/kg for plants which grew in unamended soil.

Namgay et al. (2010) studied the effects from the application of biochar (prepared from *Eucalyptus saligna* at 550°C, at three rates of 0, 5, and 15 g/kg) in the soil on the availability of As, Cd, Cu, Pb, and Zn to maize. Their results showed that adding biochar reduced the concentration of As, Cd, and Cu in maize shoots (especially at the highest rate of trace element application), whereas the effects were inconsistent on Pb and Zn concentrations in the shoots. These researchers explained that formation of stable metal-organic complexes and adsorption of the trace elements to organic matter are two main mechanisms to decrease HMs concentration in plants in soil samples, treated with biochar. Application of two types of biochars in soil had different results on pH and CEC of soils with the pH in soil samples treated with B600 after plant harvest soaring to 7.8, compared to the control pH of 7.2, whereas B300 had no significant effect on pH, staying at 7.3, with respect to the control treatment. Similarly, CEC in both of treated soil by B300 (15.5 Cmol($_+$)/kg) and B600 (17.6 Cmol($_+$)/kg) ascended significantly, as compared to the control (14.8) in post-harvest soils.

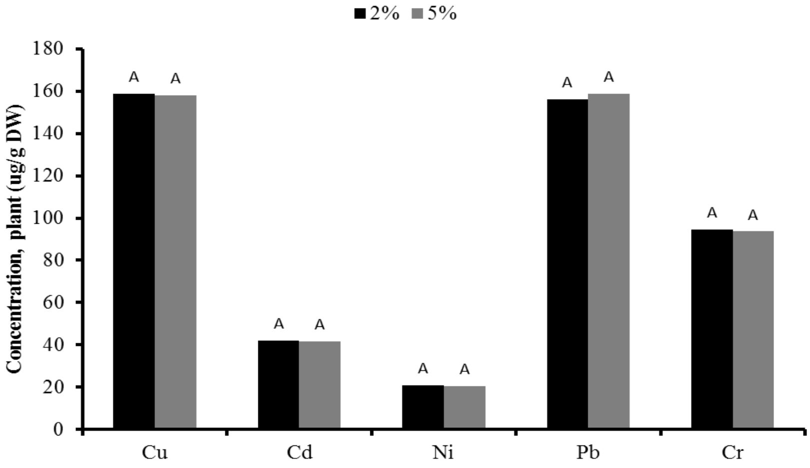

Fig. 6. Effect of amendment rates on HMs concentration by the corn shoots (ug/g DW). Different letters (in each separate group of bars) indicate significant differences among the means of different treatments (p<0.05).

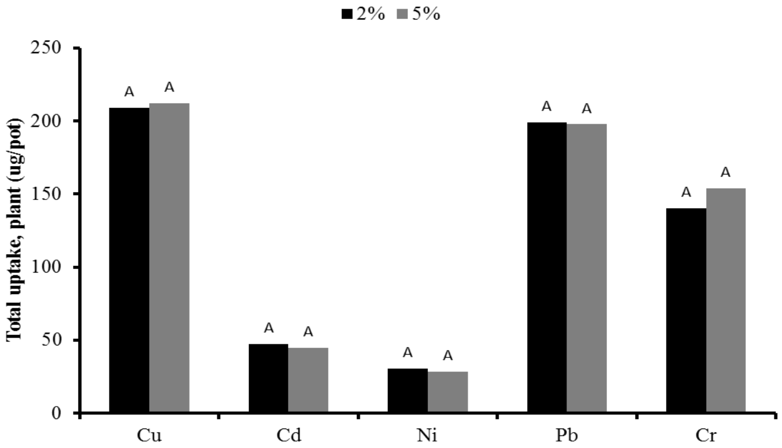

Fig. 7. Effect of amendment rates on HMs uptake by the corn shoots (ug/pot). Different letters (in each separate group of bars) indicate significant differences among the means of different treatments (p<0.05)

Similar to the results from our study, Namgay et al. (2010) and Liang et al. (2006) reported that application of biochar in soil significantly increased pH and CEC, which could be an important reason for the decreased HMs in the plant. Results of ANOVA (Table 3) showed that DTPA–extractable concentration of HMS in treated soils after plant harvest were significantly affected by the type and rate of the amendments (except for DTPA-Cr). The DTPA-extractable of Cr, Pb, and Ni in soils was considerably affected by irrigation source; however, for DTPA-extractable of Cu and Cd the influence was not significant. The interaction between the treatments did not have a clear trend (Table 3); however the different types of amendmend interactions, amendment rates, and irrigation sources (A×B×C) were significant in relation to the concentration of DTPA-extractable of all studied HMs in treated post-harvest soils. Table 4 shows the effect of the amendments application on DTPA-extractable of HMS in treated soils following plant harvest.

Table 4. DTPA–extractable concentration of HMS (mg/kg) in post-harvest soils

HMs	Amendments			
	CFA	**B300**	**B600**	**Control**
Cu	86.51^{B}	64.40^{C}	63.50^{C}	93.94^{A}
Cd	55.79^{C}	57.58^{C}	63.13^{B}	71.35^{A}
Ni	20.6^{B}	17.53^{C}	18.31^{C}	42.35^{A}
Pb	224.98^{B}	221.23^{B}	192.48^{C}	246.65^{A}
Cr	68.65^{A}	48.42^{C}	61.82^{B}	70.40^{A}

Different letters in columns indicate a significant difference at the level of 5% based on the Duncan test. The values represent the means (n = 18).

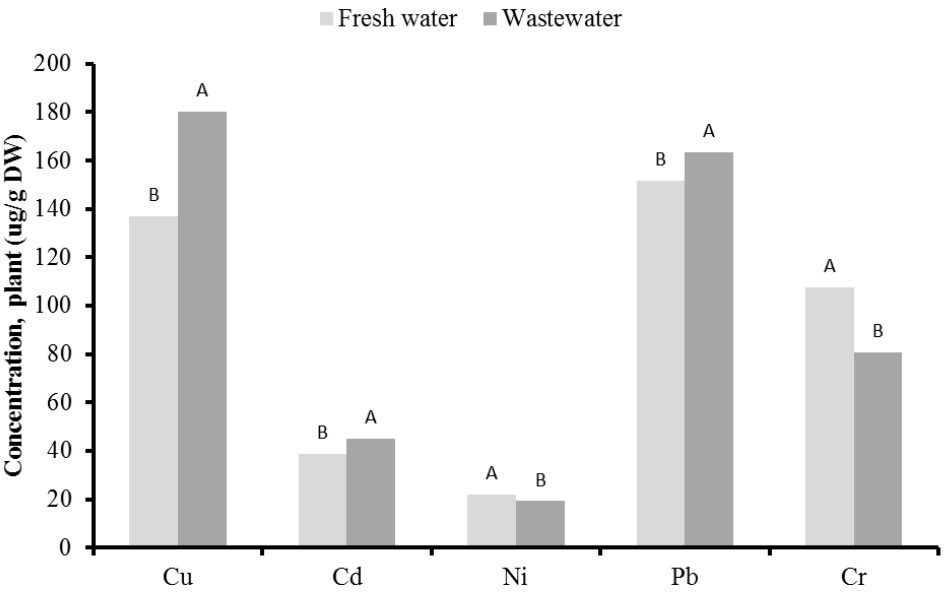

Fig. 8. Effect of irrigation sources on the HMs concentration by the corn shoots (ug/g DW). Different letters (in each separate group of bars) indicate significant differences among the means of different treatments (p<0.05)

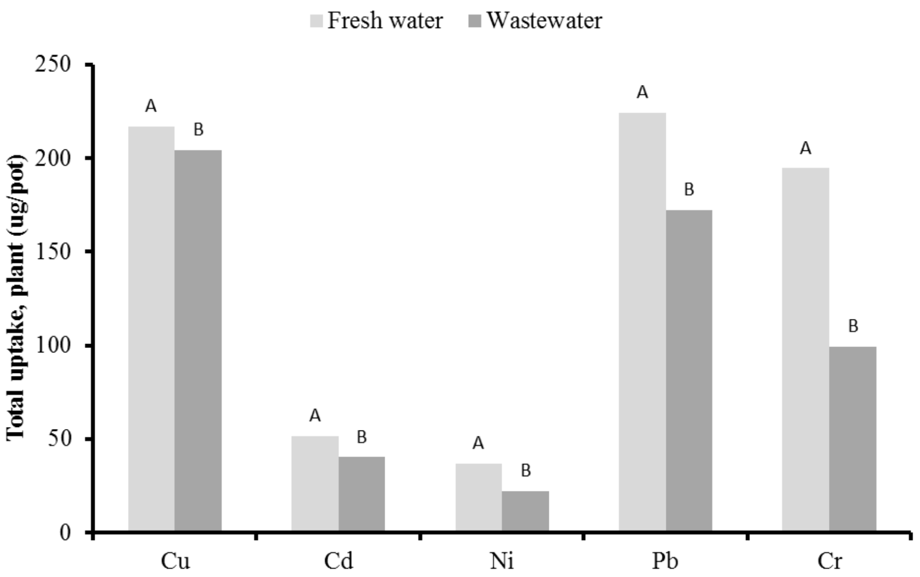

Fig. 9. Effect of irrigation sources on the HMs uptake by the corn shoots (ug/pot). Different letters (in each separate group of bars) indicate significant differences among the means of different treatments ($p<0.05$)

Table 5. Effect of amendment rates and irrigation sources on DTPA–extractable concentration of HMS (mg/kg) in post-harvest soils

Amendments rates	Cu	Cd	Ni	Pb	Cr
2%	79.99^{A}	63.09^{A}	25.57^{A}	225.48^{A}	62.35^{A}
5%	74.22^{B}	60.83^{B}	23.81^{B}	217.17^{B}	62.3^{A}
Irrigation sources	Cu	Cd	Ni	Pb	Cr
Freshwater	77.94^{A}	59.70^{B}	24.88^{A}	207.10^{B}	81.76^{A}
wastewater	76.22^{A}	64.22^{A}	24.50^{A}	235.56^{A}	42.88^{B}

Different letters in columns indicate a significant difference at the level of 5% based on the Duncan test. Values are means (n = 18).

The amount of DTPA-extractable metals was significantly decreased by the addition of amendments, with the exception of Cr in CFA-amended soils. Increasing soil pH (8.1) followed by application of CFA led to the transformation of Cr(III) to Cr(VI), ultimately increasing Cr mobility. The most significant reduction in DTPA-extractable of Cu and Pb was observed in the soil sample with B600 amendment. In contrast, application of CFA, B300, and B300 had the highest impact on reduction of DTPA-extractable of Cd, Ni, and Cr, respectively. Compared to the control, application of B600, B300, and CFA reduced the availability of Cu by 32.4%, 31.4%, and 7.9%; the availability of Ni by 56.76%, 58.6%, and 51.35%; the availability of Cd by 11.52%, 19.29%, and 21.8%; the availability of Pb by 21.96%, 10.3%, and 8.78%; and the availability of Cr by 12.18%, 31.22%, and 2.48%, respectively. Table 5 presents the effects of amendment rates and irrigation sources on DTPA-extractable of HMS.

DTPA-extractable of HMS plummeted through increasing application rates of the amendments, with the exception of Cr (Table 5). However, means of DTPA-extractable of Cd and Pb soared with application of WW, compared to FW treatment. On the other hand, WW application significantly reduced DTPA-extractable of Cr, compared to FW treatment, while addition of WW had no significant impact on DTPA-extractable of Cr and Cu. In our previous study (Saffari et al., 2016), effect of selected amendments on Pb stabilization was investigated, with its results showing that CFA and B600 were superior to B300 for stabilizing Pb in desorption experiment. It seems that the interaction among HMs led to the fact that CFA (similar to B600) could not considerably affect Pb stabilization. Additionally, in another previous study of us (Saffari et al., 2015), based on the effect of some amendments on Ni stabilization in a Ni-spiked soil, the results showed that B600 was an ineffective amendment to immobilize Ni; however, application of B300 and CFA in soil samples significantly decreased Ni desorption rate.

Figure 10 illustrates the relative distribution of HMs in untreated and amended soils. Among the chemical forms of HMs, both EX and Car fractions have the highest mobility potential. Thus, amount of EX and Car forms could be used to evaluate the effect of the amendments on HMs immobilization in this study. According to the results, chemical forms of HMs were influenced by the addition of amendments; however, the effects varied with kinds of HMs. As can be seen, Cd in the non-amended soil existed in a more mobile form than the other HMs, with 45-55 % of total Cd existing in EX and Car fractions, whereas in the non-amended soil, Ni, Cu, Pb, and Cr were mainly in FeA-OX, Mn-OX, FeA-OX, and Res fractions, respectively. Distribution of chemical forms of Cd in non-amended soil followed the order: Car> Res> Mn-OX> FeA-OX > EX> Om. Table 6 shows the amendments efficiencies on each chemical forms of HMs in post-harvest soils. As expected, addition of B600 significantly reduced EX and Car fractions, while increasing FeA-OX and Om forms of Cd. As for Mn-OX and Res of Cd, they were not affected by B600 application at all. On the other hand, application of B300 shifted Cd from Res form to the Om, Mn-OX, and FeA-OX fractions. CFA application decreased EX form but had no effect on other fractions of Cd. Cu fractions in control soil declined in the following order: Mn-OX> Car>Res> FeA-OX > FeC-OX >EX >OM. Application of both biochars (B300 and B600) increased Res and decreased EX and Car forms of Cu. In contrast, application of CFA increased and decreased EX and Car fraction of Cu, respectively. Zhang et al. (2011) investigated the effect of alkaline fly ash on heavy metal speciation in stabilized sewage sludge. Their results showed that the application of CFA to sewage sludge increased the percentage of EX form of Cu and Zn, but decreased the Res phases of Cu and Zn.

Table 6. Summary of amendment efficiencies on chemical forms of soil HMs after corn harvest, compared to control

HMs	Amendment	EX	Car	Om	Mn-OX	FeA-OX	FeC-OX	Res
Cu	CFA	+	-	*	*	-	-	+
	B300	-	-	+	*	*	*	+
	B600	-	-	+	-	+	*	+
Cd	CFA	-	*	*	+	*	Nd	*
	B300	*	-	+	+	+	Nd	-
	B600	-	-	+	*	+	Nd	*
Ni	CFA	-	-	-	*	*	*	*
	B300	+	-	+	*	-	*	+
	B600	-	-	+	*	*	+	*
Pb	CFA	-	+	-	-	-	*	*
	B300	-	+	-	-	-	+	+
	B600	-	+	*	-	-	+	+
Cr	CFA	+	+	-	*	+	*	*
	B300	-	-	-	+	*	*	+
	B600	*	-	-	+	*	-	+

Chemical forms of each HMs are (+) increased, (-) decreased, (*) not affected by selected amendments.
Nd: not detected

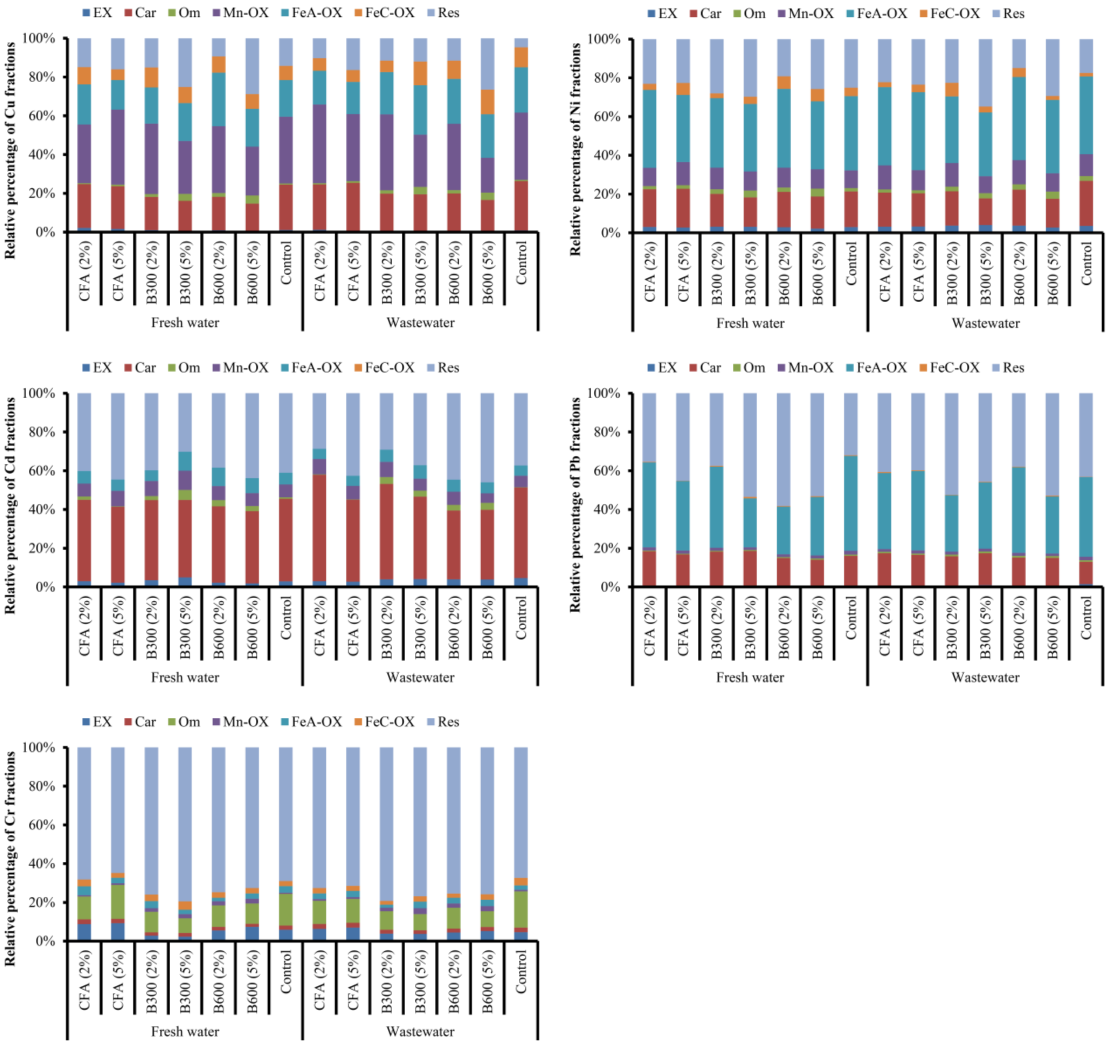

Fig. 10. Effect of amendments, amendments rates, and irrigation sources on relative distribution of chemical forms of Cu, Ni, Cd, Pb, and Cr in post-harvest soils

The amount of chemical forms of Ni in control soil was in the following order: FeA-OX> Res> Car> Mn-OX> FeC-OX> EX> Om. EX and Car fractions of Ni was decreased through application of B600 and CFA; however, addition of B300 increased EX and Res forms. Ehsan et al. (2013) investigated the impact of biochar (at the rates of 0.5%, 1%, and 2% W/W), derived from unfertilized dates (at 900°C) on the immobilization of Cd and Ni in an artificially-polluted alkaline soil (with 10 mg/kg Cd and 100 mg/kg Ni). They found that following incubation, the water-soluble Ni and NH_4NO_3-extractable of soil Cd and Ni contents were significantly lower in all biochar treatments than the control. Mean amount of Cr in untreated soils was as the following: Res> Om> EX> FeA-OX > FeC-OX> Car> Mn-OX.

Among the amendments, CFA had the highest impact on Cr mobility. CFA raised EX and Car forms. Herath et al. (2015) experimented the effect of different biochar rates of *Gliricidia sepium* (at 900 °C) on immobilization and phytotoxicity reduction of heavy metals in serpentine soil. Their results showed that application of 5% of the biochar significantly reduced the concentration of EX form in Cr, Ni, and Mn by 99%, 61%, and 42%, respectively. Mean proportions of Pb in individual fractions followed the order: FeA-OX> Res> Car> Mn-OX> Om> EX > FeC-OX. Application of three amendments shifted Pb distribution from EX to Car form. Park et al. (2011) applied biochar (from chicken manure and green waste) to reduce the bioavailability and phytotoxicity of heavy metals and found that addition of biochar substantially modified the partitioning of Cd, Cu, and Pb from the easily exchangeable phase to less bioavailable organic-bound fraction. Figure 11 shows the amendments' impact, amendment rates, and irrigation sources on MF (%) of HMs in post-harvest soils. According to the results, the lowest MF of Cu was obtained via application of B600 (5%) in the soil, irrigated with FW. In contrast, the highest MF (25.7%) of Cu was observed in the control soil, irrigated with WW. In general, irrigation with WW led to an increase in MF of Cu, compared to FW. Saffari and Saffari (2013) showed that the MF of Cu was increased in a calcareous soil smaple, treated with WW. They explained that the acidity of WW dissolved large proportions of soil calcium carbonate, increasing the EX form of Cu.

Jiang et al (2012) studied the effects of rice straw biochar on chemical fractions of Cu(II), Pb(II), and Cd(II) in an Ultisol soil. They showed that application of biochar decreased and increased acid soluble and reducible fractions, respectively. The obtained results from MF of Ni showed that irrigation with WW in control soil had the highest MF of Cu (25/8%) among all soil samples. The lowest MF of Ni was observed in application of B600 under WW treatment. Different results obtained from application of WW on MF of Ni did not lead to any conclusion about negative effects of this treatment. The MF of Cd in amended soils showed that B600-treated soils and FW had the lowest MF among all soil samples. The highest MF (54.52%) of Cd belonged to CFA and WW treatment. Totally, WW-treated soil had higher MF of Cd than the one, treated with FW. Application of B300 and FW had the highest effect on MF of Pb, resulting in the highest MF (18.61%). The lowest MF of Pb belonged to the control soil samples, treated with WW. The increase in MF of Pb in irrigated soils with WW was higher than FW. Based on its properties, CFA application raised the MF of Cr greater than other treatments.

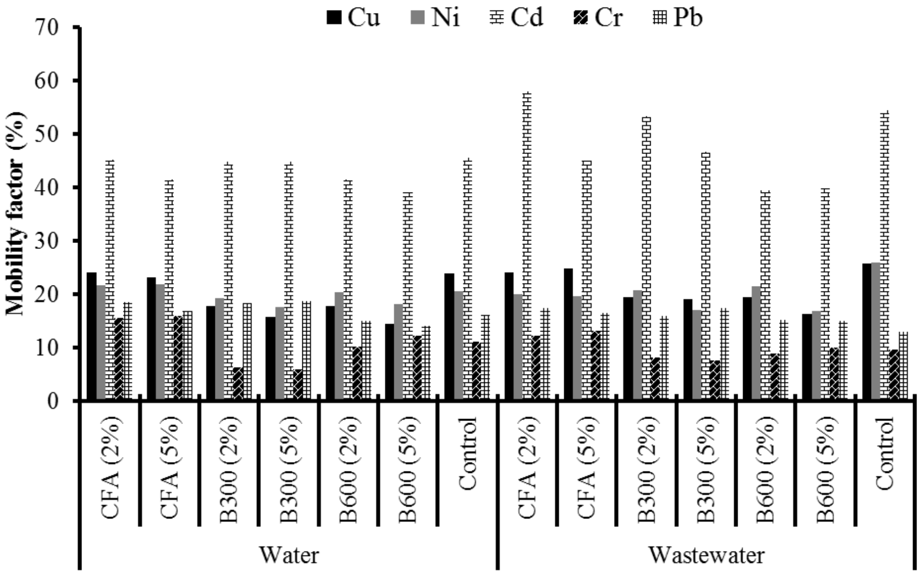

Fig. 11. Effect of amendments, amendments rates, and irrigation sources on HMs mobility factor (%) in post-harvest soils

The lowest MF of Cr was observed in treated soils with B300 and FW. Irrigation with WW decreased MF of Cr in all amended soils, higher than FW. Generally, results from amendment effects on HMs mobility showed that B600 had the highest positive effect on reduction of MF of HMs, with the exception of Cr. Also, WW application considerably increased MF of Cu and Cd.

CONCLUSION

The present study evaluated two kinds of biochar (prepared at 300°C and 600°C) and coal fly ash in order to determine their ability to decrease phytoavailability of some HMs in a multi-element contaminated soil. The application of amendments in HM-spiked soil altered phytoavailability and mobility of HMs, based on characteristics of the amendments as well as the type of HMs. Addition of B600 had the highest effect on DMY, compared to other soil amendments, thanks to the increasing specific surface area, CEC, and pH of soils. It promoted HMs sorption through surface complexation and ion exchange mechanisms. Depending on the type of HMs, applications of amendments to soil was potential to reduce HMs concentration in corn shoot (except for CFA-treatment, owing to dilution effect). In post-harvest soil samples, application of the amendments, especially at the highest application rate (5%), significantly decreased HMs concentration (with the exception of Cr in CFA-amended soils), compared to control. Evaluation of chemical forms of HMs in post-harvest soil samples indicated that addition of amendments significantly reduced mobility factor of HMs (with the exception of Cr in CFA-amended soils). In general, application of three soil amendments to this polluted soil had a considerable effect on reduction of HMs availability; however, among the studied amendments, B600 and CFA had the maximum and minimum impact on reduction of HMs availability, respectively.

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