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EFFECT OF RICE RESIDUE BIOCHAR ON BIOAVAILABILITY OF CADMIUM TO INDIAN MUSTARD UNDER COMBINED LEAD AND CADMIUM SPIKING IN A LOAMY SAND SOIL

Patil Kalyani, R Sikka* and M S Mavi

Department of Soil Science, Punjab Agricultural University, Ludhiana – 141004, Punjab

ABSTRACT

A screen house experiment was conducted to study the effect of rice residue biochar (0, 0.5, 1 and 2 % w/w) on the bioavailability and accumulation of Cd by Indian mustard (*Brassica juncea*) in a loamy sand soil spiked with three levels each of Cd (0, 10 & 25 mg Kg^{-1} soil) and Pb (0, 25 & 50 mg Kg^{-1} soil) in all possible combinations. Immobilization of Cd by rice residue biochar was assessed by DTPA-extractable Cd in soil and Cd concentrations in shoots of Indian mustard. Mean DTPA-Cd after harvest of crop decreased with increasing rates of applied rice residue biochar. Addition of biochar at its highest level of 2% decreased the mean DTPA-Cd in soil and its concentrations in plant by 59% and 36% over control, respectively. Biochar addition increased dry matter yield (DMY) of Indian mustard and a maximum increase of 18% over control was observed at a level of 1%. However, DMY decreased by 19% with addition of biochar at 2%. It was concluded that rice residue biochar can be used as an amendment for amelioration of Cd contaminated sites as its concentration in plants decreased with increasing levels of biochar application.

Keywords: Cadmium, Dry matter yield, Lead, Indian mustard, Rice residue biochar

Continuous addition of mineral fertilizers to the agricultural soils has been reported to lead to a buildup of Pb, Cd, Co, and Ni in soils (Abdelhafez *et al.,* 2012). Similarly, organic fertilizers in terms of animal manures and sewage sludge enrich the soil with heavy metals such as Mn, Zn, Cu, Co, Cr, Pb, Ni, and Cd (Verkleji, 1993). Plants grown in soils containing high levels of Cd show visible symptoms of chlorosis and growth inhibition, interference with the uptake and transport of minerals and water by plants leading to browning of root tips and finally death of the plant (Das *et al*., 1997; Toppi *et al*., 1999; Mohanpuria *et al*., 2007). However, combined effects of heavy metals have been reported to differ significantly from the effects of individual pollutants due to interactions between heavy metals (MacFarlane and Murchett 2002). According to Madwiya *et al*. (2004), the interaction between Pb and Cd means that metals can impact one another's absorption and translocation, as well as the uptake and translocation of other critical nutrients in plants.

Several remediation technologies are available for treating heavy metal contaminated soils. However, each technology has some advantages and disadvantages (Alaboudi *et al*., 2019). Common soil amendments used for metal stabilization are lime, fly ash, zeolite, phosphate amendment, cement, and rice husk ash, biochar (Houben *et al*., 2013; Ciccu *et al*., 2003; Cao *et al*., 2008; Khan *et al*., 2009; Beesley *et al*., 2015). In recent years biochar has been used as in situ soil amendment, and it was found to be effective in reducing the mobility of heavy metals in soils (Houben *et al*., 2013; Song *et al*., 2014).

Biochar potentially decreased heavy metals' mobility and bioavailability in soil (Jiang *et al*., 2012; Abdelhafez *et al*., 2014; Lu *et al*., 2014). The reason for this had been accredited to their high porous structure, high pH, more active functional groups, organic matter and CEC (Beesley *et al*., 2011; Park *et al*., 2011). The properties of biochar are dependent upon the type of feedstock and the production procedure. Biochar produced from rice material has distinctive chemical properties and higher yield due to assimilation of silica in it (Jindo *et al*., 2014). As a result, the production of rice straw-derived (RSD) biochar appeared to be a potential strategy for discussing the issue of reduced soil nutrients and C storage, as well as increased Green House Gas (GHG) emissions, that are linked with rice straw application from rice fields (Watanabe *et al.,* 2007).

The *Brassicaceae* family has received much attention due to its fast growth, elevated fully-harvestable biomass production, and high energy potential while being tolerant to high metal concentrations (Houben *et al*., 2013). Mustard green (*Brassica juncea*) is used in phytoremediation to remove heavy metals. In particular, *Brassica juncea* is an effective plant for removing Cd from soil (Schneider *et al*., 1999) and found to accumulate Pb as much as10,000 mg Pb kg^{-1} shoot dry matter (Blaylock *et al*., 1997). Due to

*Corresponding author : sikkar@pau.edu
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the fact that *Brassica juncea* is considered a metal hyperaccumulator plant especially for Cd (Jagtap *et al*., 2013). Objective of the study was planned to find effect of rice residue biochar on the bioavailability of cadmium to Indian mustard under combined lead and cadmium spiking in a loamy sand soil.

MATERIALS AND METHODS

Screen house experiment

For the pot experiment, a loamy sand soil (Typic Ustipsamments) was collected from the research farm of the Department of Soil Science, PAU, Ludhiana, Punjab and its physio-chemical properties are given in Table 1. The soil was dried and sieved through stainless steel sieve of 2 mm. Five kg of sieved soil was filled in polythene lined plastic pots. A stock solution of cadmium nitrate ($Cd(NO_3)_2 .4H_2O$) and lead nitrate ($Pb(NO_3)_2$) were used to contaminate the soil with three levels each of Cd (0, 10, 25 mg Cd kg^{-1} soil) and Pb (0, 25, 50 mg Pb kg^{-1} soil) in different combinations.The artificially Cd+Pb spiked soils were further amended with four levels of rice residue biochar (0, 0.5, 1 and 2% w/w). Fertilizers nitrogen (100 kg ha^{-1}) phosphorus (30 kg P_2O_5 ha^{-1}) was applied through urea and potassium dihurdogen orthophodphate, respectively. Each treatment was replicated thrice. The pots were arranged in CRD (completely randomized design) and were equilibrated for 30 days by alternate wetting and drying. The soil samples were collected from each pot after the equilibration period for analysis of DTPA- Cd. Ten seeds of Indian mustard (*Brassica juncea* (L.) Czern. cv PBR- 91) were sown in each pot. After a week, seedlings were thinned to five plants per pot and the uprooted plants were mixed in the same pots. The crop was raised by followiing recommeneded package of practices for rabi crops and harvested at 60 days. The harvested shoot samples were thoroughly washed with tap water followed by dilute HCl and finally with distilled water and oven-dried at 70 ^{0}C to constant weight, weighed and ground in a willey mill and stored in paper bags for chemical analysis.

Rice residue derived biochar used in this study was generated from feedstock collected after rice crop harvest in Punjab, India. Biochar was produced from the feedstock by using an intermediate pyrolysis technique. Pyrolysis, thermochemical conversion of organic materials in an oxygen depleted atmosphere that separates ash rich biochar from vapor fraction. Rice residues were pelletized into pellets of 8 mm diameter and 10-12 mm length before pyrolysis. The temperature of pyrolysis was fixed at 450-500 °C with a residence time of 5 min. The resultant biochar yields were around 35 percent (dry weight). Before the addition of biochar, the pellets were powdered, homogenized and passed through a 2 mm sieve. The properties of biochar are presented in a Table 2.

Table 1. Physio-chemical properties of soil used for screen house experiment

Soil parameter	Contents
pH (1:2)	7.13
EC (dS m^{-1})	0.50
Organic Carbon (%)	0.14
Available P (kg ha^{-1})	6.96
Available K (kg ha^{-1})	158.48
DTPA-extractable (mg kg^{-1})	
Pb	0.30
Cd	0.04
Fe	6.82
Cu	1.02
Zn	2.60
Mn	5.60
Mechanical Composition %)	
Sand	85.60
Silt	2.41
Clay	11.90
Texture	Loamy sand

Chemical analysis

The pH and EC of the biochar were measured in 1:5 suspension (biochar: water). The CHNS elemental analyzer was used to determine total C and N. Inductively Coupled Plasma- Atomic Emission Spectrophotometer (ICP) was used to examine the macronutrients (P, K, Ca, Mg) and micronutrient (Fe, Mn, Cu and Zn) content of biochar.

Soil pH, EC and available N, P & K were analysed by using standard procedures (Page *et al* 1984). The DTPA- extractable micronutrients, Pb and Cd were determined by the method given by Lindsey and Norvell, (1978).

The total concentration of Cd in the shoots of Indian mustard was analyzed by digesting 0.5 g of ground plant material with 10 ml distilled nitric acid and the digest was diluted to 50 ml. The concentration of Cd in DTPA extractants and plant digests was determined by using ICP- AES.

Statistical analysis

Statistical Analysis of the data was done using analysis of variance (ANOVA) in a factorial completely randomized design (CRD) with the help of CPCS1 (1.0) software.

Table 2. Properties of rice residue biochar

Property	Contents
pH	8.92
EC (dS m^{-1})	10.36
Total C (g kg^{-1})	432.00
Total macronutrients (g kg^{-1})	
N	9.00
P	4.89
K	43.90
Ca	3.92
Mg	2.01
Total micronutrients (mg kg^{-1})	
Fe	3814.0
Cu	115.0
Mn	540.8
Zn	2224.2

RESULTS AND DISCUSSION

Metal immobilization

The mean DTPA-Cd increased from 0.22 mg kg^{-1} soil in control to 3.67 and 11.80 mg kg^{-1} soil with the spiking of 10 and 25 mg Cd/kg soil, respectively (Table 3). There was an increase in DTPA-Cd when it was combined @ 10 and 25 mg kg^{-1} soil with combined with 25 and 50 mg Pb kg^{-1} soil. DTPA-Cd was 3.67, 3.69 and 4.44 mg kg^{-1} soil when it was spiked @ 10 mg kg^{-1} soil with 0, 25 and 50 mg Pb kg^{-1} soil, respectively. The corresponding value of DTPA-Cd when spiked @ 25 mg kg^{-1} soil were observed to be 11.80, 13.34 and 13.75 mg kg^{-1} soil, respectively (Table 3). The increase in DTPA-Cd was significant at each successive levels of Cd spiking in the presence of Pb spiking. Application of biochar had a significant effect on curtailing the DTPA-Cd. The mean DTPA-Cd decreased significantly from 7.03 mg kg^{-1} in control to 6.19, 5.14, 4.42 mg kg^{-1} soil at 0.5, 1 & 2 percent addition of biochar, respectively. The decrease at all levels of biochar was statistically significant. The interaction between combined Cd-Pb spiking and biochar addition was found to be significant.

Upon studying the preferential order of heavy metal adsorption in soil, Elliott *et al.* (1986) observed that nearly all soils had a relatively stronger preference for Pb, while weaker for Cd, indicating that when present together, Pb will be preferentially adsorbed and fixed occupying the adsorption sites, as a result of which, more Cd will be present in available form. Lead is always inferior to Cd in competition and the availability of Pb decreases in presence of Cd (Chen *et al*., 2007).

Reduction in DTPA extractable Cd on the addition of biochar might be attributed to adsorption of Cd on the surface of biochar (Beesley *et al*., 2011), precipitation, ion exchange & complexation (Park *et al*., 2011; Ahmad *et al*., 2014; Al-Wabel *et al*., 2014) and complex formation with O – containing functional groups present in biochar (Paz-Ferreiro *et al*., 2014). Awad *et al.* (2021) reported that biochar application reduced Cd by 17 per cent and it was attributed to greater surface area of biochar that enhanced formation of insoluble complexes of Cd. Ding *et al.* (2016) found that co-existence of Pb and Cd exhibited competitive adsorption on water hyacinth biochar and Pb was more adsorbed than Cd which may be due to precipitation and cation exchange complex bond. Khalid (2018) reported a reduction in TCLP extracted Cd at the highest biochar application which accounted for the adsorption on organic fractions and oxides present on biochar surface.

Other than high pH, EC and CEC, rice straw biochar have; high surface alkalinity, Si-content (Lu *et al*., 2014) and O-containing functional groups (Ahmad *et al.,* 2014) which make it more effective in reducing heavy metals from soil.

Dry matter yield (DMY)

The DMY of Indian mustard decreased sequentially significantly with increased Pb and Cd spiking (Table 4). The highest mean DMY of Indian mustard was observed in control (12.37 g pot^{-1}) which decreased to 11.31 and 10.58 g pot^{-1} with application of 25 and 50 mg Pb Kg^{-1} soil, respectively. The mean DMY further decreased to 10.72, 9.81 and 8.94 g pot^{-1} when soil was spiked with Pb at 0, 25, and 50 mg kg^{-1} soil along with 10 mg Cd kg^{-1}, respectively. The DMY further decreased to 7.40, 5.97 and 4.96 at spiking of 25 mg Cd kg^{-1} soil with 0, 25 and 50 mg Pb kg^{-1} soil, respectively. The reduction at $Cd_{25}Pb_{50}$ (4.96 g pot^{-1}) was 59.92 per cent, over control. However, addition of biochar resulted in an increase in DMY compared to control. Addition of up to 1 per cent biochar increased mean DMY but had a depressing effect at 2 per cent addition. The mean DMY of Indian mustard grown in soil increased from 8.71 g pot^{-1} in control to 9.84 and 10.61 g pot^{-1} at 0.5 and 1 percent addition of biochar, respectively. Increase in DMY of Indian mustard at 1 per cent biochar addition was mainly due to decreased toxicity of Pb and Cd and increased nutrient release from biochar. However, biochar addition at 2 per cent itself might have acted as surface sink for nutrient adsorption and decreased DMY yield of Indian mustard. Interaction effects however, remained non-significant.

Wang *et al.* (2016) and Sinha *et al.* (2006) reported that the increased Cd+Pb accumulation caused alteration in the uptake of nutrients, overall impact on photosynthetic activities, disturbed ionic homeostasis and alteration in energy metabolism. Hampering of metabolic processes of plants would negatively affect

Table 3. Effect of rice residue biochar addition on DTPA-Cd in equilibrated soil

Cd (mg kg^{-1})	Pb (mg kg^{-1})	DTPA-Cd (mg kg^{-1})				
		Biochar levels (% w/w)				
		0	0.5	1	2	Mean
0	0	0.34	0.27	0.21	0.07	0.22
0	25	0.32	0.26	0.17	0.07	0.21
0	50	0.28	0.18	0.10	0.06	0.16
10	0	4.66	4.05	3.41	2.57	3.67
10	25	4.78	4.06	3.72	2.21	3.69
10	50	5.01	4.96	4.15	3.63	4.44
25	0	15.41	12.68	9.81	9.29	11.80
25	25	16.10	14.32	12.15	10.78	13.34
25	50	16.38	14.95	12.56	11.09	13.75
Mean		7.03	6.19	5.14	4.42	
CD (5 %)		Biochar levels (A) = 0.30 Cd+Pb levels (B) = 0.45 AB=0.91				

growth and development of plants which in turn may result into reduced biomass. Awad *et al.* (2020) reported that garden waste biochar had increased dry root and shoot biomass of *Brassica juncea* due to enrichment of nutrients and more negative sites for metal binding. This impact of biochar on *Brassica juncea* was supported by

Khan *et al.* (2015), who also reported a significant increase in biomass of *Brassica rapa* L. by 44 per cent at an application of 2 per cent rice straw biochar. Houben *et al.* (2013) also reported that with addition of biochar, biomass of rapeseed (*Brassica napus*) increased significantly which was attributed not only to the alleviation of metal toxicity but also to the enhancement of soil fertility. According to Jun *et al.* (2020) excessive biochar addition not only increased water holding capacity but also reduced soil aeration, thereby decreased the root metabolism in sunflower.

Cadmium concentration in shoots of Indian mustard

The Cd content in shoot of Indian mustard increased significantly with increasing levels of Cd spiking (Table 5). The mean Cd concentration in the shoot of Indian mustard increased significantly from 0.84 mg kg^{-1} dry matter (DM) in control to 11.40 and 24.56 mg kg^{-1} DM at 10 and 25 mg kg^{-1} soil, respectively. The concentration of shoot Cd at $Cd_{10}Pb_0$, $Cd_{10}Pb_{25}$, $Cd_{10}Pb_{50}$ was 11.40, 11.52, 12.08 mg kg^{-1} DM respectively. The concentration of shoot Cd at $Cd_{25}Pb_0$, $Cd_{25}Pb_{25}$, $Cd_{25}Pb_{50}$ was 24.56, 26.35, 26.93 mg kg^{-1} DM respectively. The increase in

Table 4. Effect of rice residue biochar on dry matter yield of Indian mustard in Cd and Pb spiked soil

Cd (mg kg^{-1})	Pb (mg kg^{-1})	Dry matter yield (g pot^{-1})				
		Biochar levels (% w/w)				
		0	0.5	1	2	Mean
0	0	12.30	12.89	13.05	11.25	12.37
0	25	10.45	11.47	12.37	10.96	11.31
0	50	9.99	11.27	11.79	9.28	10.58
10	0	10.90	11.24	11.60	9.14	10.72
10	25	9.64	10.94	11.59	7.05	9.81
10	50	8.43	9.64	10.85	6.85	8.94
25	0	5.87	9.49	9.75	4.52	7.40
25	25	5.72	5.95	8.03	4.20	5.97
25	50	5.06	5.72	6.50	2.55	4.96
Mean		8.71	9.84	10.61	7.31	
CD (5%)		Biochar levels (A) = 0.79 Cd+Pb levels (B) = 1.19 AB= NS				

Table 5. **Effect of rice residue biochar on cadmium concentration in shoots of Indian mustard in Cd and Pb spiked soil**

Cd (mg kg^{-1})	Pb (mg kg^{-1})	Cd (mg kg^{-1})				
		Biochar levels (% w/w)				
		0	0.5	1	2	Mean
0	0	1.20	1.02	0.76	0.37	0.84
0	25	1.25	1.10	0.81	0.43	0.90
0	50	1.26	0.88	0.82	0.74	0.92
10	0	14.46	12.02	9.64	9.46	11.40
10	25	13.62	12.43	10.33	9.69	11.52
10	50	14.73	13.09	10.26	10.23	12.08
25	0	30.88	28.30	22.83	16.23	24.56
25	25	31.00	29.73	24.04	20.61	26.35
25	50	31.19	29.56	25.15	21.83	26.93
Mean		15.51	14.24	11.63	9.95	
CD (5 %)		Biochar levels (A) = 0.88		Cd+Pb levels (B) = 1.32	AB= 2.65	

Cd concentration with successive levels of Pb spiking in the presence of Cd spiking was significant. The addition of biochar had a significant effect on curtailing Cd concentration in shoot of Indian mustard. The mean Cd concentration decreased significantly from 15.51 mg kg^{-1} DM in control to 14.24, 11.63, 9.95 mg kg^{-1} DM at 0.5, 1, 2 percent addition of biochar, respectively. Per cent decrease of Cd kg^{-1} DM was 8.94, 33.40 and 55.80 at 0.5, 1, 2 per cent biochar addition over to control. The interaction between combined Cd-Pb spiking and biochar addition was found to be significant.

Motesharezadeh *et al.* (2012) observed that using Pb and Cd together caused maximum Cd accumulation in roots and shoots of sunflower. Liu *et al.* (2008) found that increased Pb concentration accelerated Cd accumulation in the plant while Cd could hamper Pb accumulation in shoots *of I. Balsamina*. Similar results were earlier reported by Kumar *et al.* (1995) who observed that the increased availability of Cd in soil solution in presence of Pb led to its greater absorption by plants while the competition between Cd^{2+} and Pb^{2+} ions in soil may have reduced Pb uptake when both were present together. The reduction of Cd concentration in shoots of Indian mustard on the addition of biochar might be attributed to (i) increased pH, the addition of phosphates and silicates leading to precipitation and co-precipitation of metals (Ahmad *et al*., 2014) (ii) adsorption of Pb on the surface of biochar (Elliott *et al*., 1986; Namgay *et al*., 2010; Paz-Ferreiro *et al*., 2014) and (iii) high Si concentration in rice residue biochar helped to increase immobilization of Pb and Cd in soil (Wu *et al.,* 2013; Yang *et al.,* 2015). Houben *et al.* (2013) reported that the addition of Miscanthus biochar at 5 and 10 per cent decreased Pb and Cd concentration in *Brassica napus* which was were attributed to both the immobilization of plant available metals and 'dilution effect' due to increased plant biomass. However, Majeed *et al.* (2021) observed that biochar addition reduced the Cd concentration in maize leaves by 30 per cent due to increased surface adsorption and its precipitation formed due to biochar application.

Post-harvest soil DTPA- Cd

DTPA- Cd in soil analyzed after harvest decreased as compared to its concentration in equilibrated soil (Table 6). DTPA-Cd at $Cd_{10}Pb_0$, $Cd_{10}Pb_{25}$, $Cd_{10}Pb_{50}$ was 3.67, 3.69 and 4.44 mg kg^{-1} soil of equilibrated soil (Table 3) which decreased to 1.48, 1.67, 1.83 mg kg^{-1} soil after crop harvest respectively (Table 6). The concentration of DTPA-Cd at $Cd_{25}Pb_0$, $Cd_{25}Pb_{25}$, $Cd_{25}Pb_{50}$ were 11.80, 13.34 and 13.75 mg kg^{-1} soil in equilibrated soil and it decreased to 7.39, 8.36, 10.32 mg kg^{-1} soil after harvest, respectively. The addition of rice residue biochar significantly decreased the mean DTPA-Cd. The mean DTPA-Cd was 3.79, 3.23, 2.33 mg kg^{-1} soil at harvest of the crop under 0.5, 1 and 2 per cent addition of biochar levels respectively.

Despite a decrease in postharvest DTPA-extractable Cd, there was a considerable residual availability of applied Cd.

Sikka and Nayyar (2012) also reported that after harvesting Indian mustard, the amount of DTPA-Cd in the soil was lower than that in equilibrated soil. The beneficial effects of biochar aging could explain the increase in Cd immobilization with time after biochar addition. The favorable effect of biochar addition was due to increase in the creation of surface acidic functional groups and oxygen concentration (Sui *et*

Table 6. Effect of rice residue biochar on DTPA-extractable Cd after harvest of Indian mustard in Cd and Pb spiked soil

Cd (mg kg^{-1})	Pb (mg kg^{-1})	DTPA-Cd (mg kg^{-1})				
		Biochar levels (% w/w)				
		0	0.5	1	2	Mean
0	0	0.10	0.08	0.06	0.03	0.07
0	25	0.08	0.08	0.06	0.03	0.06
0	50	0.06	0.04	0.03	0.02	0.04
10	0	2.43	1.94	1.17	0.38	1.48
10	25	2.67	2.03	1.54	0.43	1.67
10	50	2.72	2.15	1.89	0.57	1.83
25	0	9.42	8.25	6.58	5.32	7.39
25	25	10.53	8.37	7.89	6.64	8.36
25	50	12.63	11.22	9.86	7.57	10.32
Mean		4.52	3.79	3.23	2.33	
CD (5 %)		Biochar levels (A) =0.13 Cd+Pb levels (B) = 0.19 AB= 0.39				

al., 2017; Chen and Chen 2009) which helped in Cd immobilization.

Based on the results of the present study, it may be concluded that rice residue biochar immobilized Cd in soil and curtailed its absorption by plants, making it a potential remediation material for polluted soil. Further studies are also required to determine the addition rate and frequency for the long-term aging process of biochar and the fate of sequestered contaminants under field conditions.

Authors' contribution

Conceptualization of research work and designing of experiments (RS, MSM); Execution of field/lab experiments and data collection (KP); Data analysis and interpretation of results (KP, RS); Preparation of manuscript (KP, RS)

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