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BIOAVAILABILITY OF Cd AND Pb TO INDIAN MUSTARD (*Brassica juncea* (L.) CZERN.) AS INFLUENCED BY BIOCHAR

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ABSTRACT

Contamination of soils with cadmium (Cd) and lead (Pb) poses serious threat to the environment as well as human health via their entry in the food web. Exploration of the viable remedial measures has become inevitable to prevent their entry into the food chain. Thus, the present screen house study was undertaken to evaluate the effect of rice residue biochar on bioavailability to Indian mustard (*Brassica juncea* (L.) Czern) in a loamy sand soil spiked with four levels of Cd and Pb varying from 0 to 40 mg kg^{-1} and 0 to 200 mg kg^{-1} soil, respectively. The soil of the metal treated pots was further amended with four rates (0, 1%, 2% and 4%, w/w) of rice residue biochar. Crop was grown for 60 days with recommended doses of nitrogen and phosphorus. Addition of biochar up to 2% increased the above ground biomass of Indian mustard but decreased with application @ 4% as compared to unamended soil. Application of biochar significantly reduced the accumulation of Cd and Pb by the crop with increasing rates of its application. The ameliorative efficiency of biochar for Pb was more as compared to Cd. It was concluded that rice residue biochar has the potential to curtail the solubility, availability and translocation of Pb and Cd to Indian mustard.

Keywords: Bioavailability, Biochar, Contaminated soil, Heavy metals, Indian mustard

In recent decades, anthropogenic and natural activities have resulted in elevated levels of heavy metals in the atmosphere as well in the soils (Nriagu and Pacyna, 1988; Bi et al., 2006; Cloquet et al., 2006). Unlike organic contaminants, the persistence time of heavy metals in soils is longer as they do not undergo degradation (Jiang et al., 2012) and pose threat to the environment as well to the human health (Lu et al., 2014). Cadmium (Cd) is one of the most dominant toxic metal for living organisms as it may prove to be carcinogenic to humans even at low concentrations (Khan et al., 2015). Higher concentration of Cd adversely affects the uptake, transport and use of nutrients and water by plants (Das et al., 1997) and causes various ailments to animals and humans (Li et al., 2016). Next to Cd, Lead (Pb) adversely affects the soil-plant-animal-human continuum (Majer et al., 2002). All Pb containing compounds have been reported to be cumulative poisons for gastrointestinal tract and nervous system. (Oliver, 1997).

Conventional practices employed for the remediation of the sites contaminated with heavy metals include excavation and landfilling which are environmentally disruptive and economically unviable on large scale (Houben et al., 2013). Therefore, concerns about mobility and bioavailability of heavy metals have been raised because of food safety, potential health risks and their detrimental effects on the ecosystem (Uchimiyaet al., 2010). The bioavailability of toxic metals can be minimized through chemical and biological immobilization of metal(loid)s using a range of organic compounds (biosolids, straw, compost and biochar) and inorganic compounds (lime and phosphate compounds) (Chai et al., 2012). Recently, biochar has been used to bind and/or precipitate soil contaminants (heavy metals) thus lowering their mobility and bioavailability (Méndez et al., 2012; Kim et al., 2015). Biochar is a rich source of carbon produced by heating biomass or feedstock in a closed chamber at high temperature ranging from 350ºC to 1000ºC in a low oxygen thermal process referred to as pyrolysis (Liu et al., 2011). The metal binding characteristics of biochar is due to the presence of active functional groups, microporous structure, high pH, large surface area and high CEC (Jiang et al., 2012). Biochar could enhance plant growth by immobilizing toxic metals, supplying nutrients and also by improving soil physical and biological environment (Lehmann et al., 2006; Taghizadeh-Toosi *et al* 2012). Therefore, biochar could serve as an efficient alternative amendment (Arthur et al., 2005) for phytostabilization of toxic metals at contaminated sites.

Presently, disposal of rice residues is a major global issue as it constitutes about 25 per cent of total agricultural residues i.e. 0.9 Gt per year (Knoblauch et al., 2011) which is mainly subjected to on-farm burning (Pathak et al., 2010). This on farm burning of rice residues results not only to loss of carbon and plant nutrients but also pollutes the environment by release of

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greenhouse gases, smoke and suspended particulate matter. Alternatively, rice residues could be used for biochar synthesis, gasification, power generation and paper production (Roy and Kaur, 2015). Biochar derived from rice residue has been reported to have higher surface alkalinity, Si content, (Lu et al., 2014) and O-containing functional groups (Ahmad et al., 2014) and thus may be used as an amendment for contaminated sites and may prove as a viable practice for agricultural residues worldwide. Hence, it is important to evaluate rice residue biochar for reducing the risk of Cd and Pb in soil by using both a biological measure such as plant uptake and a physicochemical measure such as selective soil extraction. The present investigation was undertaken to study the effect of biochar addition on (1) immobilization and bioavailability of Cd and Pb and (2) dry matter yield of Indian mustard in relation to addition of biochar.

MATERIALS AND METHODS

Characteristics of soil and biochar

The loamy sand surface soil (0-15 cm) (Typic Usticpssamments) was collected from an uncontaminated site. It was spiked @ 0, 10, 20 & 40 mg Cd kg^{-1} soil using $Cd(NO_3)_2$ and 0, 50, 100 & 200 mg Pb kg^{-1} soil by using $Pb(NO_3)_2$. Sub portions of the soil were further amended (w/w) with four levels of rice residue biochar @ 0, 1, 2 & 4%. The chemical properties of the soil and biochar used in this experiment are given in Table 1 & 2. Biochar used in the study was formed from rice residues by using intermediate pyrolysis technique (Yang et al., 2019). Pyrolysis is a process of thermochemical conversion of organic materials in an anaerobic atmosphere separating the ash rich biochar from the vapor fraction. Prior to pyrolysis, rice residue was pelletized into pellets of size 8 mm diameter and 10 to 12 mm length. The pyrolysis temperature was fixed at 450-500°C with the residence time of 5 minutes. The resultant biochar yield was around 35% (dry weight). Before addition of biochar, the pellets were homogenized, powdered and passed through 2 mm sieve.

Pot experiment

Plastic pots were filled with 10 kg of Cd and Pb-spiked soil and amended (w/w) with four levels of rice residue biochar viz. 0, 1, 2 & 4%. Each treatment was replicated thrice and the pots were arranged in completely randomized factorial design. The amended soils were equilibrated for 1 month by alternate wetting and drying. Afterequilibrium, representative soil samples were drawn from each pot with the help of stainless-steel tube auger for the determination of DTPA-Cd and Pb. Eight seeds of Indian mustard (cultivar RLM619) were sown in each pot and the soil in the pots was fertilized with basal dose of N and P @ 45 mg N and 13 mg P_2O_5 kg^{-1} through analytical grade urea and potassium dihydrogen orthophosphate, respectively. The seedlings were thinned to 5 per pot after 7 days of germination. The pots were irrigated withdistilled water as and when required. Plants were grown for a period of 60 days and harvested by cutting the plant shoots one cm above the soil surface. Plant samples were washed sequentially with tap water, 0.1 % hydrochloric acid (HCl) and distilled water. The harvested plant material was oven dried at 65 ± 2°C to constant weight and dry matter yield (DMY) wasrecorded. Ground plant material (0.5 g) was digested with 10 ml of diacid mixture of nitric and perchloric acid (3:1) to colorless residues and final volume was made to 50 ml and the contents were filtered through Whatman No. 42 filter paper for further chemical analysis. Post-experimental soil samples were drawn with stainless steel tube auger from top to bottom of the pots, thus ensuring representative samples.

Chemical analysis

The pH of biochar was determined using biochar: water suspension in 1:5 (w/v) after occasionally stirring for 30 minutes. The electrical conductivity (EC) of biochar was measured after suspending biochar in water (1:5 w/v) overnight. Total C and N were determined using CHNS elemental analyzer (model Varion EL III). The nutrients P, K, Ca, Mg, Fe, Mn, Cu and Zn in biochar were determined on ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrophotometer) by digesting 0.5 g biochar with distilled nitric acid. Soil pH and EC, soil organic carbon available N, P and K were determined by the following standard methods of analysis (Jackson 1967; Walkley and Black 1934; Subbiah and Asija., 1956; Olsen et al., 1954; Merwin and Peech respectively). DTPA-extractable Cd and Pb were determined on AAS (Atomic Absorption Spectrophotometer) (Lindsay and Norvel, 1978).

Plant samples were analysed on ICP-AES for total Cd and Pb content.

TEM and FTIR analyses of biochar

The morphological variability of biochar particles was determined by Transmission Electron Microscope (Hitachi H-7650) in high resolution imaging mode at 80 kV acceleration voltage. Fourier Transform Infrared Spectroscopy (FTIR) of biochar was used to identify and characterize the development and alterations in functional group chemistry of biochar (Kleber et al., 2009).

Statistical analysis

The data was statistically analysed by using analysis of variance (ANOVA) in factorial completely randomized design (CRD). The significant difference between mean values was evaluated at 5 per cent ($P<0.05$) level of probability using LSD values in SPSS (version 21) statistical software.

RESULTS AND DISCUSSION

Characterization of biochar

The pH and EC of rice residue biochar were 8.91 and 11.02 dS m^{-1}, respectively, which were 1.2 and 33.4 times higher than that of the soil, respectively. The C content was 432 g kg^{-1} of biochar representing mainly the recalcitrant fractions of carbon (Lehmann et al., 2006). Total concentration of all the nutrients was higher in biochar as compared to the soil used in the study. The absorption peaks indicated the stretching and bending vibrations of specific functional groups (Fig.1). The peaks near 3300 to 3900 cm^{-1}, 3100 to 2900 cm^{-1} and 2500 to 2300 cm^{-1} were attributed to the hydroxyl (-OH) stretching aliphatic C–H ring stretching and the presence of CO_2 (O=C=O) respectively. The hydroxy (−OH) and aromatic C=O rings contributed to the alkalinity of biochar resulting in higher pH (Yuan et al., 2011). The peaks between 1500 to 1250 cm^{-1} represented stretching peaks for the aromatic C-H, C=C, C=O and C–O rings thereby indicating the aromatic nature of the group (Ozcimen and Ersoy-Mericboyu, 2010). The rice residue biochar had particles with greater morphological variability. The TEM micrographs of biochar represented variable morphologies ranging from spherical, roughly cylindrical and hexagonal particles. The average size of the particles was observed to be 60 nm with a of 50 to 550 nm. The particles in TEM micrographs appeared to be embedded in the scattered mass of melted silica carbon composite (Fig. 2).

Effect of biochar on Cd and Pb immobilization

There was a consistent and significant increase in the levels of DTPA- extractable Cd and Pb in soil with increasing levels of Cd and Pb spiking (Table 3). The DTPA- Cd increased from 0.22 in control to 4.20, 7.66 and 12.16 mg kg^{-1} soil with spiking @ 10, 20 and 40 mg kg^{-1} soil, respectively and correspondingly DTPA-Pb increased from 0.32 in control to 16.54, 25.14 and 41.47 mg kg^{-1} soil with spiking @ 50, 100 and 200 mg Pb kg^{-1} soil, respectively. However, addition of biochar significantly curtailed the bioavailability of DTPA- extractable Cd and Pb. Mean DTPA-Cd and Pb decreased by 12.9 and 15.2 per cent, respectively at 1 per cent addition of biochar whereas the corresponding decrease at 2 per cent addition of biochar was 30.4 and 27.6 per cent, respectively. The magnitude of decrease, however was much higher at 4 per cent addition of biochar registering a decline of 44.9 and 47.1 per cent for Cd and Pb, respectively. The reduction in the extraction of Cd and Pb soil amended with biochar might be due to formation of organo-metallic complexes and their adsorption on O-containing functional groups (Jiang et al. 2012). High pH, ash content, Si content, surface alkalinity and O-containing functional groups of rice residue biochar have been reported to be effective in reducing Cd and Pb mobility (Ahmad et al., 2014; Lu et al., 2014). Houben et al. (2013) also reported that addition of biochar at 5 per cent decreased the bioavailable fraction of Cd and Pb by 44 and 76 per cent, respectively. Méndez et al. (2012) observed a decrease in DTPA- and $CaCl_2$- extractable Cd, Pb and Zn on addition of sewage sludge derived biochar in a Mediterranean agricultural soil. The immobilizing capacity of biochar through precipitation of metals as their respective hydroxide, phosphates and carbonates (Cao et al., 2011) which reduced the mobility of metals. Lu et al. (2014) reported that the solubility of Cd and Pb decreased significantly with the increasing rates of biochar addition and this might be due to water soluble organic carbon present in biochar. Water soluble organic carbon, is considered as the most mobile fraction of organic ligands and it might enhance the formation of stable heavy metal-organic complexes (Cao et al. 2003). The immobilization process might also include complexation of heavy metals with functional groups of biochar either by physical adsorption or metal exchange with biochar alkali and alkaline earth cations (Lu et al., 2014). Beesley and Marmiroli (2011) concluded sorption as more important mechanism than liming effect of biochar for the immobilization of heavy metals in soil. The O-containing functional groups (–COOH and –OH) present on biochar (Park et al., 2011; Yuan and Xu, 2011) form stable complexes with Pb (II) and increase specific adsorption of metals on the surface of biochar (Uchimiya et al., 2010).

Plant growth and metal accumulation

A significant and successive decrease in DMY of Indian mustard was observed with the increasing rate of Cd and Pb spiking (Table 4). Mean DMY decreased significantly by 18.7, 35.6 and 61.6 percent with application of 10, 20 and 40 mg Cd kg^{-1} soil, and by 28.5, 45.9 and 69.7 percent with application of 50, 100 and 200 mg Pb kg^{-1} soil, respectively over control. Mean DMY increased by 11.3 and 12.5 per cent with 1 and 2 percent addition of biochar respectively in Cd spiked soils and the corresponding increase for Pb spiked soils was 18.8 and 19.6 percent. However, at 4 per cent addition of biochar the mean DMY decreased by 17.5 and 12.6 per cent as compared to control in Cd and Pb spiked soils, respectively. The increase in DMY at initial levels of biochar addition might be attributed to enhanced nutrient supply as evident from Table 4 and reduced accumulation of metal through immobilization.

It might be postulated that higher addition of biochar causes an imbalance of nutrients in the soil thereby affecting the growth of plants. Hossain et al. (2010) also observed an increase in DMY of cherry tomato by 64 per cent on addition of sludge derived biochar. Fox et al. (2014) also reported increase in yield of *Lolium* at 1 and 2 per cent addition of *Miscanthus* biochar. Lehmann et al. (2006) also observed an increase in nutrient availability and improved soil properties which might have enhanced the DMY of plants on addition of biochar. Prapagdee et al. (2014) reported increased availability of plant nutrients on application of biochar, but excessive application reduced the supply of nutrients and growth of plants because of high soil alkalinity.

Cd and Pb content of Indian mustard

Significant ($p<0.05$) main and interactive effects of biochar and Cd & Pb addition on their translocation in Indian mustard were observed (Table 5). The total mean Cd & Pb concentration of Indian mustard irrespective of the amendment increased markedly and significantly with increasing rates of their application. The mean Cd concentration increased from 0.81 in control to 12.59, 23.75 and 50.88 mg kg^{-1} shoot dry matter at 10, 20 and 40 mg Cd kg^{-1} soil whereas Pb content increased from 1.68 in control to 14.91, 22.94 and 33.25 mg kg^{-1} shoot dry matter at 50, 100 and 200 mg Pb kg^{-1} soil, respectively. The highest level of Cd (40 mg Cd kg^{-1} soil) and Pb (200 mg Pb kg^{-1} soil) spiking the mean Cd and Pb content in shoot of Indian mustard increased by 63 and 19 times, respectively as compared to control. The increase in metal content in plants was due to an increase in the available Cd & Pb in soil (Table 2). However, addition of biochar significantly decreased Cd & Pb concentration in Indian mustard and the magnitude of this decrease was higher with the increasing rates of the biochar application. Mean Cd concentration decreased by 13.8, 35.6 and 50.1 per cent as compared to control with the addition of biochar @ 1, 2 and 4 percent, respectively. The corresponding decrease for Pb was 22.7, 39.9 and 59.8 per cent, respectively. The higher percent reduction for Pb concentration indicated that rice residue biochar was more effective in curtailing its bioavailability to Indian mustard as compared to Cd.

The reduction in content of Cd and Pb in shoots of Indian mustard on biochar addition might be attributed to immobilization of metals in soil (Table 5). The magnitude of Cd and Pb reduction increased with the increase in levels of biochar addition. The biochar used in the experiment was alkaline in reaction and have O-containing functional groups which might have adsorbed the metals and curtailed their availability (Park et al. 2011; Houben et al 2013). Kim et al. (2015) reported reduction in Cd and Pb accumulation in lettuce with biochar addition and was attributed to the increased soil pH that induced concomitant decrease in bioavailable forms of metals. Khan et al. (2016) also reported decreased Cd concentration in plant shoots resulting from addition of biochar which was attributed to both the immobilization of plant available metals and 'dilution effect' due to increase plant biomass. In the present study also the reduced concentration of Cd and Pb in shoots of Indian mustard on addition of biochar could be attributed to precipitation of metals with phosphates and silicates ions and adsorption of metals on the surface of biochar.

Significant ($p<0.05$) main and interactive effects of biochar and applied metals (Cd & Pb) on their extractability in post-harvest soil were observed (Table 5). The mean DTPA-Cd decreased from 4.83 in control to 4.02, 2.66 and 2.33 mg kg^{-1} soil whereas the mean DTPA-Pb decreased from 18.93 in control to 15.38, 9.70 and 6.68 mg kg^{-1} soil, respectively at 1, 2 and 4 percent addition of biochar (Table 6). Compared to the equilibrated soil the mean DTPA-Cd decreasedby 40.6, 50.8 and 45.6 per cent at 1, 2 and 4 per cent addition of biochar whereas decrease in case of DTPA-Pb was 32.6, 50.2 and 53.1 per cent, respectively. The decrease in post-harvest Cd and Pb content of soil might be attributed partly to the immobilization of metals through transformations to relatively more stable forms and partly by plant removal (Sikka et al. 2010).

In conclusion, rice residue biochar has a potential to significantly reduce the solubility and availability of Cd and Pb in soil and thereby reducing their translocation in Indian mustard. However, the ameliorative efficiency of biochar was more for Pb as compared to Cd. As the effectiveness of biochar was found to be rate dependent, therefore, the biochar rates must be carefully monitored for higher desired efficiency under the soil and climatic condition of any contaminated site.

Authors' contribution

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

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