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Impact of Biochar and Lime on Phytoavailability of Pb and Cd in a Contaminated Soil

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ABSTRACT

Current study was conducted to evaluate the efficiency of water hyacinth (*Echhornia crassipes*) biochar and lime to remediate cadmium (Cd) and lead (Pb) contaminated soils. A pot experiment was carried out treating soil with Cd and Pb (both separately and in combination) at the rates of 3, 6, 9 mg/kg and 250, 500, 750 mg/kg, respectively. Biochar and lime were applied separately to the metal spiked soils at the rate of 5.54 g/kg and 2.77 g/kg, respectively. After growing metal accumulator plant *Eleusine indica* for five months, metal contents were examined in plant biomass (composite sample prepared mixing equal amount of both root and shoot). Water hyacinth biochar had higher efficiency (up to 72%) to reduce phytoavailability of Pb compared to Cd for *E. indica* when applied in metal spiked soil. However, lime application restricted the availability of Cd to *E. indica* up to 38.8% compared to control. Documented greater microbial biomass carbon (MBC) and chlorophyll content under application of water hyacinth biochar than lime indicates its higher efficacy for growth of plants and microbes in Pb and Cd contaminated soil due to supply of nutrient in the soil. Therefore, the water hyacinth biochar and lime have potential to reduce bioavailability of Pb and Cd in contaminated soil.

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INTRODUCTION

Heavy metals are noxious to the environment and possess pronounced threats to human and other organisms, create huge financial issues like removal costs and restoration problems (Chapman *et al.*, 2013). Heavy metals do not go through chemical and microbial degradation due to their persistent nature and their total concentrations usually do not change (Adriano *et al.*, 2004). Soil amendments are applied for remediation of heavy metal contaminated soil. The soil amendments can aid in heavy metal remediation either through (i) metal immobilization that is reducing the bioavailability to plants, animals and humans or (ii) metal mobilization that is through increasing bioavailability to plants enhancing phytoremediation or phytoextraction. The aspect of immobilization is related to the capability of the soil amendments to absorb, precipitate or formation of stable complex, causing in situ remediation of contaminated soil. Whereas the other aspect is increased bioavailability or mobilization of metal, where the phytoremediation plays a crucial role to eliminate metals from the polluted soils (Mahajan and Kaushal, 2018).

Grass species for example *Saccharum bengalense* can be used for both phytostabilization and phytoextraction purposes depending on the metal present in soil (Mishra *et al.*, 2017). *Eleusine indica*, a small annual weed found worldwide was documented to accumulate heavy metal Pb and Cd (Garba *et al.*, 2012; Hamzah, *et al.*, 2017). In the present study *Eleusine indica* plant was employed to test phytoavailability of cadmium (Cd) and lead (Pb) under application of water hyacinth biochar and lime.

Biochar is a carbon rich by-product, produce from pyrolysis of biomass in limited oxygen condition (Lahori *et al.*, 2017). Currently, biochar is earning great emphasis due to its positive influence on soil properties and reducing heavy metal hazards (Rawat *et al.*, 2019; Tate *et al.*, 2020). Biochar possess micro-pores, bigger surface area, active functional groups, which actively take part in heavy metal bioavailability and distribution in soil. Kim *et al.* (2015) documented efficiency of rice hull-derived biochar prepared at 500 °C pyrolysis temperature in absorption of heavy metal Cd, Pb, Cu and Zn from soil. In another study, Puga *et al.* (2015) reported reduction of available Cd, Pb and Zn in mine contaminated soil leading to lowered plant uptake of the metals causing reduction in phytotoxicity on application of sugarcane-straw derived biochar. Water hyacinth is a well-known baleful weed, produce huge biomass due to unstoppable colonization, which cannot be eliminated completely. This can be utilized for producing biochar and compost to improve soil nutrient and may help in remediation of metal contamination. Although, reports are available on water hyacinth biochar to adsorb Cd (Li *et al.*, 2016) and trivalent chromium (Hashem *et al.*, 2020) from wastewater, information are scarce on use of phytoaccumulator plant and water hyacinth biochar for immobilization of Cd and Pb in acidic sandy loam soil. Lime is a soil amendment basically applied to raise soil pH (Anderson *et al.*, 2013). pH is a crucial regulator for metal distribution in soil. By virtue of increase soil pH, lime can improve absorption of heavy metal ions to soil and promote formation of metal hydroxide or carbonate mineral precipitate leading to minimizing bioavailability of heavy metals in soil (Wang *et al.*, 2012; Xiao *et al.*, 2017). Lime can increase negative charge in variable charge soil, can form strongly bound metal hydroxyl species and can sequester metal due to enhanced microbial activity (Bolan *et al.*, 1999; Bolan *et al.*, 2003). Many experiments demonstrated the capability of lime to reduce heavy metal uptake by plants (Hong *et al.*, 2007; Kibria *et al.*, 2011; Xiao *et al.*, 2017). Under these contexts, the present study aimed to evaluate the effect of water hyacinth biochar and lime (calcium carbonate) on reducing phytoavailability of Cd and Pb in laboratory contaminated soil with single and mixture of two metals. The study hypothesised that the soil amendments will behave differently to the different metals and mixed metal contaminated soil.

METHODOLOGY

Preparation of experimental soil and amendments

The primary soil (0-15 cm depth) was collected from agricultural field of Khalihamari, Napaam Goan, Tezpur, Assam (26°42´ N and 92°50´ E). The soil was dried under natural sunlight and sieved to remove the debris. For preparation of biochar fresh biomass of water hyacinth was collected and air dried for few days. The biomass was pyrolyzed conventionally at 250 ± 10 °C temperature with a heating rate of ≈ 30 °C/min for 3 hour utilizing iron kiln, clay, and digital infrared thermometer (Meco IRT 550 P). Lime (calcium carbonate) was purchased from Napaam market. The basic characteristics of soil and the biochar, such as pH, water holding capacity, total carbon and nitrogen content, organic carbon content, available nitrogen, phosphorus and potassium content were analysed initially. pH was measured using pH meter (AN ISO 9001:2008, B.D. Instrumentation, India) in suspension of soil and water at ratio of 1:5 and biochar and water at a ratio of 1:10. Water holding capacity was determined following the method of Tripathi (2009). For estimating total nitrogen and carbon content CHNS-O analyser (Thermo Scientific, FLASH 2000) was utilized. Organic carbon content was analysed according to Walkey and Black (1934) with slight modification. Available nitrogen, phosphorous and potassium content were estimated following the methods given by Subbiah and Asija (1956), Bray and Kurtz (1945) and Jackson (1973) respectively. Metal concentrations in experimental soil and water hyacinth biochar were measured by digesting the samples in tri-acid mixture (HNO_3-HF-$HClO_4$) at a ratio of 5:1:1 (Shentu *et al.*, 2008). Digested samples were analysed in atomic absorption spectrometer (AAS-ICE 3500) to determine concentrations of the metals.

Pot experiment

The experiment was carried out for a period of five-month form December 2018 to April 2019 in the Department of Environmental Science, Tezpur University (26°69′ N and 92°82′ E). The pot experiment was conducted using lead (Pb) and cadmium (Cd) spiked soil. Sources of Pb and Cd were $Pb(NO_3)_2$ and $CdCl_2.H_2O$ respectively. To maintain uniformity, the soils were mixed well with the respective metals salt solution prior to filling the soil to the pots. Cadmium was added at the rate of 3, 6, 9 mg/kg (Cd_3, Cd_6, Cd_9) and lead at the rate of 250, 500, 750 mg/kg (Pb_{250}, Pb_{500}, Pb_{750}) with another one set each combining both the metals (Pb_{250} + Cd_3, Pb_{500} + Cd_6, Pb_{750} + Cd_9). The tested levels of Cd and Pb were on or above the world health organization (WHO) maximum permissible levels for the metals in soil (Chiroma *et al.*, 2014). Thoroughly sieved (≤ 2 mm) biochar and lime were mixed to the soil at the rate of 5.54 g/kg (10 t/ha) and 2.77 g/kg (5 t/ha) respectively. One set of pots were kept as experimental control without the soil amendments. A total of ninety pots were arranged in completely randomized design to accommodate three replications. Similar age group young seedlings of *Eleusine indica* were uprooted from the university campus and planted in prepared pots. Pots were kept under natural sunlight. Watering was done regularly throughout the growing period. Excess watering was avoided to restrict metal leaching from the pots.

Determination of pigment and metal content in plant and physico-chemical properties of soil

Plant pigment, the leaf total chlorophyll content was estimated during February 2019 on the third month of plantation following the method of Anderson and Bordman (1964). After growing the plants for five months on April 2019 the plants were uprooted carefully and metal content in the plant tissue (composite sample prepared combining equal weight of both root and shoot) was estimated. Composite sample of 100 mg dried weight was digested in 10 ml of di-acid mixture of HNO_3 and $HClO_4$ at a ratio of 9:4 (AOAC 1990). The digested samples were then analysed in an atomic absorption spectrometer (AAS-ICE 3500) to estimate metal content. pH of the rhizospheric soil samples were estimated using pH meter (AN ISO 9001: 2008, B.D. Instrumentation, India) in suspension of soil and water at ratio of 1: 5. Microbial biomass carbon was estimated following chloroform fumigation incubation method given by Vance *et al.* (1987).

Statistical analysis

Obtained data were analysed statistically using analysis of variance (ANOVA) method. Significant difference between the means were estimated following Duncan's multiple-range test (DMRT) at $p \leq 0.5$. Data were presented as means plus or minus standard deviation. All Statistical analyses were performed using SPSS (version 16.0; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Properties of experimental soil and biochar

The experimental soil was sandy loam in texture and slightly acidic in nature with a pH of 6.27 (Table 1). The soil had a water holding capacity of 42.5%. The nutrient status of the soil was moderate depicted by available nitrogen of 201.9 mg/kg, available phosphorus of 34.1 mg/kg and available potassium of 116.5 mg/kg (Sanchez *et al.*, 1997; Espinoza *et al.*, 2006). While the heavy metal content (Cd, Cu, and Pb) in the experimental soil was within the range of world health organization (WHO) permissible limit of metals in soils (Table 1). The tested water hyacinth biochar had an alkaline pH of 9.11 (Table 1). The biochar had a higher water holding capacity of 71%, thus may improve the water retention capacity of soil and reduce stress due to low water in soil (Wang *et al.*, 2014). The heavy metal Cd, Cu and Pb concentration of the biochar was observed lesser (Table 1), which lower the risk of soil contamination through the application of this biochar.

Change in soil pH

pH plays crucial role in regulating mobility of heavy metal in soil. Increased soil pH was documented on application of the amendments (Table 2). An increased pH of 0.8 and 1.9 units were recorded under water hyacinth biochar and lime application respectively. Similarly, Bian *et al.* (2016) also recorded decrease in soil acidity on application of wheat straw biochar at the rate of 20 and 40 t/ha in soil. Alkaline soil pH facilitates absorption and precipitation of metal in soil, thus, encourage metal immobilization in soil (Xiao *et al.*, 2017).

Table 1: Selected properties of experimental soil and water hyacinth biochar (n = 4)

Parameters	Value	Water hyacinth biochar	Reference/Method
Texture	Sandy loam	--	Piper, 1966
pH	6.27 ± 0.05	9.11 ± 0.13	pH meter
WHC (%)	42.51 ± 1.17	71.32 ± 2.56	Tripathi, 2009
Available N (mg/kg)	201.9 ± 2.13	44.62 ± 1.13	Subbiah and Asija, 1956
Available P (mg/kg)	34.1 ± 0.72	102.63 ± 1.57	Bray and Kurtz, 1945
Available K (mg/kg)	116.5 ± 1.27	341.56 ±2.14	Jackson, 1973
Total N (%)	0.36 ± 0.03	1.03 ± 0.04	CHNS-O analyser
Total C (%)	2.88 ± 0.06	32.41 ± 0.38	CHNS-O analyser
OC (%)	1.09 ± 0.01	21.11 ± 0.32	Walkley and Black, 1934
Cd (mg/kg)	ND	ND	Shentu *et al.*, 2008
Cu (mg/kg)	38.1 ± 0.89	33.6 ± 0.92	Shentu *et al.*, 2008
Pb (mg/kg)	77.1 ± 0.81	16.57 ± 0.80	Shentu *et al.*, 2008

Data are mean ± SD; WHC = Water Holding Capacity; OC = Organic Carbon; ND = Not Detectable at ppm level

Table 2: pH of soil after the experimental period

Treatments	Soil pH		
	Control	**WHBC**	**Lime**
M_0	6.36 ± 0.06a	7.28 ± 0.14ab	8.31 ± 0.16a
Pb_{250}	6.13 ± 0.07cd	7.14 ± 0.26b	7.74 ± 0.08c
Pb_{500}	6.06 ± 0.08de	7.50 ± 0.14a	8.15 ± 0.18ab
Pb_{750}	6.26 ± 0.14abc	7.19 ± 0.10b	7.53 ± 0.04d
Cd_3	6.31 ± 0.04ab	6.83 ± 0.16cd	8.34 ± 0.02a
Cd_6	6.26 ± 0.02abc	7.09 ± 0.08bc	8.33 ± 0.06a
Cd_9	6.22 ± 0.07abc	7.32 ± 0.18ab	8.06 ± 0.08b
Pb_{250} + Cd_3	6.20 ± 0.07bc	6.60 ± 0.30d	8.04 ± 0.14b
Pb_{500} + Cd_6	6.32 ± 0.08ab	6.70 ± 0.06d	8.31 ± 0.08a
Pb_{750} + Cd_9	5.97 ± 0.05e	6.78 ± 0.06d	8.30 ± 0.06a
LSD ($p \leq 0.05$)	0.061	0.136	0.084
Mean total	6.21 ± 0.20	7.04 ± 0.45	8.11 ± 0.31

Data are mean ± SD (n = 4); Treatments Pb is lead and Cd is cadmium. Subscript numbers 3, 6, 9, 250, 500, 750, are levels of applied metal concentration in mg/kg. Mean values followed by the same letter in a column are not significantly different at $P \leq 0.5$

Effect of amendments on phytoavailability and immobilization of metal

Metal uptake by plant increased with elevated metal concentration in the soil both under amended and non-amended soil condition (Figure 1). Application of both water hyacinth biochar and lime reduced the uptake of Pb and Cd by the plant. Maximum reduction in Pb uptake was noted from application of water hyacinth biochar in both single and mixed Pb treatments compared to control (Figure 1A). Water hyacinth biochar reduced phytoavailability of Pb up to 72.2% under single Pb treatments, while in mixed metal treatments it reduced up to 67.6%. However, application of lime reduced the phytoavailability of Pb up to 25.3% and 21%, respectively in single metal and mixed metal treated soils compared to control. Furthermore, highest reduction of Cd uptake was noted on application of water hyacinth biochar under single metal treatments followed by application of lime under mixed metal treatments and single metal treatments. Maximum reduction (40%) in plant Cd content was observed at treatment Cd_3 from application of water hyacinth biochar followed by treatment Pb_{750} + Cd_9 (38.8%) under application of lime. Water hyacinth biochar had more potentiality to reduce phytoavailability of Pb to *E. indica* compared to Cd. The efficiency of water hyacinth biochar to reduce phytoavailability of heavy metals may be due to biochar properties to induced hike in soil pH, surface sorption of metals in the pore spaces, precipitation or formation of organometallic compounds (Beesley *et al.*, 2011; Fellet *et al.*, 2014; Baruah *et al.*, 2020).

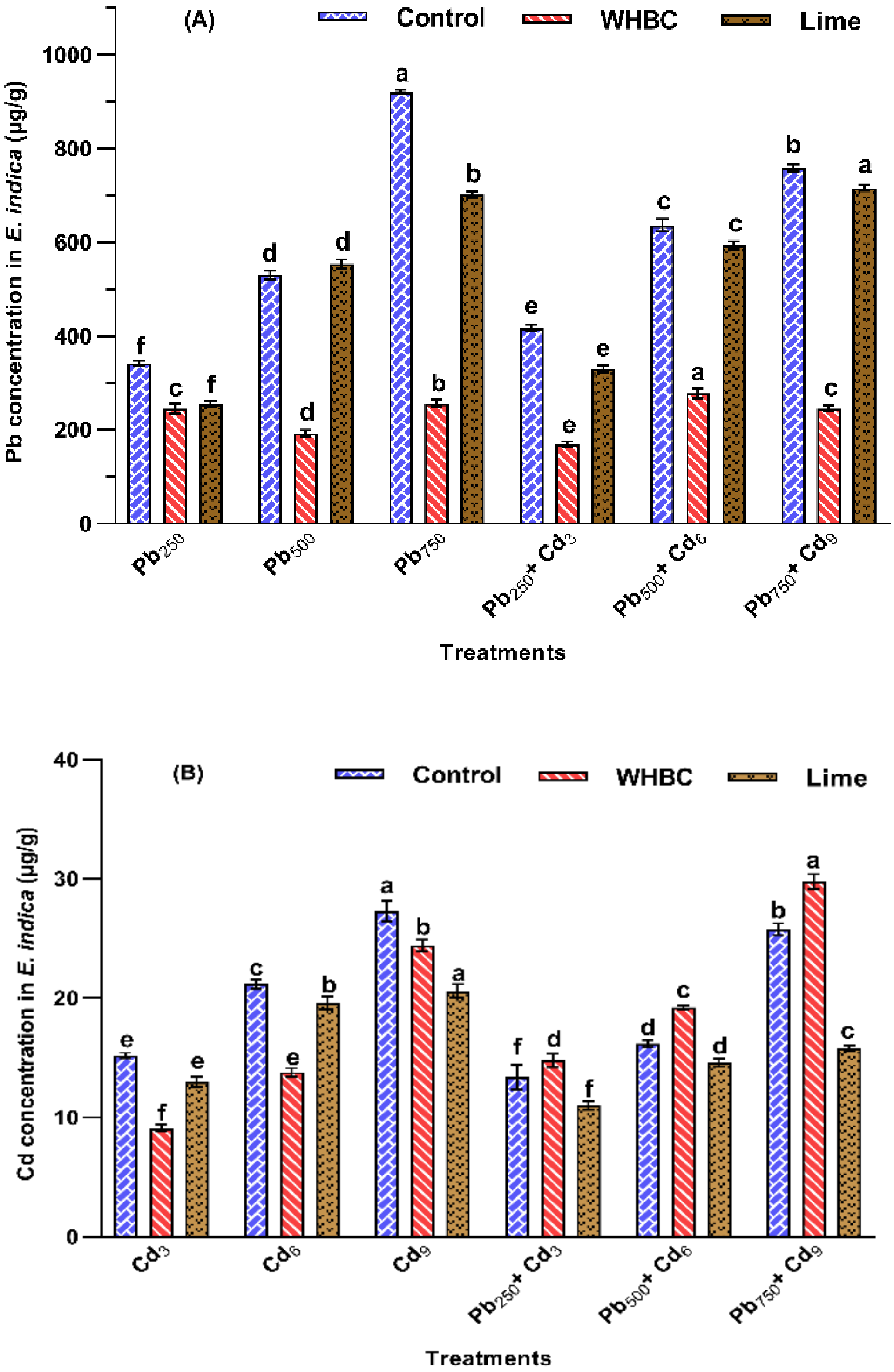

Figure 1. Lead (A) and cadmium (B) concentration in *E. indica* plant under water hyacinth biochar (WHBC) and Lime application in Pb and Cd spiked soil. Treatments Pb is lead and Cd is cadmium. Subscript numbers 3, 6, 9, 250, 500, 750, are levels of applied metal concentration in mg/kg. Data are means of three replicates. Mean values followed by the same letter for single amendment are not significantly different at $P \leq 0.05$

Similarly, mechanism involved in decreased metal mobility under lime application can be attributed to precipitation and adsorption of metal in soil because of changing soil pH. Soil pH has great role in metal immobilization. The diminution in metal mobility under higher soil pH obey the principle of Le Chatelier's. According to Le Chatelier's principle if a dynamic equilibrium is disturbed by changing the conditions, the equilibrium position moves to counteract the change. Thus, the processes that remove either protons or ligand ions from reaction site can increase dissociation processes like formation of metal-ligand bonds or absorption of ligand ions by soils and the equilibrium moves to other direction in the opposite cases (Elkhatib *et al.*, 2006). Earlier reports are also available on reduction in Cd and Pb uptake in rice under biochar amendment (Cui *et al.*, 2011; Hamid *et al.*, 2018). Similarly, Karalić *et al.* (2013) and Malinowska (2017) documented decreased mobility or availability of metals on lime application. Contrarily to the single metal treatments, addition of water hyacinth biochar could not reduce the Cd content in mixed metal treated plants (Figure 1B). The inefficiency of the water hyacinth biochar to immobilize Cd in mixed metal treatments may be associated with its structural and physicochemical properties such as biochar's specific surface area, pore volume and cation exchange capacity (Ahmad *et al.*, 2014; Touray *et al.*, 2014; Tomczyk *et al.*, 2020). Similarly, the type of interfering ions, type of biochar, rate of biochar application, type of soil, metal concentration in soil and soil pH has important role in effectiveness of metal removal (Bradl, 2004; Bogusz *et al.*, 2015; Tomczyk *et al.*, 2019).

Effect of amendments on soil microbial biomass carbon

Microbial growth was affected negatively due to presence of heavy metal in soil. A significant reduction in microbial biomass carbon was observed in metal spiked soils compared to control soil without external metal application (Figure 2). The highest reduction in soil microbial biomass carbon was observed at treatment Pb_{750} + Cd_9 (54.5%) followed by treatment Cd_9 (52.8%) and Cd_6 (43.5%). Earlier reports are also there on reduction of microbial soil biomass carbon in soil incubated with Pb and Cd treatment (Oijagbe *et al.*, 2019). This reduction in microbial biomass carbon could be due to effect of the heavy metals on microbial community structure caused by disruption of essential functions leading to death of cells or change in viability causing alteration in population size (Sethi and Gupta, 2014). Application of soil amendments increased the microbial biomass carbon compared to control. Microbial growth was higher on application of biochar compared to lime.

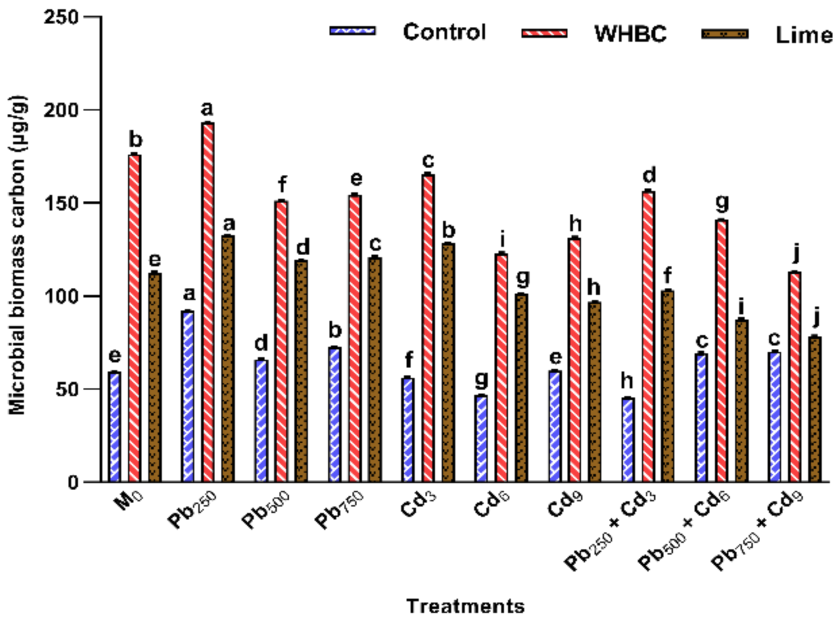

Figure 2. Microbial biomass carbon of the Pb and Cd contaminated soil after the experimental period. Treatments Pb is lead and Cd is cadmium. Subscript numbers 3, 6, 9, 250, 500, 750, are levels of applied metal concentration in mg/kg. Data are means of three replicates. Mean values followed by the same letter for single amendment are not significantly different at *P* ≤ 0.05

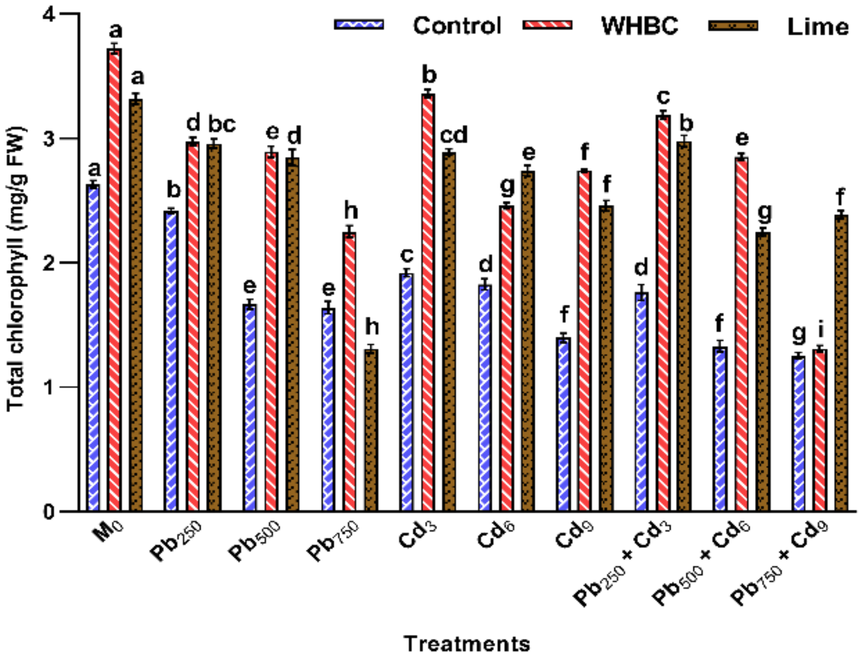

Figure 3. Total chlorophyll content of *E. indica* under Cd and Pb exposure during third month of plantation. Treatments Pb is lead and Cd is cadmium. Subscript numbers 3, 6, 9, 250, 500, 750, are levels of applied metal concentration in mg/kg. Data are means of three replicates. Mean values followed by the same letter for single amendment are not significantly different at $P \leq 0.05$.

Highest microbial biomass carbon (193.3 µg/g) was observed from application of water hyacinth biochar under Pb exposure at treatment Pb_{250} followed by Cd exposure at treatment Cd_3 (165.5 µg/g) and mixed metal exposure at treatment Pb_{250} + Cd_3 (156.5 µg/g) (Figure 2). The increased in microbial biomass carbon on application of water hyacinth biochar could be associated with supplement of nutrients by the biochar (Table 1) (Luo *et al.*, 2013; Irfan *et al.*, 2019). Xu *et al.* (2018) also reported potentiality of macadamia nutshell derived biochar to improve microbial biomass carbon in Cd and Pb spiked soil. While lime application increased microbial biomass carbon at treatment Pb_{250} (132.4 µg/g) followed by treatment Cd_3 (128.3 µg/g). Similarly, Filep and Szili-Kovács, (2010) documented increase in microbial biomass carbon on application of lime.

Effect of amendments on plant pigment

The plant pigment content, total chlorophyll was documented to reduce on exposure to the heavy metals (Figure 3). Maximum reduction in total chlorophyll content was noted on exposure to mixed metal treatments followed by single Cd and Pb treatments. Earlier study documented reduction of chlorophyll content in rice plant on exposure to 400, 800 and 1200 ppm of Pb (Ashraf *et al.*, 2017). Likewise, Hussain *et al.* (2013) recorded significant decline in photosynthetic pigment content in Maize seedling on exposure to 3, 6, 9 and 12 mg $CdCl_2$/kg sand. The soil amendments were effective in maintaining the pigment content of the plants (Figure 3). Total chlorophyll content was higher (55.6%) under application of water hyacinth biochar relative to lime (46.5%). Thus, both the soil amendments can be applied to increase tolerance ability of the test plant under heavy metal contaminated soil.

CONCLUSIONS

The present study demonstrates the efficiency of water hyacinth biochar and lime for reducing phytoavailability of Pb and Cd to *E. indica*. Water hyacinth biochar can reduce phytoavailability of Pb up to 72% for *E. indica* when applied in metal spiked soil. Similarly, lime application restricted the availability of Cd up to 38.8%. Moreover, the tested amendments improve plants health by maintaining photosynthetic pigment content. Thus, the amendments have potentiality to improve tolerance capacity of plants when grown in heavy metal contaminated soil. Likewise, the reduction in phytoavailability of Pb and Cd on application of water hyacinth biochar could be attributed to the biochar properties to induced hike in soil pH, sorption of metals on pore spaces, organometallic compound formation or precipitation. Lime decreased metal mobility possibly due to adsorption of metal in soil and precipitation caused by change in soil pH. However, the recorded efficiency of the amendments may reduce in naturally contaminated soil. Therefore, future study is necessary to evaluate the practical field utility of the biochar and lime. Moreover, further studies are required to find out the mechanism of the biochar and lime on heavy metal immobilization.

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