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

R. M. Boro, N. Baruah and N. Gogoi*

Plant Physiology and Biochemistry Laboratory, Department of Environmental Science, Tezpur University, Assam, India.

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.

*Corresponding author: nirmalievs@gmail.com
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 Walkley and Black (1934) with slight modification. Available nitrogen, phosphorus 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 (HNO₃-HF-HClO₄) 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(NO₃)₂ and CdCl₂.H₂O 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 (Cd₃, Cd₆, Cd₉) and lead at the rate of 250, 500, 750 mg/kg (Pb₂₅₀, Pb₅₀₀, Pb₇₅₀) with another one set each combining both the metals (Pb₂₅₀ + Cd₃, Pb₅₀₀ + Cd₆, Pb₇₅₀ + Cd₉). 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 HNO₃ and HClO₄ 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 ≤ 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).

| 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

| Treatments | Soil pH | Control | WHBC | Lime |
|------------|---------|---------|------|------|
| M₀         | 6.36 ± 0.06a | 7.28 ± 0.14ab | 8.31 ± 0.16a |
| Pb₂₅₀      | 6.13 ± 0.07cd | 7.14 ± 0.26b | 7.74 ± 0.08c |
| Pb₅₀₀      | 6.06 ± 0.08de | 7.50 ± 0.14a | 8.15 ± 0.18ab |
| Pb₇₅₀      | 6.26 ± 0.14abc | 7.19 ± 0.10b | 7.53 ± 0.04d |
| Cd₃        | 6.31 ± 0.04ab | 6.83 ± 0.16cd | 8.34 ± 0.02a |
| Cd₆        | 6.26 ± 0.02abc | 7.09 ± 0.08bc | 8.33 ± 0.06a |
| Cd₉        | 6.22 ± 0.07abc | 7.32 ± 0.18ab | 8.06 ± 0.08b |
| Pb₂₅₀ + Cd₃| 6.20 ± 0.07bc | 6.60 ± 0.30d | 8.04 ± 0.14b |
| Pb₅₀₀ + Cd₆| 6.32 ± 0.08ab | 6.70 ± 0.06d | 8.31 ± 0.08a |
| Pb₇₅₀ + Cd₉| 5.97 ± 0.05e | 6.78 ± 0.06d | 8.30 ± 0.06a |
| LSD (p ≤ 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 ≤ 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 Cd₃ from application of water hyacinth biochar followed by treatment Pb₇₅₀ + Cd₉ (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).

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**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 physiochemical 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 Pb750 + Cd9 (54.5%) followed by treatment Cd9 (52.8%) and Cd6 (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](image-url)
Highest microbial biomass carbon (193.3 μg/g) was observed from application of water hyacinth biochar under Pb exposure at treatment Pb\textsubscript{250} followed by Cd exposure at treatment Cd\textsubscript{3} (165.5 μg/g) and mixed metal exposure at treatment Pb\textsubscript{250} + Cd\textsubscript{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\textsubscript{250} (132.4 μg/g) followed by treatment Cd\textsubscript{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\textsubscript{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.

AKNOWLEDGEMENT

The authors are thankful to Sophisticated Analytical Instrument Centre (SAIC), Tezpur University, Assam, India for the instrumentation facilities.

REFERENCES

Adriano, D. C., Wenzel, W. W., Vangronsveld, J. and Bolan, N. S. (2004). Role of assisted natural remediation in environmental cleanup. Geoderma. 122, 121-142.

Ahmad, M., Rajapaksha, A.U., Lim, J.E., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S.S. and Ok, Y.S. (2014). Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere. 99, 19-33.

Anderson, J. M. and Boardman, N. K. (1964). Studies on the greening of dark-grown bean plants II. Development of photochemical activity. Australian Journal of Biological Sciences. 17, 93-101.

Anderson, N. P., Hart, J. M., Sullivan, D. M., Horneck, D. A., Pirelli, G. J. and Christensen, N. W. (2013). Applying lime to raise soil pH for crop production (Western Oregon). Oregon State University, USA. Available online at https://catalog.extension.oregonstate.edu/em9057

Ashraf, U., Kanu, A. S., Deng, Q., Mo, Z., Pan, S., Tian, H. and Tang, X. (2017). Lead (Pb) toxicity; physio-biochemical mechanisms, grain yield, quality, and Pb distribution proportions in scented rice. Frontiers in Plant Science. 8, 259.

Association of Official Analytical Chemists (1990). Official Methods of Analysis: Changes in Official Methods of Analysis Made at the Annual Meeting. Supplement (Vol. 15). Association of Official Analytical Chemists.

Baruah, N., Gogoi, N. and Farooq, M. (2020). Influence of biochar and organic soil amendments on bioavailability and immobilization of copper and lead to common cocklebur in acidic sandy loam soil. Journal of Environmental Chemical Engineering. 104480.

Beesley, L. and Marmiroli, M. (2011). The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environmental Pollution. 159, 474-480.

Bian, R., Li, L., Bao, D., Zheng, J., Zhang, X., Zheng, J., Liu, X., Cheng, K and Pan, G. (2016). Cd immobilization in a contaminated rice paddy by inorganic stabilizers of calcium hydroxide and silicon slag and by organic stabilizer of biochar. Environmental Science and Pollution Research. 23, 10028-10036.

Bogusz, A., Oleszczuk, P. and Dobrowolski, R. (2015). Application of laboratory prepared and commercially available biochars to adsorption of cadmium, copper and zinc ions from water. Bioresource Technology. 196, 540-549.

Bolan, N. S., Adriano, D. C., Mani, P. A. and Duraisamy, A. (2003). Immobilization and phytoavailability of cadmium in variable charge soils. II. Effect of lime addition. Plant and Soil. 251(2), 187-198.

Bolan, N. S., Naidu, R., Syers, J. K. and Tillman, R. W. (1999). Surface charge and solute interactions in soils. Advances in agronomy. 67, 87-140.

Bradl, H.B. (2004). Adsorption of heavy metal ions on soils and soils constituents. Journal of colloid and interface science. 277(1), 1-18.

Bray, R.H. and Kurtz, L.T. (1945). Determination of total, organic and available forms of phosphorus in soils. Soil Science. 23, 39-45.

Chapman, E. E. V., Dave, G. and Murimboh, J. D. (2013). A review of metal (Pb and Zn) sensitive and pH tolerant bioassay organisms for risk screening of metal-contaminated acidic soils. Environmental Pollution. 179, 326-342.

Chiroma, T. M., Ebeweke, R. O. and Hymore, F. K. (2014). Comparative assessment of heavy metal levels in soil, vegetables and urban grey waste water used for irrigation in Yola and Kano. International Refereed Journal of Engineering and Science. 3(2), 01-09.
Cui, L., Li, L., Zhang, A., Pan, G., Bao, D. and Chang, A. (2011). Biochar amendment greatly reduces rice Cd uptake in a contaminated paddy soil: a two-year field experiment. BioResources. 6, 2605-2618.

Elkhatib, E. A., Saleh, M. E., Mahdy, A. M. and Barakat, N. H. (2006). Effects of Organic Ligands and pH on Copper Extractability from Soils of Arid Region. Alexandria Science Exchange. 27(1), 84.

Espinoza, L., Slaton, N. A. and Mozaffari, M. (2006). Understanding the numbers on your soil test report. Cooperative Extension Service, University of Arkansas, US Department of Agriculture, and county governments cooperating.

Fellet, G., Marmiroli, M. and Marchiol, L. (2014). Elements uptake by metal accumulator species grown on mine tailings amended with three types of biochar. Science of the Total Environment. 468, 598-608.

Filep, T. and Szili-Kovács, T. (2010). Effect of liming on microbial biomass carbon of acidic arenosols in pot experiments. Plant, Soil and Environment. 56(6), 268-273.

Garba, S. T., Osemeahon, A. S., Maina, H. M. and Barminas, J. T. (2012). Ethylenediaminetetraacetate (EDTA)-assisted phytoremediation of heavy metal contaminated soil by Eleusine indica L. Gearth. Journal of Environmental Chemistry and Ecotoxicology. 4(5), 103-109.

Hamid, Y., Tang, L., Wang, X., Hussain, B., Yaseen, M., Aziz, M. Z. and Yang, X. (2018). Immobilization of cadmium and lead in contaminated paddy field using inorganic and organic additives. Scientific Reports. 8, 1-10.

Hamzah, A., Hapsari, R. I. and Priyadarshini, R. (2017). The potential of wild vegetation species of Eleusine indica L., and Sonchus arvensis L. for phytoremediation of Cd-contaminated soil. Journal of Degraded and Mining Lands Management. 4, 797.

Hashem, M.A., Hasan, M., Momen, M.A., Payel, S. and Nur-A-Tomał, M.S. (2020). Water hyacinth biochar for trivalent chromium adsorption from tannery wastewater. Environmental and Sustainability Indicators. 5, 100022.

Hong, C. O., Lee, D. K., Chung, D. Y. and Kim, P. J. (2007). Liming effects on cadmium stabilization in upland soil affected by gold mining activity. Archives of Environmental Contamination and Toxicology. 52(4), 496-502.

Hussain, I., Akhtar, S., Ashraf, M. A., Rasheed, R., Siddiqi, E. H. and Ibrahim, M. (2013). Response of maize seedlings to cadmium application after different time intervals. ISRN Agronomy. 2013.

Irfan, M., Hussain, Q., Khan, K.S., Akmal, M., Ijaz, S.S., Hayat, R., Khalid, A., Azeem, M. and Rashid, M. (2019). Response of soil microbial biomass and enzymatic activity to biochar amendment in the organic carbon deficient arid soil: a 2-year field study. Arabian Journal of Geosciences. 12(3), 95.

Jackson, M.L. (1973). Soil chemical analysis, Prentice Hall of India Pvt. Ltd., New Delhi. pp 498.

Karalić, K., Lončarić, Z., Popović, B., Zebec, V. and Kerovec, D. (2013). Liming effect on soil heavy metals availability. Poljoprivreda. 19(1), 59-64.

Kibria, M. G., Osman, K.T., Ahammad, M. J. and Alamgir, M. D. (2011). Effects of farm yard manure and lime on cadmium uptake by rice grown in two contaminated soils of Chittagong. Journal of Agricultural Science and Technology. 5, 352-358.

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

Lahori, A. H., Zhanyu, G. U. O., ZHANG, Z., Ronghua, L. I., Mahar, A., Awasthi, M. K., Feng, S.H.E.N., Sial, T.A., Kumbhar, F., Ping, W.A.N.G. and JIANG, S. (2017). Use of biochar as an amendment for remediation of heavy metal-contaminated soils: prospects and challenges. Pedosphere. 27(6), 991-1014.

Li, F., Shen, K., Long, X., Wen, J., Xie, X., Zeng, X., Liang, Y., Wei, Y., Lin, Z., Huang, W. and Zhong, R. (2016). Preparation and characterization of biochars from Eichornia crassipes for cadmium removal in aqueous solutions. PLoS One. 11, e0148132.

Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q. Devonshire, B. J. and Brookes, P. C. (2013). Microbial biomass growth, following incorporation of biochars produced at 350 C or 700 C, in a silty-clay loam soil of high and low pH. Soil Biology and Biochemistry. 57, 513-523.

Mahajan, P. and Kaushal, I. (2018). Role of phytoremediation in reducing cadmium toxicity in soil and water. Journal of Toxicology. 2018.

Malinowska, E. (2017). The effect of liming and sewage sludge application on heavy metal speciation in soil. Bulletin of Environmental Contamination and Toxicology. 98(1), 105-112.
Mishra, T., Pandey, V. C., Singh, P., Singh, N. B. and Singh, N. (2017). Assessment of phytoremediation potential of native grass species growing on red mud deposits. Journal of Geochemical Exploration. 182, 206-209.

Oijagbe, I.J., Abubakar, B. Y. and Edogbanya, P. R. O. (2019). Effects of heavy metals on soil microbial biomass carbon. Journal of Biology and Medicine. 4(1), 30-32.

Piper, C.S., 1966. Soil and Plant Analysis. Hans Publisher, Bombay. p 368. Pp

Puga, A. P., Abreu, C. A., Melo, L. C. A., Paz-Ferreiro, J. and Beesley, L. (2015). Cadmium, lead, and zinc mobility and plant uptake in a mine soil amended with sugarcane straw biochar. Environmental Science and Pollution Research. 22(22), 17606-17614.

Rawat, J., Saxena, J., and Sanwal, P. (2019). Biochar: a sustainable approach for improving plant growth and soil properties. In: V. Abrol and P. Sharma (Eds.), Biochar-An Imperative Amendment for Soil and the Environment. IntechOpen. Available at https://doi.org/10.5772/intechopen.82151

Sanchez, P.A., Shepherd, K.D., Soule, M.J., Place, F.M., Buresh, R.J., Izac, A.M.N., Uzo Mokwunye, A., Kwasiga, F.R., Ndiritu, C.G. and Woomer, P.L. (1997). Soil fertility replenishment in Africa: an investment in natural resource capital. Replenishing Soil Fertility in Africa. 51, 1-46.

Sethi, S. and Gupta, S. (2014). Optimization of cultural parameters for cellulase enzyme production from fungi. BioLife. 2, 989-996.

Shentu, J., He, Z., Yang, X. E. and Li, T. (2008). Accumulation properties of cadmium in a selected vegetable-rotation system of southeastern China. Journal of Agricultural and Food Chemistry. 56, 6382-6388.

Subbiah, B.V. and Asija,G.L. (1956). A rapid process for the determination of available nitrogen in soils. Current Science. 25, 259-260.

Tate, J. O., Teknikio, J. B. and Abhunu, O. E. (2020). Particle size effect of locally pyrolyzed biochar on the remediation of some heavy metals in crude oil contaminated soils. Pochvoznanie, agrokhimiya i ekologiya/Bulgarian Journal of Soil Science, Agrochemistry and Ecology. 54, 3-11.

Tomczyk, A., Boguta, P. and Sokółwska, Z. (2019). Biochar efficiency in copper removal from Haplic soils. International Journal of Environmental Science and Technology. 16(8), 4899-4912.

Touray, N., Tsai, W. T., Chen, H. R. and Liu, S. C. (2014). Thermochemical and pore properties of goat-manure-derived biochars prepared from different pyrolysis temperatures. Journal of Analytical and Applied Pyrolysis. 109, 116-122.

Vance, E.D., Brookes, P.C., and Jenkinson D.S. (1987). An extraction method for measuring soil microbial biomass carbon. Soil Biology and Biochemistry. 19, 703-707.

Walkley, A. and Black, I.A. (1934). An examination of the method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science. 34, 29-38.

Wang, D. Y., Yan, D. H., Song, X. S., and Wang, H. (2014). Impact of biochar on water holding capacity of two Chinese agricultural soil. Advanced Materials Research, 941, 952-955.

Wang, L., Xu, Y., Liang, X., Sun, G., Sun, Y. and Lin, D. (2012). Remediation of contaminated paddy soil by immobilization of pollutants in the Diaojiang catchment, Guangxi Province. Journal of Ecology and Rural Environment. 28(5), 563-568.

Xiao, R., Huang, Z., Li, X., Chen, W., Deng, Y. and Han, C. (2017). Lime and phosphate amendment can significantly reduce uptake of Cd and Pb by field-grown rice. Sustainability. 9(3), 430.

Xu, Y., Seshadri, B., Sarkar, B., Wang, H., Rumpel, C., Sparks, D., Farrell, M., Hall, T., Yang, X. and Bolan, N. (2018). Biochar modulates heavy metal toxicity and improves microbial carbon use efficiency in soil. Science of the Total Environment. 621, 148-159.