REMEDIATION OF HEAVY METAL POLLUTED SOILS BY MICROBES AND BIOCHAR

S. Sultana*, H.M. Naser, M.R. Khatun, M.B. Banu, M. Akter
Soil Science Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh
**Corresponding author: sultanasarmin1979@yahoo.com*

Abstract

Heavy metal contamination of soil and its subsequent uptake by crops are a great concern in question of food quality and safety. A study was undertaken to remediate soil contaminated with heavy metals by use of microbes and biochar. A pot culture experiment was performed by growing amaranth (*Amaranthus oleraceus* L.) in metal contaminated soil (10 kg pot^{-1}) treated with and without microbes and biochar. Results showed that lead (Pb), cadmium (Cd), and nickel (Ni) uptake by plants increased in pots receiving microbes. On the other hand, biochar immobilized the metals in soil and hence decreased the metal content in plants, except chromium (Cr). Metal uptake by amaranth had increased by 2.15-29.9%, 10.9-24.7%, and 7.43-14.2%, respectively for application of *Rhizobium, Azotobacter* and P solubilizing bacteria (PSB), respectively. While metal uptake had decreased for water hyacinth, barnyard grass and fern plant-based biochar by 25.8-43.4%, 30.9-48.2% and 23.9-46.7%, respectively. The highest value of transfer coefficient was found for Ni (0.55) in plants grown in *Azotobacter* treated soils and the lowest value for Cd (0.12) in water hyacinth biochar. The effectiveness of microbes and biochar on heavy metal uptake by amaranth plants depends on the nature and type of amendments. It is apparent that microbe helps mobilization of metals for plant uptake, and biochar causes metal immobilization, however both processes help remediation of heavy metals contaminated soils.

Keywords: Azotobacter, Cadmium, Lead, PSB, Rhizobium

1. Introduction

Globally heavy metal contamination of soils and crops has been a great environmental concern (Reddy, 2014). Soil contamination with heavy metals is a serious threat that has arisen from various human activities such as mining (Kumar *et al.,* 2017), industries (Liu *et al.,* 2018) and agriculture practices (Abdelhafez *et al.,* 2012). Heavy metals are one of the most prevalent contaminants causing public health problems, entering the body through consumption of food, ingestion of soil and inhalation of dust (Wilson and Pyatt, 2007). The build-up of heavy metal levels in agricultural soils leads to soil contamination and increases

heavy metals uptake by growing plants (Abdelhafez *et al.,* 2012), which in turn affects food quality and safety (Muchuweti *et al.,* 2006). Vegetables that are cultivated on soil contaminated with heavy metals, particularly leafy vegetables, tend to accumulate more metals than vegetables produced in uncontaminated soils due to irrigation water pollution (Naser *et al.,* 2018).

There are various methods of remediating metal polluted soils-physical, chemical and biological methods. Most physical and chemical methods are expensive on one hand and on the other hand, it hardly makes the soil fit for plant culture (Marques *et al.,* 2009). Bioremediation involves the use of microorganisms, green plants and vegetations in ameliorating or detoxifying the pollution that results from heavy metals. Microorganisms that have the capability of growing in heavy metal-polluted environment and also have a significant metal uptake are used in bioremediation (Shakoori *et al.,* 2004). Microbial biomass of bacteria, fungi and yeast is reported to be used in bioremediation (Morales-Barrera and Cristiani-Urbina, 2008). Biochar influences a number of biogeochemical processes and in general there has been reported a positive effect on plant productivity (Liu *et al.,* 2013). Stabilization/solidification (S/S) technologies are recognized by Environmental Protection Agency (EPA) as the best demonstrated available technology (BDAT) for the remediation of heavy metal contaminated soils (Singh *et al.,* 2010). Abdelhafez *et al.* (2014, 2016) illustrated the beneficial effect of biochar for soil improvement and Pb remediation in a shooting range and metal smelter contaminated soils. Numerous studies have shown that biochar has the potential to remediate soils contaminated with heavy metals and seems to be an attractive alternative to standard materials used in in-situ soil remediation (Beesley and Marmiroli, 2011; CAO *et al.,* 2011; Ahmad *et al.,* 2012; Von *et al.,* 2017).

In the last decade there has been an increasing interest in the use of biochar and microbes to tackle soil heavy metal pollution. The role of organic amendments on the metal absorption, transportation and assimilation is known from literature but a little is known about accumulation of heavy metal through biochar and microbes uses. Based on this reason this study was undertaken. The objectives of the study are: (i) to evaluate the effectiveness of microbes and biochar as a bioaccumulator for heavy metals in contaminated soil; (ii) to determine the uptake pattern of heavy metal in the root and shoot system of crop as influenced by microbes and biochar; and (iii) to reduce heavy metal concentrations in food crops, thus to improve food quality and safety.

2. Materials and Methods

2.1 Soil Sampling

Polluted soils were collected from Kalakoir, Konabari and Gazipur areas. These sites are irrigated by water from the Turag river which is highly contaminated with industrial effluents, sewage sludge, municipal waste water and urban runoff. Six composite topsoil samples (0-20 cm depth) from six farmers' fields were collected at random (10 individual samples per field). The collected soil was air-dried and passed through a 2-mm sieve to obtain homogeneous particle size. The physical and chemical properties of the initial soil which used in pot experiment are presented in Table 1. Table shows heavy metal status

Table 1. Initial properties of the soil samples that used in pot culture experiment

Soil Properties	Texture	pH	OM %	Ca	Mg	K	Total N (%)	P	S	B	Cu	Fe	Zn
				meq 100g^{-1}				mg kg^{-1}					
Result	Sandy clay loam	7.9	1.23	2.35	1.41	0.12	0.07	16.2	48.4	0.14	1.84	20.3	1.82
Critical level	-	-	-	2	0.5	0.2	-	7.0	10	0.2	0.2	4.0	0.6

Table 2. Heavy metal status of the industrial effluent contaminated soil that used in pot culture experiment

Soil heavy metal	Heavy metals content (µg g^{-1}) of soil samples			
	Pb	Cd	Ni	Cr
Result	9.23±1.70	1.81±0.25	22.6±1.75	40.0±2.33
MPL [a]	100	3	50	100
MPL [b]	50	1	1	30

a Ewers, (1991); b Bowen, (1966); MPL = Maximum Permissible (or Accessible) Limit

2.2 Biochar preparation

Water hyacinth, barnyard grass, and fern were collected from the field and air-dried at room temperature for one week. They, they were cut into small pieces and then placed in biochar making devices (made in the Division of Soil Science, BARI) and pyrolyzed under limited oxygen conditions. The temperature of pyrolysis was elevated to 650^{0}C at a rate of about 20^{0}C per minute and kept constant for 1 h (Lehmann and Joseph, 2009; Park *et al.,* 2011). The biochar was then allowed to cool down to room temperature and ground to pass through a 0.25-mm sieve.

2.3 Microbes preparation

Rhizobium, Azotobacter and Phosphate solubilizing bacterial (PSB) strains were collected from Soil Microbiology Laboratory of BARI which were previously cultured on YEMA, Jensens's and Pikovskaya's media, respectively. Peat based *Rhizobium, Azotobacter* and PSB bacterial inoculum were used containing 10^8 cells g^{-1} inoculant. Before sowing, amaranth seeds were mixed thoroughly with the peat-based inoculum at the rate of 50g inoculum kg^{-1} seed and six seeds were sown each pot.

2.4 Treatments

Soil samples were mixed by adding biochar materials at a rate of 2 g kg^{-1} soil. The microbe treatments were carried out as stated above. There were seven treatments comprising three types of microbes and three types of biochar along with a control. The treatments were: (i) Contaminated soil, no amendment i.e. control, (ii) contaminated soil + *Rhizobium* (iii) contaminated soil + Azotobacter (iv) contaminated soil + amendment with phosphorus solubilizing bacteria, (v) contaminated soil + water hyacinth biochar, (vi) contaminated soil + barnyard grass biochar, (vii) contaminated soil + fern plant biochar.

2.5 Experiment setup

The experiment consisted of a total 21 plastic pots, each containing 10 kg soil. Pots were placed in a completely randomized design with three replications for each of seven treatments in a shade house of Soil Science Division, BARI, Gazipur. Amaranth (*Amaranthus oleraceus* L.) seeds were sown directly in pots at a density of 15 seeds per pot on 05 April 2023. Twelve days after sowing the seedlings were thinned to eight plants per pot. All the pots were fertilized two days before sowing with N: 90 mg kg^{-1} soil, P: 75 mg kg^{-1} soil, K: 140 mg kg^{-1} soil, S: 30 mg kg^{-1} soil, Zn: 2 mg kg^{-1} soil, B: 1 mg kg^{-1} soil. Urea, triple superhosphate, muriate of potash, gypsum, zinc sulphate monohydrate ($ZnSO_4$. H_2O) and boric acid were used as a source of N, P, K, S, Zn and B, respectively. Nitrogen was applied in two equal splits, the first split before sowing and the remaining split at 8-10 leaf of plants. Wetting cycles (at field capacity) and air-drying every week were performed, during 2-month period.

The plant was cut after two months of seed sowing when it attained flowering stage. Soil was removed from the roots carefully and plants were washed with tap water followed by deionized water.

2.6 Preparation and preservation

The clean plant samples were air-dried and placed in an electric oven, dried at 85°C for 72 h, weighed for dry biomass. The dry plant samples were homogenized by grinding using a ceramic coated grinder and used for metal analysis. Samples of contaminated soils were spread on plastic trays and allowed to dry at ambient temperature for 8 days. The dry

samples of soils were ground by a ceramic coated grinder and sieved through a nylon sieve. The final samples were kept in labeled polypropylene containers at ambient temperature before analysis.

2.7 Digestion and analytical procedure

One gram of each soil and plant sample was weighed into a 50-ml beaker, followed by an addition of 10 ml mixture of analytical grade acids HNO_3: $HCIO_4$ in ratio 5:1 ratio, and left overnight for complete contact of material. Next day, the digestion was performed at a temperature of about 190^0C for 1.5 h. After cooling, the samples were transferred into 100 ml volumetric flask and solution was made up to a final volume raised up to the mark with distilled water. The metal concentrations were determined by atomic absorption spectrometry using a VARIAN model AA2407 Atomic Absorption Spectrophotometer (AAS). Analysis of each sample was carried out three times to obtain representative results and the data are reported in μg g^{-1} (on a dry matter basis).

2.8 Determination of Transfer Factor (TF)

The Transfer Coefficient was calculated by dividing the concentration of heavy metals in vegetables by the total heavy metal concentration in the soil (Mirecki *et al.,* 2015). TF = Cplant / Csoil; where, Cplant = metal concentration in plant tissue (μg g^{-1} dry weight) and Csoil = metal concentration in soil (μg g^{-1} dry weight).

2.9 Statistical analysis

The experiment was designed in a completely randomized (CRD) with seven treatments and three replications. Treatment effects were determined by analysis of variance with the help of statistical package STATISTIX-10 and mean separation was tested by Tukey HSD.

3. Results and Discussion

The results of initial soil analysis are presented in Table 1. The concentration of nickel (Ni) or chromium (Cr) was higher than that of lead (Pb) and cadmium (Cd) (Table 2). The concentration of Pb was 9.23 μg g^{-1}, Cd 1.81 μg g-1, Ni 22.6 μg g^{-1} and Cr 40.0 μg g^{-1}. However, these levels (Pd, Cd, Ni and Cr) were below the reported value of Ewers (1991) and were extremely high when compared with the levels of these metals in uncontaminated soil reported by Bowen (1966), except Pb.

The effects of the microbes and biochar material applications on the uptake levels of Pb, Cd, Ni and Cr by amaranth from the contaminated soil samples are given in Table 3. The results indicated that application of biochar increased Cr uptake, but decreased Pb, Cd, and Ni uptake by amaranth. On the other hand, microbes decreased Cr uptake, but increased Pb, Cd, and Ni uptake. If plant uptake levels adequately described the effectiveness of metal

immobilization, the results in Table 2 suggest that the effectiveness of immobilization varied in the order of water hyacinth biochar > fern plant biochar > barnyard grass biochar.

The increased uptake of metal in amaranth with addition of microbes compared with contaminated control were Pb 14.2 to 24.7%, Cd 8.82 to 29.9%, Ni 7.43 to 14.7% and Cr 2.15 to 45.0% (Table 4). On the other hand, the Cr uptake had increased by 38.1 - 45.0 % in amaranth plants due to biochar application. Addition of biochar to contaminated soil did not totally restrict the uptake of metal by amaranth plants. Water hyacinth biochar, barnyard grass biochar and fern plant biochar addition led to decreased Pb, Cd, and Ni content in amaranth, and this decrease can be better expressed by 25.8 - 43.4 %t for water hyacinth, 30.9 - 48.2 % for barnyard grass, 23.9 - 46.7% for fern plant. In case of metal uptake, it was 43.4 - 48.2%, 28.9 - 36.8%, 23.9 - 31.5% and 38.1 - 45.0%, respectively for Pb, Cd, Ni and Cr.

Table 3. Effects of microbes and biochar amendment on metal concentration of amaranth from contaminated soil

Microbe/biochar application	Metal concentration of amaranth (μg g^{-1} of dry wt.)			
	Pb	Cd	Ni	Cr
Root				
Contaminated control	5.01a	0.42ab	14.6abc	15.7b
Rhizobium	5.73a	0.61a	16.6a	15.6b
Azotobacter	6.16a	0.50ab	15.9a	15.4b
Phosphorus solubilizing bacteria	5.44a	0.46ab	15.3ab	16.2b
Water hyacinth biochar	2.81b	0.26b	11.0c	23.1a
Barnyard grass biochar	2.57b	0.30b	11.2c	23.7a
Fern plant biochar	2.60b	0.28b	11.8bc	22.1a
CV (%)	14.1	22.0	10.0	8.07
Shoot				
Contaminated control	2.73ab	0.26ab	7.21ab	12.5b
Rhizobium	3.35a	0.27a	8.68a	13.3b
Azotobacter	3.49a	0.29a	8.38a	12.3b
Phosphorus solubilizing bacteria	3.41a	0.28a	8.15a	13.7b
Water hyacinth biochar	1.47bc	0.17c	5.16b	16.7a
Barnyard grass biochar	1.54c	0.17c	3.78c	17.3a
Fern plant biochar	1.53c	0.21abc	4.77b	17.0a
CV (%)	15.12	12.9	8.90	4.15
Total plant				
Contaminated control	3.87a	0.34ab	10.9a	14.1b
Rhizobium	4.54a	0.44a	12.5a	14.4b
Azotobacter	4.83a	0.40a	12.1a	13.9b
Phosphorus solubilizing bacteria	4.42a	0.37a	11.7a	14.9b
Water hyacinth biochar	2.19b	0.21c	8.10b	19.9a
Barnyard grass biochar	2.01b	0.24b	7.48b	20.5a
Fern plant biochar	2.07b	0.24b	8.32b	19.5a
CV (%)	10.9	11.4	6.4	4.7

Mean values in the same column followed by the same letters are not significantly different ($P< 0.05$)

Table 4. Metal uptake increased/decreased in amaranth compared with contaminated control

Microbe/biochar applaication	Pb	Cd	Ni	Cr
	Metal uptake increased (%) compared with contaminated control			
Metal aspect	14.2-24.7	8.82-29.9	7.43-14.7	2.15-45.0
	Rhizobium	Azotobacter	P solubilizing bacteria	-
Microbes	2.15-29.9	10.9-24.7	7.43-14.2	-
	Metal uptake decreased (%) compared with contaminated control			
Metal aspect	43.4-48.2	28.9-36.8	23.9-31.5	38.1-45.0
	Water hyacinth	Barnyard grass	Fern plant	-
Biochar	25.8-43.4	30.9- 48.2	23.9-46.7	-

Table 5. Transfer factor of heavy metals from soil to amaranth plant as influenced by microbes and biochar applications

Microbe/biochar applaication	Name of metal			
	Pb	Cd	Ni	Cr
Contaminated control	0.42	0.19	0.48	0.35
Rhizobium	0.49	0.24	0.55	0.36
Azotobacter	0.52	0.22	0.54	0.35
Phosphorus solubilizing bacteria	0.48	0.20	0.52	0.37
Water hyacinth biochar	0.25	0.12	0.36	0.50
Barnyard grass biochar	0.22	0.13	0.33	0.51
Fern plant biochar	0.22	0.13	0.37	0.49

Among the metal contents there were significant differences between the application of microbes and biochar. The use of microbes resulted in the removal of heavy metals from the soil by plant uptake, while the use of biochar resulted in comparatively less uptake compared to uncontaminated (control) soil. Alaboudi *et al.* (2019) reported that the addition of biochar gradually decreased the availability of Pb and Cd in soil, due to which the amount of Pb and Cd uptake by the growing plants was decreased. Comprehensive reviews by several authors have described the potential value of biochar as an effective agent in immobilization of metals and organic pollutants (Mohan *et al.,* 2014; Ahmed *et al.,* 2016; Rizwan *et al.,* 2016; Yuan *et al.,* 2017). The metal concentrations decreased in amaranth plant with the addition of biochar, which might have immobilized the metal through adsorption, complexation, and precipitation phenomena, resulting in reduced accumulation in plants (Cao *et al.,* 2003; Seaman *et al.,* 2003). Metals (Pb and Cd) are adsorbed on organic matter, which generate stable forms and lead to their accumulation in organic horizons of soil (Kabata Pendias, 2001). The opposite trend was observed for Cr, and the addition of biochar increased the uptake of Cr in the plant. The Cr uptake by application of biochar in this study is in agreement with the findings of Alaboudi *et al.* (2019). For example, chromium exists as two predominant species in the environment: trivalent chromium (III) and hexavalent (chromium (VI)). Chromium (III) is generally non-toxic and is strongly bound to soil

particles, whereas chromium (VI) is extremely toxic and highly mobile (Wang *et al.,* 2018).

Lead, Cd and Ni contents of amaranth were elevated by the application of microbes in this study. Some metal-tolerant bacterial strains associated with hyper-accumulating plants have been shown to mobilize metals in soils, and consequently increase the phytoavailable metal fraction in the soil, as well as plant uptake and accumulation (Kidd, 2018). Soil microbial biomass plays indirect roles in phytoremediation of heavy metals by indirectly acting in the plants rhizosphere and influence chelated or complexed metals into soluble forms that are readily available for plant uptake. Naees *et al.* (2011) found a certain bacterium (*Burkholderia* spp.) to increase the bioavailability of Pb and Cd and increase their uptake by amaranth and tomato plants. Moreover, microbial activity can result in metal mobilization or immobilization depending on the mechanism involved and the microenvironment where the organism(s) are located (Violante *et al.,* 2008; Ehrlich & Newman, 2009; Gadd, 2010).

It was found that the roots of the amaranth plant have taken a dense metal from soil compared to shoot. Some authors have reported accumulation of heavy metals mainly in the roots of sunflower with little movement from the roots to the above ground mass (Madejón *et al.,* 2003; Lin *et al.,* 2003; Marchiol *et al.,* 2007), while others reported effective movement from the roots to above ground mass (Adesodun *et al.,* 2010; Herrero *et al.,* 2003). The concentrations of Pb, Cd, Ni and Cr increased in the plant biomass; especially the roots (Ojuederie & Babalola, 2017).

Soil-to-plant transfer ratio (amount of metal in plant to the pseudo-total amount in soil) or transfer coefficient (TC) is an important aspect of phytoextraction. The effect of the soil amendment on the TC of metals from the contaminated soil to amaranth is shown in Table 5. As with the amounts of the metal uptake by amaranth, the proportions of the metals in the soil that were absorbed by the plants decreased with applications of biochar. The potential of different biochar followed the order fern plant biochar > barnyard grass biochar > water hyacinth biochar. The highest value of TC (Ni 0.55) was found in plant grown in Azotobacter treatment and lowest (Cd 0.12) was in water hyacinth biochar.

4. Conclusions

Biochar treated soils adsorbed heavy metals, thereby reducing their mobility and bioavailability to amaranth plants. On the contrary, in case of Cr, biochar application exhibited opposite result showing mobilization of Cr in soil for ready uptake by plants. Phytoremediation coupled with microbes could be a solution towards the recovering soil quality, underlining the role of the rhizosphere and microbes associated with hyperaccumulator plants in metal accumulation. Evaluation of their potential, however, requires further study of the effect of microbes and biochar amendments on metal remediation in field conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

References

Abdelhafez, A.A., Abbas, H.H., Abd-El-Aal, R.S., Kandil, N.F., Li, J., Mahmoud, W. 2012. Environmental and health impacts of successive mineral fertilization in Egypt. Clean. 40: 356–363.

Abdelhafez, A.A., Abbas, M.H.H., Hamed, M.H. 2016. Biochar: a solution for soil lead (Pb) pollution. In: The 8th International Conference for Development and the Environment in the Arab World, pp. 89–103.

Abdelhafez, A.A., Li, J., Abbas, M.H.H. 2014. Feasibility of biochar manufactured from organic wastes on the stabilization of heavy metals in a metal smelter contaminated soil. Chemosphere. 117: 66–71.

Adesodun, J.K., Atayese, M.O., Agbaje, T., Osadiaye, B.A. Mafe, O., Soretire, A.A. 2010. Phytoremediation potentials of sunflowers (*Tithonia diversifolia* and *Helianthus annuus*) for metals in soils contaminated with zinc and lead nitrates. Water Air Soil Pollut. 207:195–201.

Ahmad, M., Soo, S., Dou, X., Mohan, D., Sung, J., Yang, J., Sik, Y. 2012. Effects of pyrolysis temperature on soybean stover- and peanut shell-derived biochar properties and TCE adsorption in water. Bioresour. Technol. 118:536.

Ahmed, M.B., Zhou, J.L., Ngo, H.H. Guo, W., Chen, M. 2016. Progress in the preparation and application of modified biochar for improved contaminant removal from water and wastewater. Bioresour. Technol. 214:836–851.

Alaboudi, A.A, Berhan, A., Brodiec, G. 2019. Effect of biochar on Pb, Cd and Cr availability and maize growth in artificial contaminated soil. Ann. Agricult. Sci. 64:95–102.

Beesley, L., Marmiroli, M. 2011. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environ. Pollut. 159:474.

Bowen, H.J.M. 1966. Trace Elements in Biochemistry (p. 241). New York: Academic.

Cao, X., Ma, L.Q., Shiralipour, A. 2003. Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyper accumulator, Pteris vittata L. Environ. Pollut. 126:157–167.

Cao, X., Ma L., Liang, Y., Gao, B., Harris, W. 2011. Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ. Sci. Technol. 45:4884. doi:10.1007/s11270-007-9520-z

Ehrlich, H.L., Newman, D. K. 2009. Geomicrobiology, 5th edn. CRC Press, Taylor & Francis, Boca Raton, FL.

Ewers, U. 1991. Standards, guidelines and legislative regulations concerning metals and their compounds. In: Merian E, ed. Metals and Their Compounds in the Environment: Occurrence, Analysis and Biological Relevance. Weinheim: VCH, pp: 458–468.

Gadd, G.M. 2010. Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology. 156: 609-643.

Herrero, E., Lopez-Gonzalvez, A., Ruiz, M., Lucas-Garcia, J., Barbas, C. 2003. Uptake and distribution of zinc, cadmium, lead and copper in Brassica napus var. Oleifera and Helianthus annus grown in contaminated soils. Int. J. Phytoremed. 5: 153-167.

Kabata-Pendias, A., Pendias, H.. 1992. Trace elements in soil and plants (2nd ed., P. 365). Boca Raton: CRC.

Kidd, P.S. 2018. The Role of the Rhizosphere and Microbes Associated with Hyperaccumulator Plants in Metal Accumulation. Agromining: Farming for Metals, 2018, Mineral Resource Reviews, 978-3- 319-61898-2. 10.1007/978-3-319-61899-9. hal-02008731

Kumar, A., Maiti, S.K., Tripti, Prasad, M.N.V., Singh, R.S. 2017. Grasses and legumes facilitate phytoremediation of metalliferous soils in the vicinity of an abandoned chromite–asbestos mine. J. Soils Sedim. 17: 1358–1368.

Lehmann, J., Joseph, S. 2009. Biochar for environmental management: an introduction. In: Lehmann J, Joseph S (eds) Biochar for environmental management-science and technology. Earthscan, London, pp 1–9.

Lin, J., Jiang, W., Liu, D. 2003. Accumulation of copper by roots, hypocotyls, cotyledons and leaves of sunflower (Helianthus annuus L.). Bioresour. Technol. 86: 151–155.

Liu X, Zhang A, Ji C, Joseph S, Bian R, et al., 2013. Biochar's effect on crop productivity and the dependence on experimental conditions- a meta-analysis of literature data. Plant Soil. 373:583-594.

Liu, J., Liu, Y.J., Liu, Y., Liu, Z., Zhang, A.N. 2018. Quantitative contributions of the major sources of heavy metals in soils to ecosystem and human health risks: a case study of Yulin, China. Ecotoxicol. Environ. Saf. 164: 261–269.

Madejón, P., Murillo, J.M., Marañón, T. Cabrera, F., Soriano, M. 2003. Trace element and nutrient accumulation in sunflower plants two years after the Aznalcollar mine spill. Sci. Total Environ. 307: 239–257.

Marchiol, L., Fellet, G., Perosa, D., Zerbi, G. 2007. Removal of trace metals by Sorghum bicolor and Helianthus annuus in a site polluted by industrial wastes: A field experience. Plant Physiol. Biochem. 45: 379–387.

Marques, A.P.G.C., Rangel, A.O.S.S. and Castro, P.M.L. 2009. Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology," Crit. Rev. Environ. Sci. Technol. 39(8): 622–654.

Mirecki, N., Agič, R., Šunić, L., Milenković, L., Ilić, Z.S. 2015. Transfer factor as indicator of heavy metals content in plants. Fresenius Environmental Bulletin. © by PSP Volume 24 – No 11c. 4212-4219. https://www.researchgate.net/publication/285589331.

Mohan, D., Sarswat, A., Ok, Y.S., Pittman, C.U. 2014. Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent—A critical review. Bioresour. Technol. 160: 191–202.

Morales-Barrera, L., Cristiani-Urbina, E. 2008. Hexavalent chromium removal by a Trichoderma inhamatum fungal strain isolated from tannery effluent. Water Air Soil Pollut. 187:327–336.

Muchuweti, M., Birkett, J.W., Chinyanga, E., Zvauya, R., Scrimshaw, M.D., Lester, J.N. 2006. Heavy metal content of vegetables irrigated with mixture of wastewater and sewage sludge in Zimbabwe: implications for human health. Agric. Ecosyst. Environ. 112: 41–48.

Naees, M, Ali, Q., Shahbaz, M. and Ali, F. 2011. Roles of rhizobacteria in phytoremediation of heavy metals: an overview. Int. Res. J. Plant Sci. 2(8): 220–232.

Naser, H. M., Rahman, M.Z., Sultana, S., Quddus, M.A., Hossain, M.A. 2018. Heavy metal accumulation in leafy vegetables grown in industrial areas under varying levels of pollution. Bangladesh J. Agril. Res. 43(1): 39-51.

juederie, O.B. Babalola, O.O. 2017. Microbial and Plant-Assisted Bioremediation of Heavy Metal Polluted Environments: A Review. Int. J. Environ. Res. Public Health. 14: 1-26.

Park, J.H., Choppala, G.K., Bolan, N.S., Chung, J.W., Chuasavathi, T. 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil. 348: 439–451.

Reddy, K.R. 2014. Evolution of geoenvironmental engineering. Environ. Geotech.1(3):136–141.

Rizwan, M., Ali, S., Qayyum, M.F., Ibrahim, M., Zia-ur-Rehman, M., Abbas, T., Ok, Y.S. 2016. Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: A critical review. Environ. Sci. Pollut. Res. 23:2230–2248.

Seaman, J.C., J.M. Hutchison, B.P. Jackson, Vulava, V.M. 2003. In situ treatment of metals in contaminated soils with phytate. J. Environ. Qual. 32:153–161.

Shakoori, A.R., Rehman, A., Haq, R.U. 2004. Multiple metal resistance in the ciliate protozoan, Vorticella microstoma, isolated from industrial effluents and its potential in bioremediation of toxic wastes. Bull. Environ. Contamin. Toxicol. 72: 1046–1051.

Singh, B., Singh, B.P., Cowie, A.L. 2010. Characterization and evaluation of biochars for their application as a soil amendment. Aust. J. Soil Res. 48: 516–525.

Violante, A., Huang, P.M., Gadd, G.M. (Eds.). 2008. Biophysicochemical processes of heavy metals and metalloids in soil environments. Wiley, Chichester.

Von, G.K., Alam. M., Hubmann. M., Ok Y., Konhauser, K., Alessi, D. 2017. Modified sequential extraction for biochar and petroleum coke: Metal release potential and its environmental implications. Bioresour. Technol. 236: 106.

Wang, Y, Wang, H.S., Tang, C.S., Gu, K., Shi, B. 2018. Remediation of heavy-metal-contaminated soils by biochar: a review. Environ. Geotech: https://doi.org/10.1680/jenge.18.00091

Wilson, B., Pyatt, F.B. 2007. Heavy metal dispersion, persistence, and bioaccumulation around an ancient copper mine situated in Anglesey, UK. Ecotoxicol. Environ. Saf. 66:224–231.

Yuan, Y., Bolan, N., Prévoteau, A., Vithanage, M., Biswas, J.K., Ok, Y.S., Wang, H. 2017. Applications of biochar in redox-mediated reactions. Bioresour. Technol. 246: 271–281.