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Biochar-Assisted Bacillus Inoculation Reduces Cadmium And Lead Bioavailability And Enhances Maize Growth In Contaminated Soil

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ABSTRACT

Cadmium (Cd) and lead (Pb) in agricultural soil are harmful and interfere with the growth of the crop, with the possibility of increasing the amount of metal in the food chain. To assess the growth promoting potential of heavy-metal-tolerant Bacillus strains (HMTBS) and the possible reduction of Cd and Pb bioavailability by combining HMTBS with biochar, a glasshouse pot experiment was carried out at the Department of Soil and Environmental Sciences, The University of Agriculture, Peshawar. Three bacterial strains (Bacillus pumilus, Bacillus proteolyticus and Bacillus paramycoides) were used along with four different rates of biochar (0, 1, 1.5 and 2%). Cultivar of maize, Azam, was cultivated under Cd and Pb stress and plant growth, AB-DTPA extractable soil metals, metal concentration of the shoots and roots, metal uptake and translocation factors were determined. The application of

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biochar always showed a positive effect on growth and reduced metal availability. In both bacterial strains, the plant height under 2% biochar grew from 87 cm to 102 cm, shoot dry biomass grew from 82.2 to 94.0 g pot-1 and root dry weight increased from 3.31 to 5.21 g pot-1 in comparison with 0% biochar. This 2% rate of biochar decreased the concentration of AB-DTPA extractable Cd from 7.16 to 1.92 mg kg-1 and Pb from 8.15 to 3.00 mg kg-1. Shoot Cd and Pb decreased from 8.34 to 3.37 mg kg-1 and from 8.44 to 3.21 mg kg-1, respectively. The tested strains showed varying abilities to decrease Cd and Pb concentration in the tissues of plants and soil; B. paramycoides had the highest reduction potential and B. proteolyticus had the highest mean shoot biomass. The results suggest that the use of metal tolerant Bacillus spp. with 2% biochar is a potential, low cost, and environmentally friendly method for enhancing maize growth in Cd and Pb polluted soil.

Keywords: Biochar; Bacillus; Cadmium; Lead; Maize; PGPR; Contaminated Soil

Introduction

Heavy metals contamination of agriculture soil is a long-term environmental and food safety issue since some metals like Cd and Pb can persist in the environment for long period and are bioavailable. Cd and Pb in contaminated soils can inhibit germination, growth of roots and shoots, photosynthesis and uptake of nutrients, and decrease crop productivity. Metal stress in soils of maize growing areas is of agronomic and environmental concern as it is an important crop in Pakistan and other agricultural areas.

Biochar has been highlighted as a remedy for metal contaminated soils due to its porous structure, surface functional groups, and overall alkaline properties, which can lead to adsorption, precipitation, complexation, cation exchange and pH-mediated immobilization of the toxic metals (El-Naggar et al., 2020; Irfan et al., 2021; Haider et al., 2022). Recent research with maize and other crops demonstrates that biochar application can decrease AB-DTPA-extractable metals and the levels of metals in plant tissues, and improve growth parameters (Irfan et al., 2021; Ma et al., 2024). Farooq et al. (2026) found that biochar applied with P also enhanced maize growth, yield, P uptake and phosphorus-use efficiency in maize production systems, which also corroborates the role of biochar in enhancing the performance of the crops under nutrient limited field conditions.

Plant growth promoting rhizobacteria (PGPR) like metal tolerant strains of Bacillus sp. can also be beneficial under metal stress because of their ability to produce growth promoting metabolites, enhance nutrient availability, change the rhizosphere chemistry and by immobilizing metal ions. Some bacteria have been reported to immobilize heavy metals and also to solubilize phosphate that can help reduce the uptake of Cd and Pb in the plant and stimulate plant growth under polluted soil conditions (Ahmad et al., 2016; Wang et al., 2022; Qin et al., 2023). Bacteria can be used to further stabilize metals and increase plant tolerance, and biochar can boost soil properties and create microbial habitat, thus offering complementary benefits when used together. Analogously, A. Khan et al. (2026) observed that biochar-microbe combination had the potential to mitigate the Cd and Pb in the contaminated soil samples and had positive effects on maize growth, similar to biochar.

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Thus, the present study aimed at assessing the effect of BFT and various rates of biochar on the growth of maize, bioavailability of Cd and Pb, metal uptake and translocation in the contaminated soil.

Materials and Methods

Experimental Site and Design

The experimental site and design are specified.The experimental site and design is defined.

The pot experiment was done in the glass house of the Department of Soil and Environmental Sciences, The University of Agriculture, Peshawar. The experiment was conducted in a completely randomized design with a 2 factor treatment plan – bacterial strain x biochar rate. Bacillus pumilus, B. proteolyticus and B. paramycoides were tested. Biochar was applied at 0, 1, 1.5 and 2%.

Table 1 Treatment structure used in the pot experiment.

Bacterial strain	Biochar rate (%)	Treatment number
Bacillus pumilus	0	1
Bacillus pumilus	1	2
Bacillus pumilus	1.5	3
Bacillus pumilus	2	4
Bacillus proteolyticus	0	5
Bacillus proteolyticus	1	6
Bacillus proteolyticus	1.5	7
Bacillus proteolyticus	2	8
Bacillus paramycoides	0	9
Bacillus paramycoides	1	10
Bacillus paramycoides	1.5	11
Bacillus paramycoides	2	12

Initial Soil Properties

The experimental soil was analyzed for some physical and chemical properties prior to application of the treatment. Soil type in the field was silt loam and pH was alkaline with low organic matter. The presence of some basic physicochemical parameters like pH and EC is significant as these are used as the most common water quality indicators and are of importance to interpret the environmental quality and changes in water quality associated with contamination (S. Khan & B. Khan, 2026).

Table 2 Physical and chemical characteristics of soil before the experiment.

Property	Unit	Value
Sand	%	22
Silt	%	68
Clay	%	10
Textural class	-	Silt loam

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Property	Unit	Value
pH (1:5)	-	7.67
EC (1:5)	dS m-1	0.19
Organic matter	%	0.55
Lime	%	12.50
Total N	%	0.017
AB-DTPA extractable P	mg kg-1	4.36
AB-DTPA extractable Cd	mg kg-1	0.04
AB-DTPA extractable Pb	mg kg-1	3.31

Crop Establishment and Measurements

Azam cultivar of maize was used. Inoculation was done by using the respective bacterial strains by soaking the seeds in 10% sugar solution prior to adding inoculum, then shade drying. Urea 120kg/ha N, single super phosphate 90kg/ha P2O5 and sulphate of potash 60kg/ha K2O were used as basal fertilizers. Normal cultural practices were maintained in crop development.

The height of the plant, the dry weight of the shoots and the root dry weight were measured. After harvest, soil was air-dried, sieved through a 2 mm mesh and analyzed for AB-DTPA-extractable Cd and Pb following Soltanpour and Schwab (1977). Shoots and roots of the plants were subjected to wet acid digestion according to AOAC (1990) and the concentrations of Cd and Pb measured by atomic absorption spectrophotometry. Metal concentration and dry biomass were used to determine metal uptake and translocation factors were used to evaluate metal movement from roots to shoots.

Statistical Analysis

The data were analyzed using Statistix 8.1. The significant treatment effects were separated by using the least significant difference (LSD) at $P < 0.05$, and the means which were significantly different were marked by alphabetical letters (Steel et al., 1997). The treatment means were converted into figures; this was done using Python code in this manuscript.

Results and Discussion

Maize Growth Response

Both bacterial strain and biochar rate influenced the growth of maize under Cd and Pb stress. Increasing biochar concentration from 0% to 2% resulted in a 17.2% increase in plant height, a 14.4% increase in shoot dry biomass and a 57.4% increase in root dry weight, across the bacterial strains. B. paramycoides plus 2% biochar had the highest individual plant height (112 cm) and root dry weight (5.67 g pot-1) whereas B. proteolyticus plus 2% biochar had the highest shoot dry biomass (98.2 g pot-1). The results in this study indicated that under metal stress, biochar added to the rhizosphere enhanced growth recovery and conditions, and bacterial inoculation also helped to enhance growth recovery.

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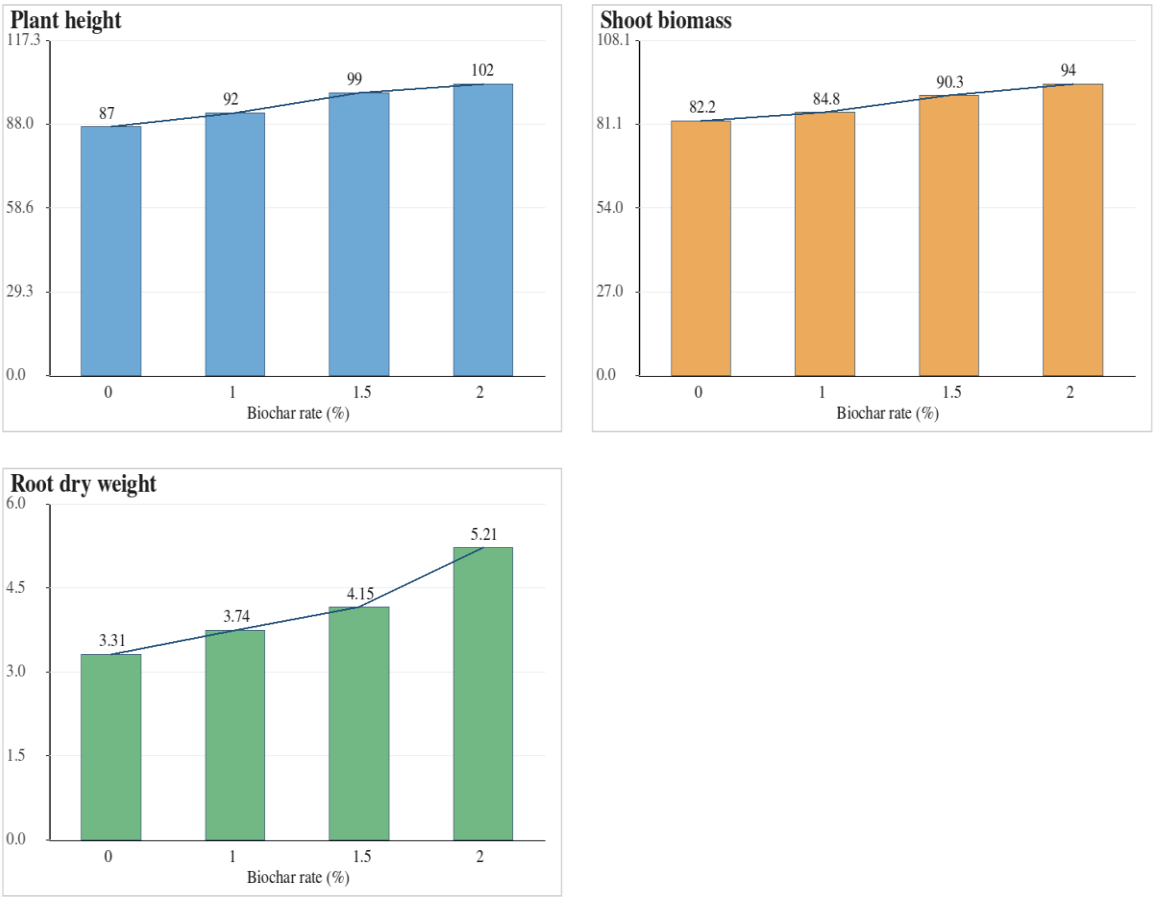

Figure 1 Effect of biochar rate on maize growth traits averaged across Bacillus strains.

This positive growth response of maize is in accordance with previous studies, in which biochar has been reported to enhance soil organic matter, nutrient retention and plant physiology in metal polluted soils (Irfan et al., 2021; Haider et al., 2022). The Bacillus inoculation response is also in line with reports that metal tolerant PGPRs can increase plant growth under metal stress by decreasing the oxidative damage, increasing nutrient availability and promoting root growth (Ahmad et al., 2016; Wang et al., 2022).

Soil Cd and Pb Bioavailability

The removal of Cd and Pb in AB-DTPA extractable fraction decreased significantly with the increase of biochar rate. Across the bacterial strains, the concentration of Cd in the soil decreased from 7.16 mg kg-1 at 0% biochar to 1.92 mg kg-1 at 2% biochar which corresponds to 73.2% reduction in soil Cd concentration. The soil Pb reduced from 8.15 to 3.00 mg kg-1 which is 63.2 % reduction. Generally, the soil Cd and Pb values were lower for B. paramycoides, suggesting that its immobilization potential is higher than the other strains.

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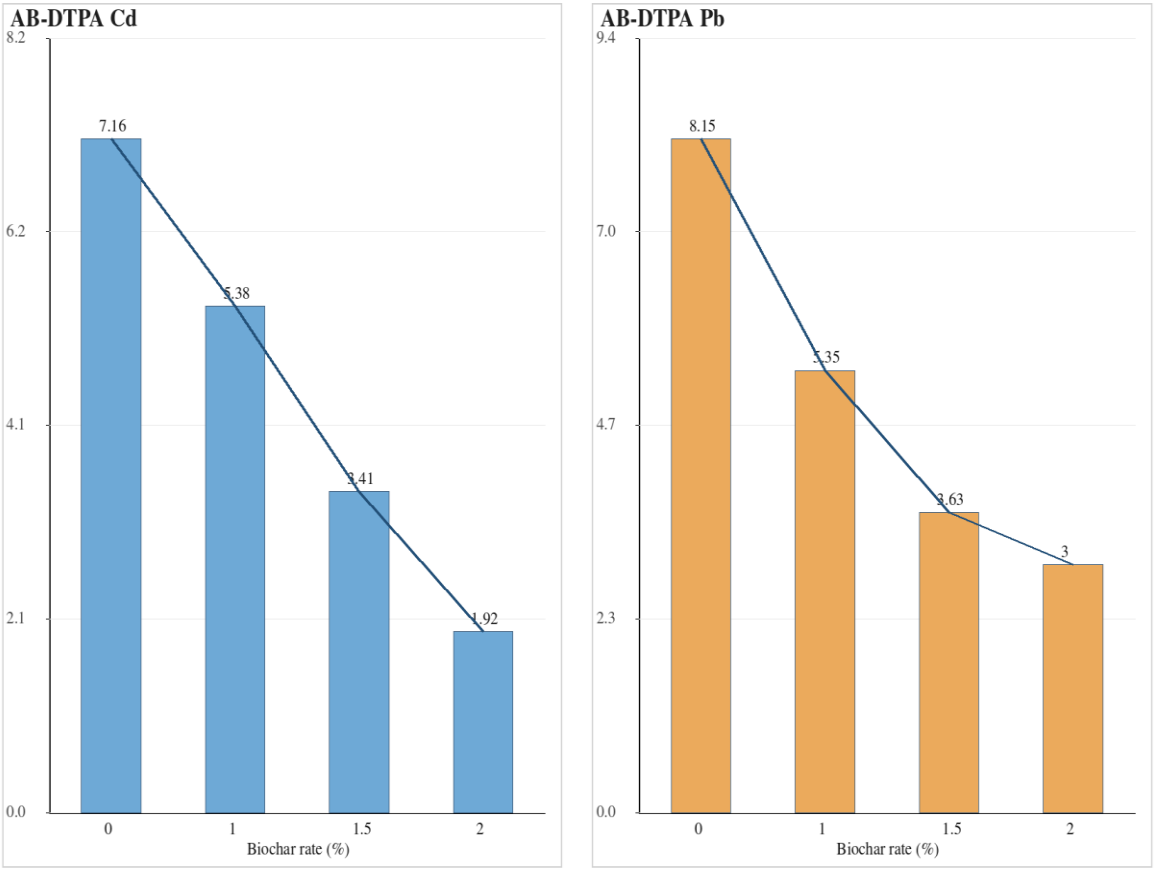

Figure 2 Effect of biochar rate on AB-DTPA-extractable Cd and Pb in soil averaged across Bacillus strains.

This decrease in AB-DTPA-extractable metals is probably due to the processes of adsorption, precipitation and complexation by biochar, as well as rhizobacterial metal immobilization processes. The same mechanism was observed by El-Naggar et al. (2020), Chen et al. (2019) and Zhang et al. (2021) with biochar applied soils and by Qin et al. (2023) with phosphate-solubilizing and metal-immobilizing bacteria.

Cd and Pb Concentration in Maize Shoots and Roots

Soil metal bioavailability decreased as reflected by plant tissue concentrations. In all the bacterial strains, the biochar reduced the shoot Cd concentration from 8.34 mg kg-1 to 3.37 mg kg-1 and shoot Pb concentration from 8.44 mg kg-1 to 3.21 mg kg-1. Root Cd declined from 9.79 to 5.44 mg kg-1, while root Pb declined from 9.45 to 4.27 mg kg-1. Overall, B. paramycoides had the lowest mean Cd and Pb concentration in both shoots and roots than the other two tested species (B. pumilus and B. proteolyticus).

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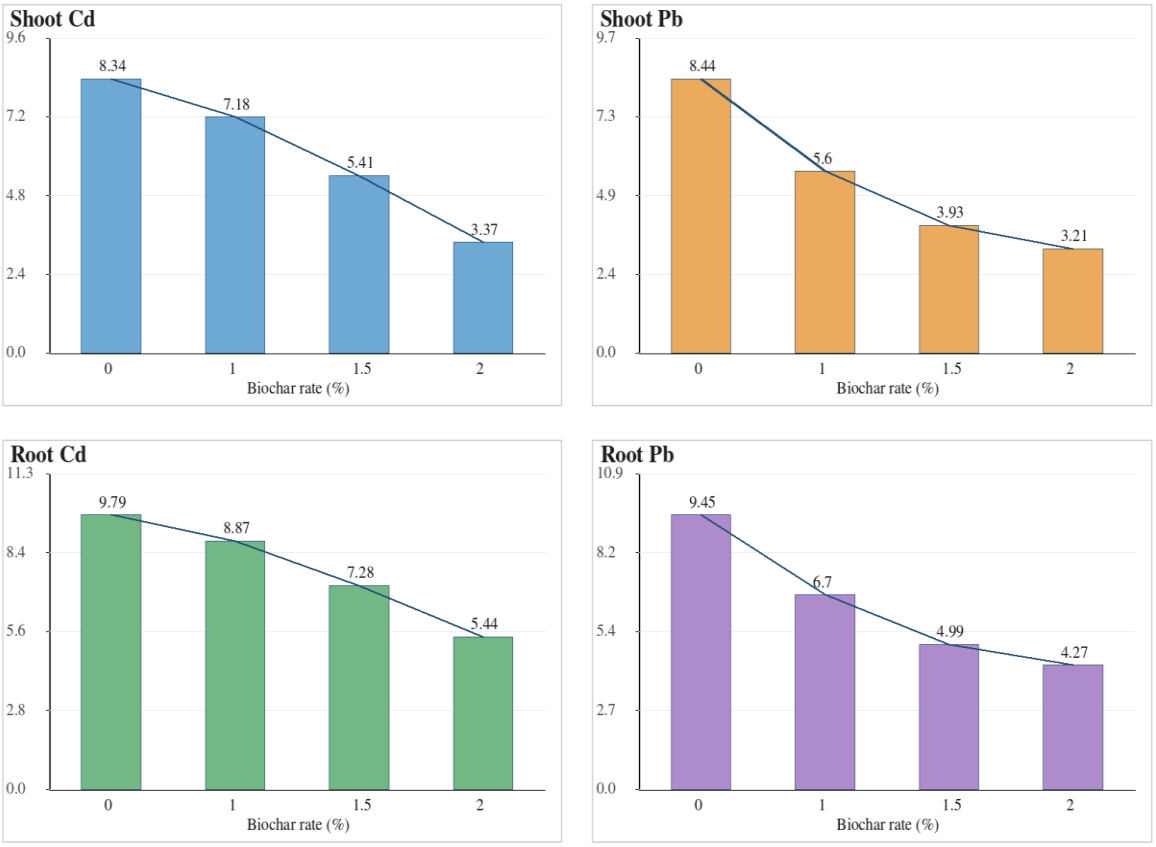

Figure 3 Cd and Pb concentrations in maize shoots and roots averaged across Bacillus strains.

The reduced concentrations of metals in shoots and roots is another piece of evidence that biochar and Bacillus inoculation decreased metal mobility from soil to plant. Low concentrations of metals in shoots are especially relevant as it reflects the lower risk of these metals progressing further up the food chain and into above ground biomass. The findings are consistent with the recent studies that biochar and heavy metal-immobilising bacteria are effective in lowering Cd and Pb uptake by the crops under contaminated environment (Wang et al., 2022; Qin et al., 2023; Ma et al., 2024).

Metal Uptake and Translocation

Biochar also decreased Cd and Pb uptake and translocation. The level of uptake of Pb decreased from 5.85 to 3.55 mg pot-1 between 0 and 2% biochar. The highest mean Cd uptake (6.00 mg pot-1) was found at 1% while at 2% biochar it decreased to 3.74 mg pot-1. The translocation factors for Cd and Pb decreased from 0.95 at 0% biochar to 0.80 at 2% biochar and 0.89 to 0.75, respectively, for 0 and 2% biochar. The lowest individual Pb translocation factor (0.74) was observed with B. paramycoides plus 2% biochar.

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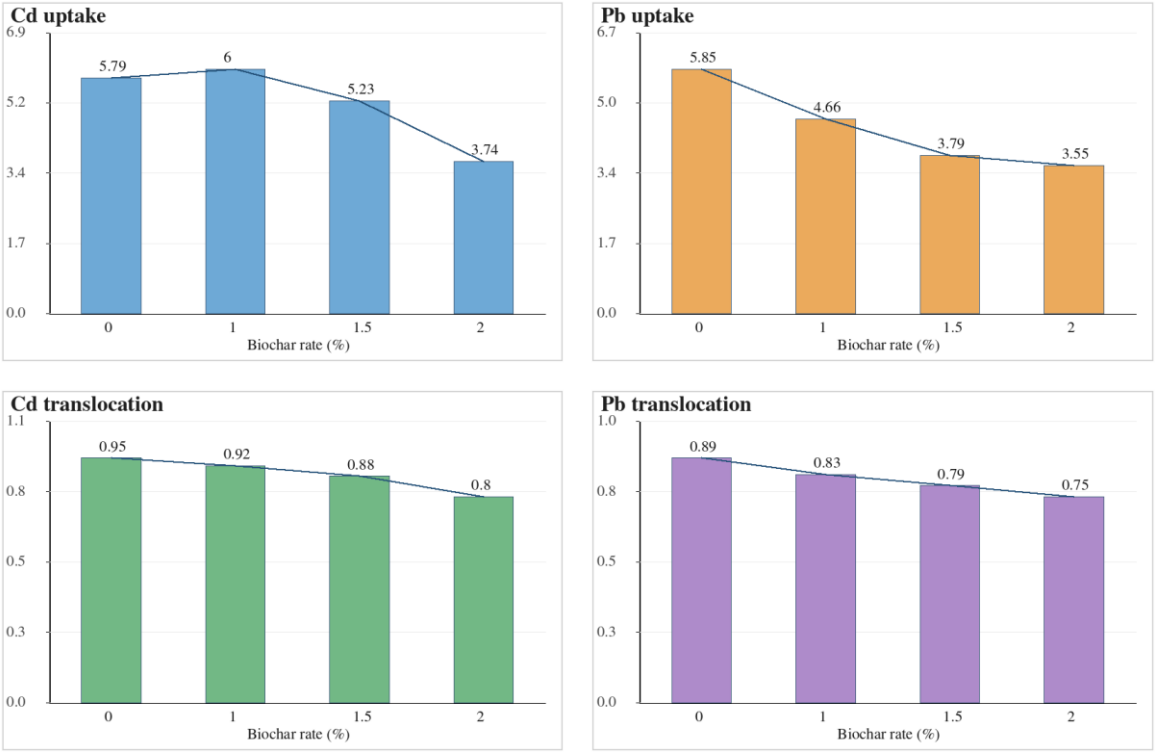

Figure 4 Effect of biochar rate on Cd/Pb uptake and translocation factor averaged across Bacillus strains.

This decrease in translocation indicates that the translocation of Cd and Pb from roots to shoots was restricted by the biochar and bacterial inoculation. This response is agronomically useful as it can be beneficial in terms of reducing the amount of metal accumulating above ground whilst at the same time improving crop growth. The current amendment regime is therefore more sensible than only a remediation measure in the case where contaminated soils are required to be in agricultural production.

Summary of Major Treatment Effects

Table 3 Main response of measured variables to 2% biochar compared with 0% biochar, averaged across Bacillus strains.

Response variable	0% biochar mean	2% biochar mean	Change at 2% biochar
Plant height (cm)	87	102	17.2% increase
Shoot dry biomass (g pot-1)	82.2	94	14.4% increase
Root dry weight (g pot-1)	3.31	5.21	57.4% increase
Soil AB-DTPA extractable Cd (mg kg-1)	7.16	1.92	73.2% reduction
Soil AB-DTPA extractable Pb (mg kg-1)	8.15	3	63.2% reduction

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Response variable	0% biochar mean	2% biochar mean	Change at 2% biochar
Shoot Cd concentration (mg kg-1)	8.34	3.37	59.6% reduction
Shoot Pb concentration (mg kg-1)	8.44	3.21	62.0% reduction
Root Cd concentration (mg kg-1)	9.79	5.44	44.4% reduction
Root Pb concentration (mg kg-1)	9.45	4.27	54.8% reduction
Cd uptake (mg pot-1)	5.79	3.74	35.4% reduction
Pb uptake (mg pot-1)	5.85	3.55	39.3% reduction
Cd translocation factor	0.95	0.8	15.8% reduction
Pb translocation factor	0.89	0.75	15.7% reduction

Conclusion

Heavy metal tolerant strains of Bacillus spp and biochar when applied together enhanced the growth of maize plants and decreased the bioavailability, uptake and translocation of Cd and Pb in contaminated soil. Application of biochar at 2% was the most efficient amendment rate in reducing the concentrations of AB-DTPA extractable Cd and Pb, as well as reducing the TFs and concentrations of metals in the maize tissues. The bacterial strains generally had a good immobilization capability for the metals, and B. paramycoides showed the best ability, while B. proteolyticus showed the highest shoot dry biomass. Overall, the best treatment for reducing Cd and Pb risk without compromising maize growth in contaminated soils is the B. paramycoides with 2% biochar treatment.

Recommendations

The use of metal-resistant Bacillus strains in combination with 2% biochar is suggested as an eco-friendly approach to deal with Cd- and Pb-polluted agricultural soil. Field-scale trials on other soil textures, contamination levels and cropping seasons should be performed to validate the long-term efficacy of metal immobilization and its impact on yield and food safety. Further research is also required to assess the properties of biochar and the survival of bacteria when added to soil, to understand how this reduction in metal uptake is achieved.

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