

Mitigating Heavy Metal Toxicity In Maize Under Contaminated Soil Using Spent Mushroom Substrate Biochar And Growth-Promoting Rhizobia

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ABSTRACT

Soil heavy metal toxicity has been rising worldwide, and bioremediation via sustainable and recalcitrant materials has been put into the spotlight in recent years. A pot experiment was carried out in the glass house of the Department of Soil and Environmental Sciences, The University of Agriculture, Peshawar to investigate the effect of spent mushroom substrate (SMS) biochar and plant growth promoting rhizobia (PGPR) under Cd and Pb contaminated soil. The experimental design was completely randomized design with seven treatments and three replications. The treatments consisted of contaminated soil with Cd (20mg kg⁻¹ soil) + contaminated soil with Pb (20mg kg⁻¹ soil) + PGPR + SMS biochar (0.5, 1, 1.5 and 2% with PGPR). The use of PGPR with 2 % SMS biochar resulted in significant increase in plant height 38.7 %, shoot dry weight 37.5 % and root dry weight 67.8 % than the contaminated control. The concentration of Cd in roots and shoots was higher in contaminated soil without biochar; root and shoot Cd concentrations were reduced by 54 and 73% respectively with PGPR amended with 2% SMS biochar and 62 and 67% respectively in contaminated soil without PGPR. The chlorophyll content also significantly improved with PGPR and SMS biochar by 5-43% over the contaminated

control. Moreover, application of 2% biochar in combination with PGPR decreased the AB-DTPA extractable Cd and Pb in soil by 52 and 44%, respectively. The soil organic matter and nutrient contents were also enhanced under PGPR and biochar. The results offer a sustainable solution to decontaminate Cd and Pb toxic soils and enhance the growth of maize and soil fertility.

Keywords: Spent Mushroom Substrate; Biochar; PGPR; Cadmium; Lead; Maize; Contaminated Soil; AB-DTPA

Introduction

Maize (*Zea mays* L.) is an important cereal crop used for animal feed, human nutrition and industrial products. Maize is a C4 crop which is efficient at assimilating nutrients that have been absorbed and converting them into grain and biomass but its development may be limited by the ability of low levels of nutrient uptake and physiological activity due to soil contamination. Heavy metal contamination of agricultural soil is therefore a very important concern as the presence of heavy metals, like cadmium (Cd) and lead (Pb) may inhibit plant growth, impact soil biological activity, and pose a risk to soil-to-plant transfer of metals (Irfan et al., 2021; Shahid et al., 2017).

Spent mushroom substrate (SMS) is a partially degraded agricultural waste product that remains from mushroom growing. High in organic matter and nutrients and will enhance soil physical, chemical and biological characteristics when recycled as an amendment. The biochar derived from SMS has the potential to be further enriched by its use due to its many attributes such as high surface area, porosity, functional groups, and the capacity to immobilize heavy metals, which also contribute to soil fertility (Roy et al., 2015; Lou et al., 2017; Huang et al., 2021; Chen et al., 2022).

Plant growth promoting rhizobacteria (PGPR) are bacteria that live in the soil around plants and help them grow by fixing nitrogen, making phosphorus more available, making plant growth hormones, producing siderophores and providing mechanisms for tolerating stresses. In combination with biochar, PGPR can work better due to the better soil habitat, nutrient retention and microbial survival provided by biochar. The application of biochar and PGPR from SMS has been previously demonstrated to promote plant growth and biochemical response under stress conditions (Guan et al., 2023; Širić et al., 2022; Wang et al., 2023).

According to Park et al. (2011), biochar has been found to have a positive effect on the adsorption and immobilization process to decrease the bioavailability and phytotoxicity of soil heavy metals. Al-Wabel et al. (2015) reported that biochar amendment could reduce the availability and uptake of heavy metals by maize plants and Irfan et al. (2021) concluded that biochar and compost application led to better growth of maize and immobilization of Pb, Cd and Cr from contaminated soil. Similarly, Širić et al. (2022), Guan et al. (2023) and Wang et al. (2023) reported that the combined use of SMS-derived biochar and plant growth-promoting rhizobacteria can improve plant growth, soil nutrient status and heavy-metal stress tolerance.

The real physicochemical assessment of the polluted medium is essential for the proper evaluation of environmental quality. The principles of physicochemical monitoring are also applied in drinking water quality assessment for Durbakheil,

Tehsil Halimzai, District Mohmand, Pakistan, before interpreting the contamination risk, which is also important (Khan and Khan, 2026). The effect of both SMS biochar and PGPR were tested under Cd and Pb stress on growth parameters of maize, soil parameters after harvest, and metal uptake in maize.

The objectives were: (i) to find out the effect of SMS-derived biochar and PGPR on growth of maize under Pb and Cd stress; (ii) to find out the effect of SMS-derived biochar and PGPR on concentration of Pb and Cd in plant shoot and root and (iii) to find out the effect of SMS-derived biochar and PGPR on soil AB-DTPA extractable Pb and Cd in the soil.

Materials and Methods

Experimental design and setup

A pot experiment was arranged in glass house of The University of Agriculture, Peshawar. The experimental design used was completely randomized design with three replications. Soil was taken from a research farm field, dried and passed through a 2 mm sieve. Soil was sieved and put in pots of 40 cm length and 30 cm diameter each containing 10 kg of soil. Pollution was carried out by adding nitrate of Pb and Cd at 20 mg kg⁻¹ soil each to the soil. SMS biochar was applied at 0.5%, 1%, 1.5% and 2% and PGPR consortium (20 mg pot⁻¹) was mixed with distilled water to prepare biofertilizer inoculum and then applied to the upper soil surface. Basal NPK dose rates of 120, 90 and 60 kg ha⁻¹ of urea, single super phosphate and muriate of potash, respectively, were used. The seeds were planted at a rate of 5 seeds/pot and all agronomic practices were the same. A composite soil sample was first analysed to determine the physicochemical properties of the soil, as per the soil and environmental assessment procedures (Thomas, 1996; Rhoades, 1996; Khan and Khan, 2026).

Table 1 Treatment details used in the pot experiment.

S. No.	Treatments
1	Control (without Pb and Cd)
2	Contaminated control (with Pb and Cd)
3	PGPR only (20 mg pot ⁻¹)
4	Biochar 0.5% + PGPR (50 g biochar per 10 kg soil)
5	Biochar 1% + PGPR (100 g biochar per 10 kg soil)
6	Biochar 1.50% + PGPR (150 g biochar per 10 kg soil)
7	Biochar 2% + PGPR (200 g biochar per 10 kg soil)

Note: BC = biochar; PGPR = plant growth-promoting rhizobia; C-control = contaminated control.

Crop data and plant analysis

Two plants were randomly selected in each pot and plant height was measured from the base of the plant to the top. Maize plants were uprooted, brought to the laboratory, roots were separated from shoots, plant were washed with distilled water and fresh shoot and root weight was recorded. Roots were air-dried, put in paper bags and oven dried for 48 h at 70°C. Chlorophyll contents (SPAD) were taken at the tasseling stage

at the mid point of the youngest fully expanded leaves, and a mean value was calculated for each pot as recommended by Costa et al. (2003).

Root and shoot samples of 0.5 g were separately digested in concentrated HNO₃ and HClO₄ till the digest became an off white colour. The digests were cooled, filtered through Whatman No. 42 filter paper and diluted to 100 mL. Cd and Pb concentrations were determined by the atomic absorption spectrophotometry (AAS) after standard elemental analysis methods (Walsh and Beaton, 1973). Translocation factor (TF) and bioaccumulation factor (BAF) were calculated as per Ahmed and Slima (2018).

Soil analysis

The soil samples were taken from each of the pots after harvest, dried, ground and sieved through 2 mm mesh. Soil pH was determined using a 1:5 soil-water suspension (Thomas, 1996) and electrical conductivity was measured after the instrumental calibration with standard solutions (Rhoades, 1996). Soil organic matter was calculated using the method of dichromate oxidation (Nelson and Sommers, 1983). The AB-DTPA extractable lead and cadmium were extracted with 20 mL AB-DTPA extracting solution for 15 min with 10 g soil and measured by atomic absorption spectrophotometry (Soltanpour and Schwab, 1977).

Total nitrogen was analysed after digestion and distillation as described by Bremner (1996). The available P and K were extracted by AB-DTPA and measured by colorimetric method and Atomic Absorption Spectrometry (Murphy and Riley, 1962; Soltanpour and Schwab, 1977). Analysis of variance and the least significant difference (LSD) test were used for the analysis of the data and separation of the treatment means, respectively, at $P \leq 0.05$ according to standard statistical procedures (Steel and Torrie, 1980).

The data was visualized and prepared for graphs using Pandas for tabular data handling (McKinney, 2010) and Matplotlib for publication quality figures (Hunter, 2007) both of which are python packages. Experimental data, treatment names, units, percentage responses remained unchanged (except for the numerical values used for the graphs, which are directly from the original paper tables).

Results and Discussion

Initial soil physicochemical characteristics

The physicochemical properties of the soil sample were determined before the experiment from the composite soil sample of the depth 0-20 cm. The soil was silty clay loam, non-saline, slightly alkaline with an initial detectable concentration of Cd and Pb (Table 2).

Table 2 Physico-chemical properties of soil under investigation.

Characteristics	Unit	Values
Sand	%	14.5
Silt	%	56.3
Clay	%	29.2
Textural class	-	Silty clay loam

Characteristics	Unit	Values
EC(1:5)	dS m-1	0.209
pH(1:5)	-	7.60
Organic matter	%	0.35
ABDTPA extractable Cd	mg kg-1	0.3
ABDTPA extractable Pb	mg kg-1	1.1

Note: Soil sample was collected before treatment application from 0-20 cm depth.

Agronomic parameters

Maize growth was highly affected by heavy metal contamination, when compared to original control (Figure 1). The negative impact of Cd and Pb on plant development was suggested from the reduction in plant height in the contaminated control. The treatments of PGPR alone resulted in an increase in plant height by 9.6% compared with the contaminated control, whereas the plant height in the treatments of PGPR + 0.5, 1, 1.5 and 2% biochar was increased by 14.5, 22.5, 29.0 and 38.7%, respectively compared to the contaminated control. As per this pattern, when both PGPR and biochar were applied, plant growth under heavy metal stress was improved, likely due to their ability to immobilize the metals, promote nutrient availability and boost microbial activity (Irfan et al., 2021; Dawar et al., 2023; Guan et al., 2023).

Shoot and root biomass also increased with the increasing level of SMS biochar. Shoot dry weight (39.6 g pot-1) and root dry weight (4.71 g pot-1) were the highest when applied with 2% biochar + PGPR. The enhanced soil structure, organic matter, and nutrient retention by biochar can promote root growth, shoot biomass, and stress tolerance, and PGPR can help with nutrient uptake. This growth enhancement in contaminated soils has been observed in previous studies, where biochar was applied to contaminated soils (Ali et al., 2017; Younis et al., 2016; Wang et al., 2023).

Maize growth response to PGPR and SMS biochar

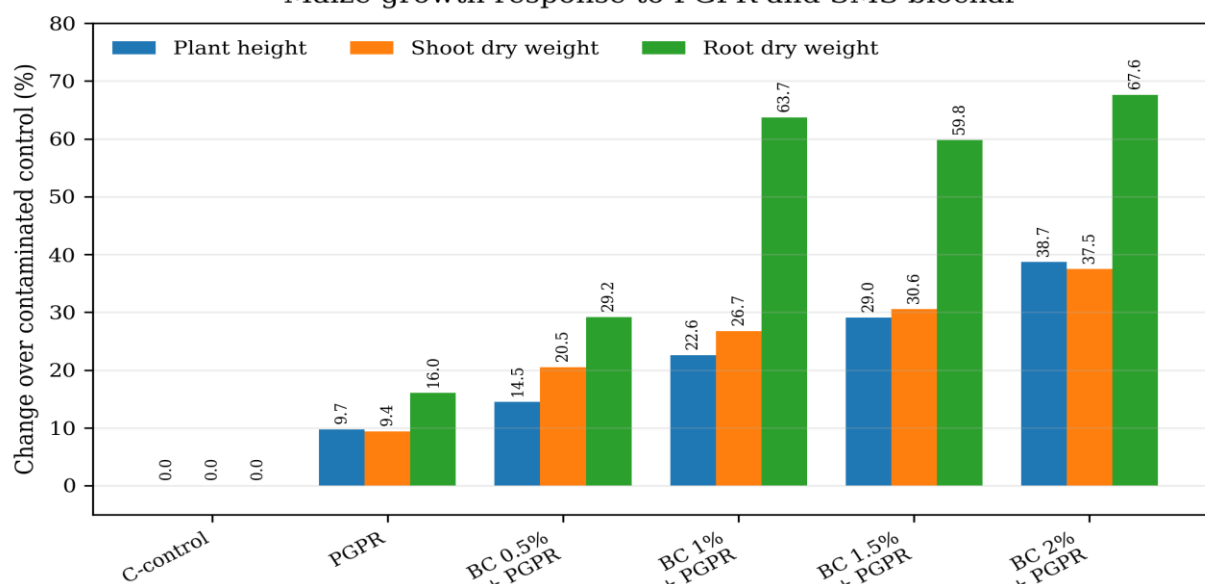


Figure 1 Growth response of maize under Cd- and Pb-contaminated soil as influenced by PGPR and SMS biochar. Bars show percentage change over contaminated control.

Cadmium concentration in maize roots and shoots

The highest concentration of cadmium was found in the root and shoot of maize in the contaminated control, while the lowest was found in the uncontaminated control (Figure 2). Cd concentration in the roots and shoots was reduced by 9.0% and 8.1% respectively by using PGPR alone. The effect of both PGPR and SMS biochar-application in a combined way was dose dependent for Cd uptake. The reduction of Cd content in roots and shoots was 54.0% and 73.5% respectively at 2% biochar amended with PGPR with respect to the contaminated control. Under metal stress, Biochar can reduce bioavailability of Cd through adsorption, surface complexation, precipitation and improvement of soil pH, and PGPR can help plant tolerate metal stress (Park et al., 2011; Al-Wabel et al., 2015; Bashir et al., 2020; Liu et al., 2018).

Cadmium concentration in maize tissues

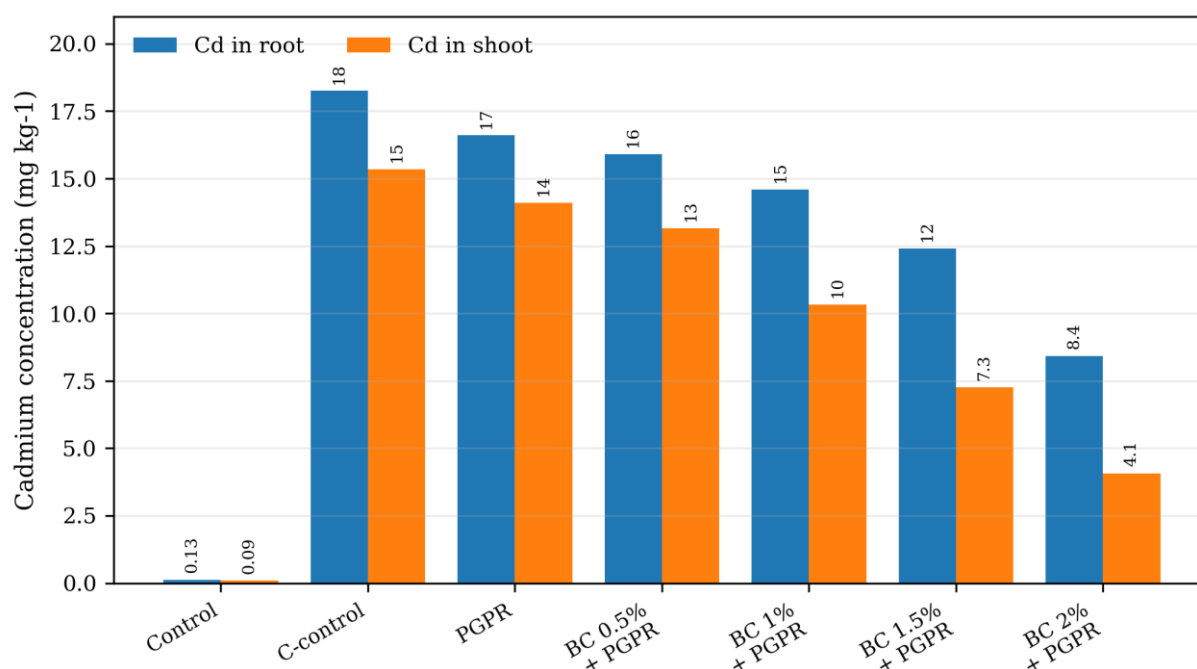


Figure 2 Cadmium concentration in maize root and shoot under PGPR and SMS biochar treatments.

Lead concentration in maize roots and shoots

The application of PGPR and SMS biochar was also seen to have a prominent effect on lead concentration (Figure 3). The highest concentration of Pb in roots and shoots was found in the contaminated control, and the lowest concentration was found in the control. The 2% biochar with PGPR treatment showed the lowest concentration of Pb in the maize tissues, while increasing the biochar level with PGPR, reduced the concentration of Pb in the tissues of maize plants. Biochar surface adsorption, precipitation and complexation reactions, cation exchange reactions have been proposed to be responsible for the reduction in Pb uptake, as these processes restrict the mobility and translocation of Pb in the plant tissues (Cao et al., 2011; Wang, Feng and Yang, 2019; Liang et al., 2021; Rees et al., 2020).

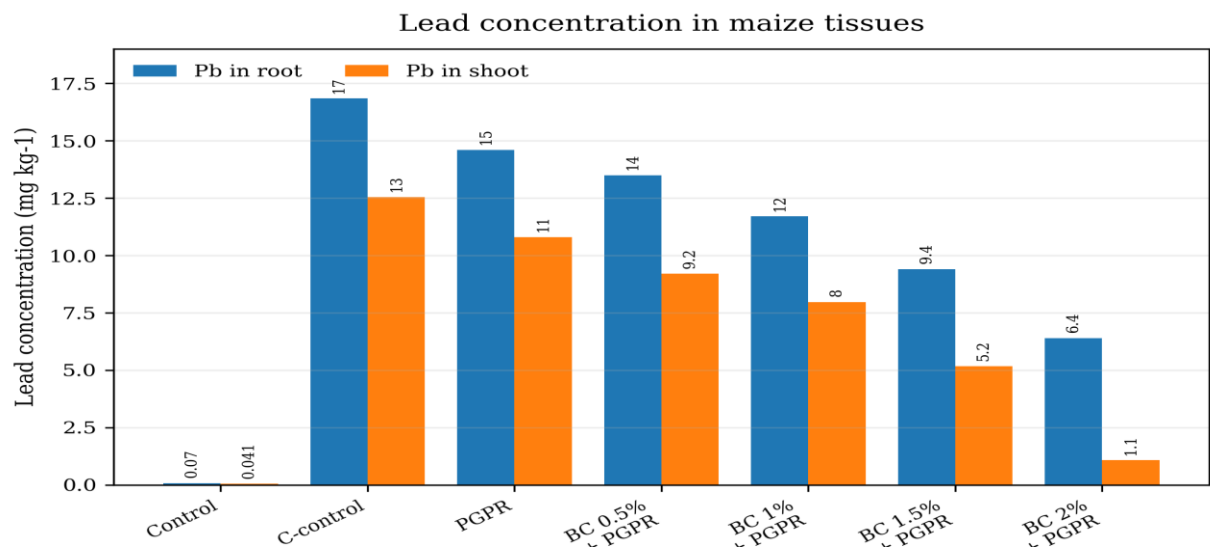


Figure 3 Lead concentration in maize root and shoot under PGPR and SMS biochar treatments.

Leaf area and chlorophyll content

The growth and physiology of plants was affected by the presence of heavy metals but there was considerable enhancement of leaf area and chlorophyll content by PGPR and SMS biochar (Figure 4). The application of PGPR alone produced 5.0 per cent increase in leaf area over the contaminated control, and the application of PGPR with 2 per cent biochar produced 19.0 per cent increase in leaf area over the contaminated control. The chlorophyll content ranged from 38.0 (contaminated control) to 54.6 SPAD with 2% biochar with PGPR. Improved photosynthetic capacity and lower heavy metal toxicity may be linked to better nutrient availability, water retention, and reduced metal uptake in biochar amended soil (Minotta and Pinzauti, 1996; Yang et al., 2022; Huang et al., 2024) which is reflected by the chlorophyll response.

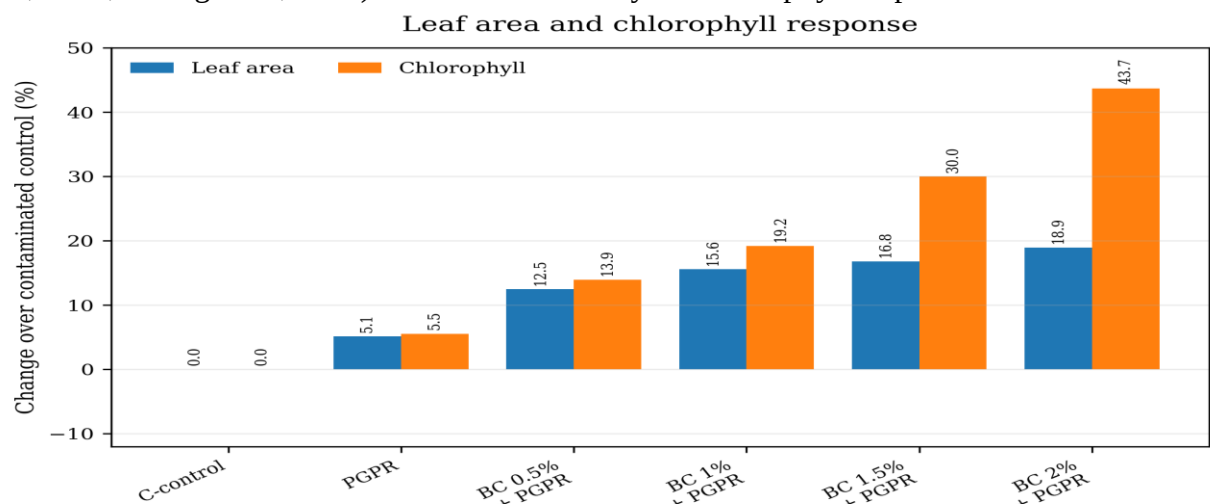


Figure 4 Leaf area and chlorophyll response of maize under PGPR and SMS biochar treatments. Bars show percentage change over contaminated control.

Translocation factor of Cd and Pb

The movement of Cd and Pb from root to shoot was the highest in the contaminated control and decreased with the increasing SMS biochar level (Figure 5; translocation factor). The 2% biochar with PGPR treatment had the lowest Cd TF (0.48) and Pb TF (0.55) values. The results suggest that increasing SMS could be used to decrease the mobility of metals from roots to shoots, which may be due to the effect of biochar on immobilizing metals in the rhizosphere and reducing their bioavailability for upward translocation (Beesley et al., 2011; Chen, Zhang and Luo, 2018; Zhang, Li and Wang, 2019).

Cd and Pb translocation factor from root to shoot

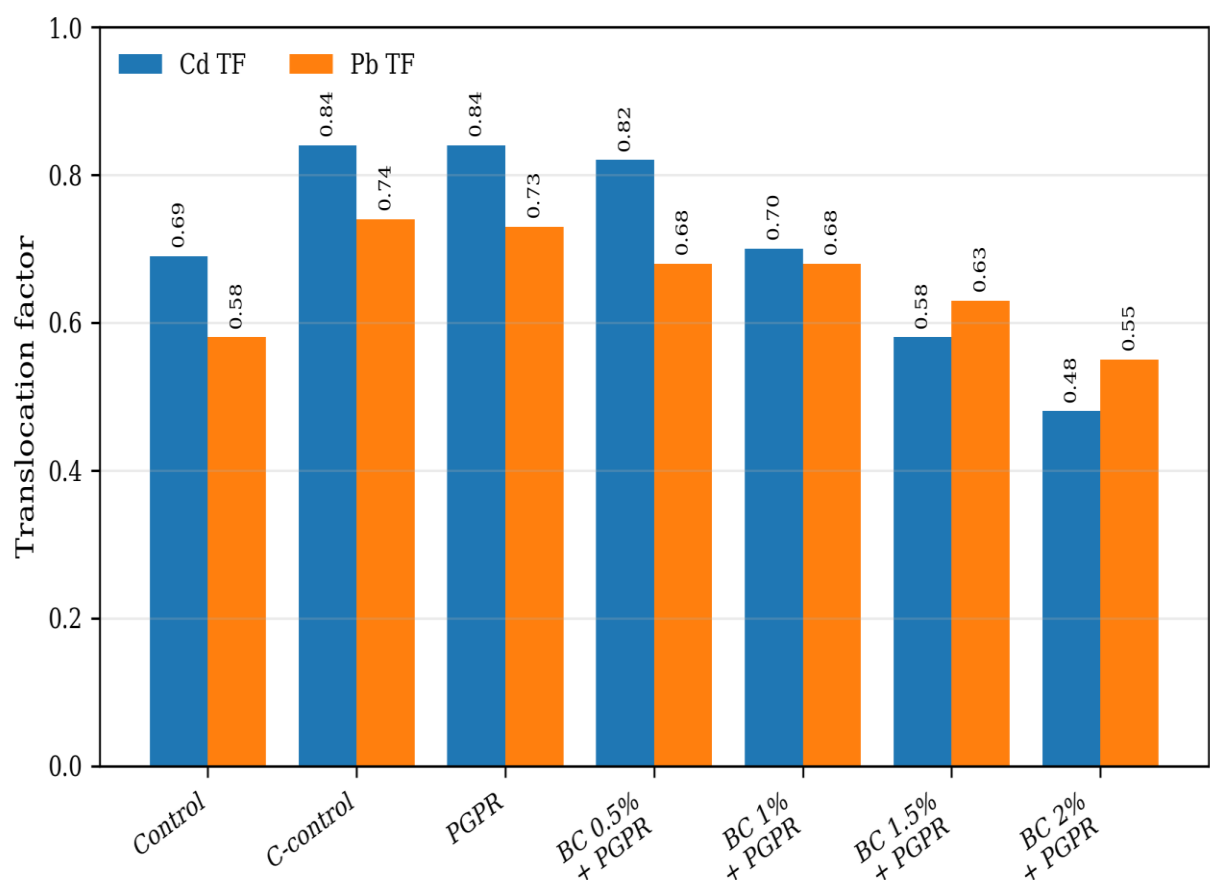


Figure 5 Translocation factor of Cd and Pb from root to shoot of maize.

Post-harvest soil pH, EC and organic matter

The post-harvest soil pH, EC and organic matter were significantly affected by PGPR and SMS biochar (Figure 6). Under 2% biochar with PGPR, the soil pH was raised from 7.48 (in the contaminated control) to 7.62. The highest EC (0.24 dS m⁻¹) was also observed with 2% biochar with PGPR whereas the OM was raised from 0.72% (contaminated control) to 1.30% (under 2% biochar with PGPR). The higher organic matter points to the potential of SMS biochar as a carbon source and soil conditioner, which is beneficial for improving soil fertility and immobilizing heavy metals (Glaser et al., 2002; Lou et al., 2017; Wang et al., 2023).

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Post-harvest soil pH, EC and organic matter response

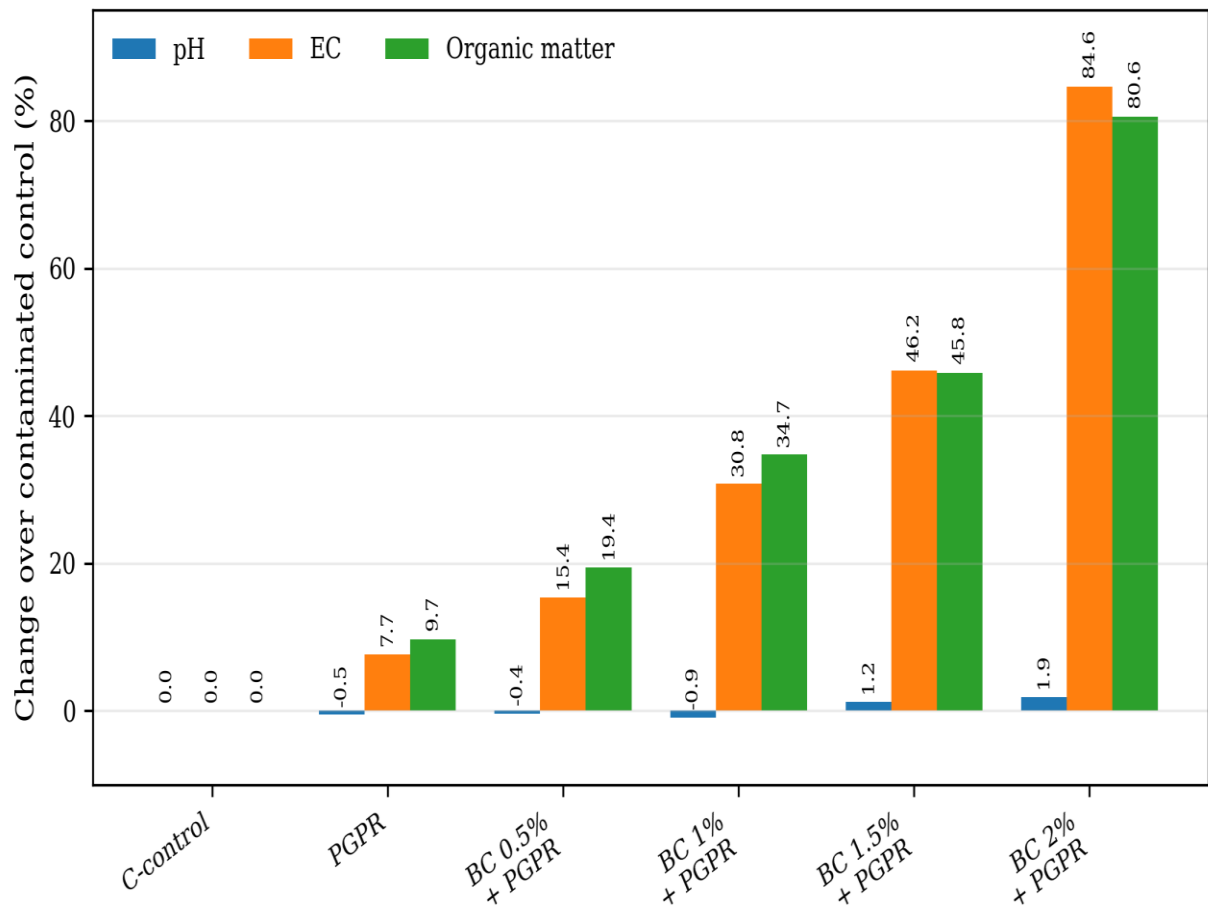


Figure 6 Post-harvest soil pH, EC and organic matter response under PGPR and SMS biochar treatments. Bars show percentage change over contaminated control.

Post-harvest soil N, P, K, Cd and Pb

The use of PGPR and SMS biochar led to an increase in the nutrient contents of the post-harvest soil (Figure 7 and 8). The highest total N (0.13%), available P (8.16 mg kg⁻¹) and K (108.14 mg kg⁻¹) were observed under 2% biochar with PGPR. During the same treatment, the AB-DTPA extractable Cd and Pb concentrations were found to be 2.34 and 2.46 mg kg⁻¹, respectively, whereas 6.32 and 7.06 mg kg⁻¹ of Cd and Pb were obtained from contaminated control, respectively. The reductions were evidence that simultaneous application of SMS biochar and PGPR reduced the bioavailability of Cd and Pb, and enhanced the soil nutrient status. The reductions in availability of heavy metals after biochar addition have been previously reported as a result of adsorption, precipitation, cation exchange and complexation mechanisms in contaminated soils (Park et al., 2011; Ali et al., 2017; Irfan et al., 2021; Huang et al., 2021).

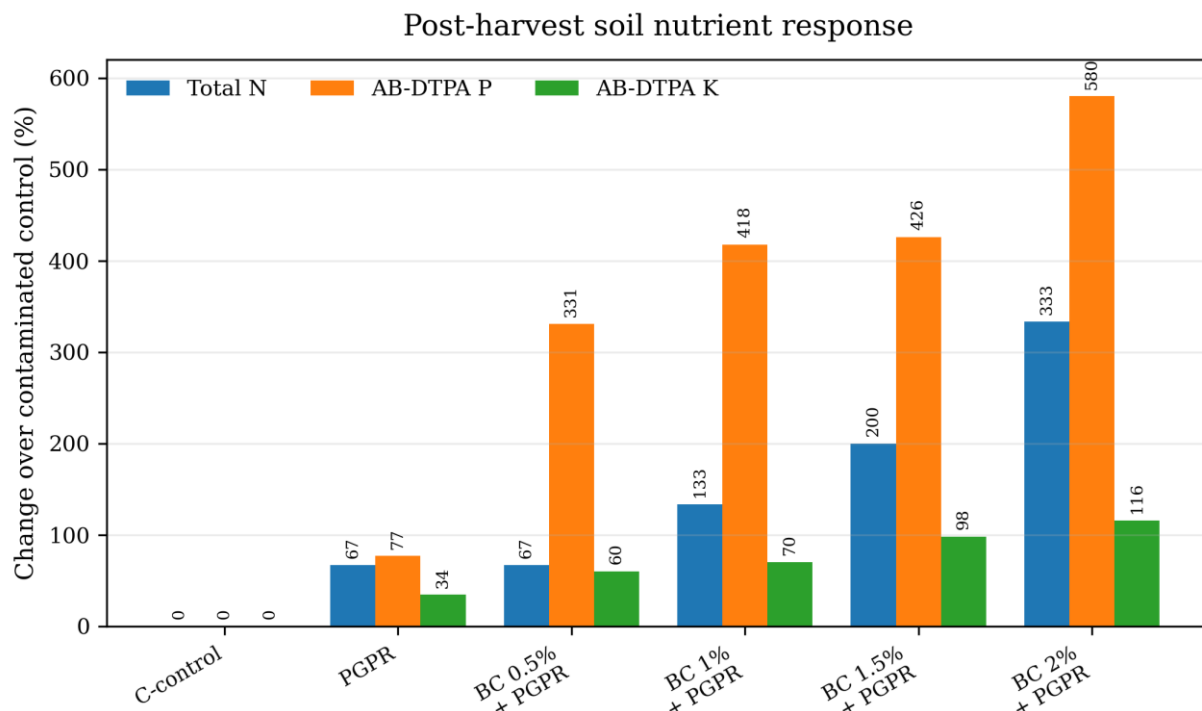


Figure 7 Post-harvest soil nutrient response under PGPR and SMS biochar treatments. Bars show percentage change over contaminated control.

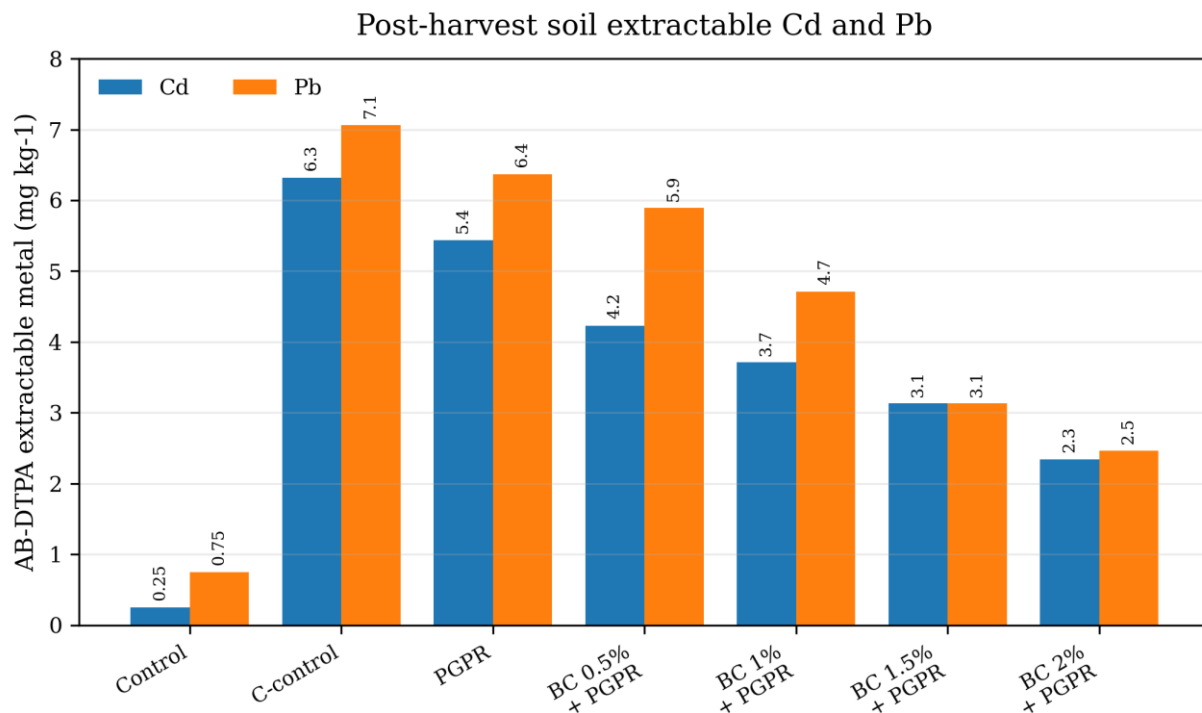


Figure 8 AB-DTPA extractable Cd and Pb in post-harvest soil after PGPR and SMS biochar application.

Conclusions and Recommendations

The use of plant growth-promoting rhizobia (PGPR) together with spent mushroom substrate (SMS)-derived biochar was effective in the alleviation of Cd and Pb phytotoxicity in maize under contaminated soil. Heavy metal contamination caused a decrease in plant height, increase in Cd and Pb accumulation in root and shoot, and decrease in shoot and root biomass, leaf area and chlorophyll content. PGPR alone was effective in enhancing maize growth and decreased uptake of metals, but the highest growth response was observed when 2% SMS biochar was added with PGPR. This treatment has led to enhanced plant growth, shoot and root dry matter content, leaf area, soil nutrient status (AB-DTPA), the amount of chlorophyll, and a decrease in the concentrations of Cd and Pb in the plants. In general, 2% SMS biochar application supplemented by PGPR could be regarded as a sustainable and feasible amendment to boost maize growth and decrease the bioavailability of Cd and Pb in contaminated agricultural soils.

From the results obtained from this research, the supplementary application of 2% biochar derived from SMS in combination with PGPR is suggested for decreasing production of bioaccumulated heavy metals in maize under contaminated soil condition. It is also recommended that the use of SMS biochar is an eco-friendly solution that can be utilized to recycle mushroom wastes to provide value added soil amendments. Additional field-scale investigations are required to support its broader adoption in the field, however, under varying soil types, levels of contamination, climatic conditions and cropping systems. Before recommending this technology for widespread use in agriculture, further studies are needed exploring the mechanisms of metal immobilization, the survival of microorganisms, nutrient dynamics, and the accumulation of metals in grains and the economic viability of the technology.

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