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Bioremediation potential of biochar, compost and *Bacillus* sp. N18 for lead contaminated soil and improving physiological and morphological attributes of maize

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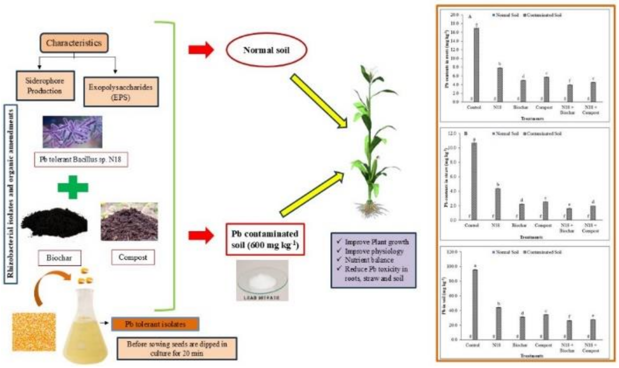
Graphical abstract



Abstract

Heavy metals like Pb, Cd, As, and Ni are becoming major environmental pollutants affecting crop productivity. The current study focused on the integration of biochar, compost, and Pb-tolerant *Bacillus* sp. (N18) to mitigate the hazardous impacts of Pb and improve maize growth under normal and Pb-spiked contaminated soil. There are six treatments in both normal and contaminated soil, arranged in CRD (completely randomized design) under a factorial setting with three replications. The findings confirmed the synergistic role of the combined application of biochar, compost, and *Bacillus* sp. (N18) in improving the growth of maize under both soils. The use of biochar+ *Bacillus* sp. (N18) in contaminated soil significantly enhanced maize growth *viz.* shoot, root length and their dry weight, antioxidant activity like SOD, POD, CAT, APX by 50.9, 82.6, 73.3, 46.7, 51.6, 42.5, 35 and 45.4%, respectively, over control. In contrast, significant reduction in the Pb uptake in maize root and straw under combined use of biochar and *Bacillus* sp. (N18), with values of 61.9 and 65.5%, respectively, while after harvest, extractable Pb contents in soil were also reduced by 79.7% over control. The bioaccumulation and translocation factors were also decreased by the use of biochar + *Bacillus* sp. (N18), which is 44.7 and 35.2% as compared with control. This dual behavior by synergizing organic amendments (compost and biochar) and *Bacillus* sp. (N18) can efficiently bioremediate the Pb toxicity in soil and improve maize production, which can help developing sustainable bioremediation methods.

Keywords: Biochar; compost; bioremediation; *bacillus* sp.; lead.

1. Introduction

Heavy metals (HMs) contamination is a serious issue for the soil, which can degrade the agroecosystem, food security, and serious human and animal health issues in the food chain (He *et al*., 2022). Due to their persistent and toxic nature, heavy metals are the universal pollutants that have great consequences for human and environmental health (Horn *et al*., 2019; Kaur *et al*., 2019). The higher amounts of various heavy metals i.e., lead (Pb), arsenic (As), and cadmium (Cd) were studied to cause various neurological issues and expression of genes for various diseases/problems in humans (Saddique *et al*., 2018). In the environment, these metals can enter by different anthropogenic as well as natural activities like crumbling, eroding, smelting, wastewater, agro-chemicals, etc., and cause harmful impacts on microorganisms that exist in soil, also disturbing the chemical and physical attributes of soil (Ali *et al*., 2019). In the soil environment, lead is a highly toxic substance that affects the soil flora and fauna. Lead contamination in soil comes from different activities such as smelting and mining activities, municipal sludge disposal, usage of lead-containing products such as paints and dyes, pulp and paper, as well as from explosives and gasoline, which can create lead toxicity in the environment (Raj and Maiti, 2020). Maize crop production was reduced in soil that is contaminated

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by lead because lead toxicity severely decreases the absorption of essential nutrients and chlorophyll contents in plants (Zanganeh *et al*., 2021). Also, lead contamination has negative impacts on plant growth, morphology, and photosynthetic processes. `Moreover, a higher level of lead reduces enzyme activities, water balance disruption, change in membrane permeability, and reduction in minerals nutrition (Fan *et al*., 2020). Furthermore, exposure to lead increases oxidative stress by enhancing the synthesis of ROS (reactive oxygen species) reported by Maneechakr and Mongkollertlop (2020).

Globally, different biological and physiochemical methods are applied to eliminate heavy metals from contaminated environments (Zulfiqar *et al*., 2023b; Rasee *et al*., 2023). But any method adaptation relies on its feasibility, reliability along with its applicability (Mustafa *et al*., 2023; Sarwar *et al*., 2023a). In contrast, the method of bioremediation in which plant growth-promoting bacteria was used could be an effective strategy with an eco-friendly and cost-effective nature (Sarwar *et al*., 2023b; Shahid *et al*., 2024). The PGPR is present and resides in plants' roots and increases plant growth through various mechanisms of growth promotion (Shabaan *et al*., 2023). However, PGPR efficiently induces resistance in response to heavy metal toxicity in crops by metal complexation and immobilization by releasing different growth-promoting hormones and exopolysaccharides (Yaashika *et al*., 2022; Mahmood *et al*., 2024). Their mechanism either leads to extraction (Konkolewska *et al*., 2020) or heavy metal stabilization (Ke *et al*., 2021; Raag Harshavardhan *et al*., 2022). Soil amended with organic matter enhances the microbial activities in rhizosphere soil (Shahbaz *et al*., 2017) by supplying organic carbon sources (Zulfiqar *et al*., 2023b). Furthermore, biochar has positive effects on the detoxification of heavy metals beyond its ability to increase crop and soil growth (Rahim *et al*., 2024; Qian *et al*., 2023). Also, it was studied that the application of biochar to metal-contaminated soil has a differential impact on plant growth by increasing soil properties such as soil pH, water holding capacity, absorption of essential nutrients, and decrease in the mobility of heavy metals due to alkaline nature soil (Yang *et al*., 2021; Salam *et al*., 2019). However, compost is also used as a natural fertilizer in agricultural soils to reduce the use of synthetic fertilizers. The use of compost improves the chemical and physical soil properties, soil fertility, and microbial population, which improves crop biomass, plant growth attributes, and also acts as a binding agent for soil aggregates (Calleja-Cervantes *et al*., 2015; Palanisamy *et al*., 2023). Composting can reduce the failure of crops and economic loss caused by metal pollution and also decrease human health issues (Ayilara *et al*., 2020). However, organic amendments to soil increase soil properties like fertility (Bonanomi *et al*., 2020), structure of soil (Rahman *et al*., 2017), porosity (Luna *et al*., 2018), cation exchange capacity (Domingues *et al*., 2020) and different other quality characters (Teodoro *et al*., 2020). In soil, it readily transforms and immobilizes heavy metals and reduces them into un-available for the uptake of plants (Sarwar *et al*., 2023a, 2023b). Different research activities have demonstrated the effect of various types of compost and biochar alone in decreasing the availability of heavy metals and improving soil fertility (Saleem *et al*., 2023). Therefore, the present investigation used a novel strategy of integrating biochar, compost, and bacteria which has not been studied earlier. Moreover, this study also providesa comparison of sole and integrated application of *Bacillus* sp. (N18), compost, and biochar to minimize the negative impacts associated with Pb toxicity in maize, bioremediation of Pb-contaminated soil, and improve the maize growth, physiology, and nutritional status.

2. Materials and methods

2.1. Collection of biochar, compost, and bacterial strain (N18)

Exopolysaccharide-producing pre-isolated, characterized, and identified lead tolerant bacterial strain *Bacillus* sp. (N18) with accession number MK999911 (Seher *et al*., 2020) was obtained from Soil Microbiology and Biotechnology Laboratory, Department of Soil Science, The Islamia University of Bahawalpur, Pakistan. Biochar and compost were obtained from Environmental Microbiology Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan.

2.2. Analysis of biochar, compost and soil

Analysis of biochar, compost, and soil was done at the Soil Microbiology and Biotechnology Laboratory, Department of Soil Science, The Islamia University of Bahawalpur, by using standard procedure described by Ryan *et al*., (2001). For phosphorus and potassium determination, samples were digested with H_2SO_4 and H_2O_2 (McGill and Figueiredo 1993). Potassium and phosphorus in digested samples were examined by flame photometer and spectrophotometer, respectively. Nitrogen contents in samples are found with the Kjeldahl digestion and distillation unit (Jackson 1973). EC (electrical conductivity) and pH of 1:20 m/v ratio of samples in distilled water were recorded by using EC and pH meter respectively. Following the procedure described by Yeomans and Bremner (1988), carbon concentration was estimated. By using the procedure described by Chaturvedi and Sankar (2006), the soil sample was digested with HNO_3:$HClO_4$ mixture, and the reading was measured using an atomic absorption spectrophotometer (Model 240FS AA, Agilent Technologies Australia.). Chemical properties of compost, biochar, and soil are presented in Table 1.

2.3. Seed inoculation and experiment management

Hybrid maize seeds were obtained from the local market of Bahawalpur and disinfected by dipping for 2 minutes in ethanol 95% followed by 0.2% solution of $HgCl_2$ for 4 min then washed 5 to 6 times with sterilized distilled water. After that, seeds were immersed for 20 min in a broth culture of Pb-tolerant bacterial strains *Bacillus sp*. (N18) (Khalid *et al*., 2004; Russel *et al*., 1982). Treatments are comprised into two sets, i.e., normal soil set (T0=Control, T1 = *Bacillus* sp. (N18), T2 = Biochar, T3 =Compost, T4 = *Bacillus* sp. (N18) + Biochar, T5 = *Bacillus* sp. (N18) +

Compost) and same treatments are applied in lead-contaminated soil set.

At the wirehouse of the Department of Soil Science, a pot study was planned to find the impact of *Bacillus* sp. (N18), compost and biochar on the growth of maize under lead stress with the latitude 29.372053 and longitude 71.770679. Physio-chemical attributes of soil were calculated before experimenting (Table 1). About 20 kg of soil is filled in pots that are free from leaves, stems, and stones. Organic amendments like compost were applied @ of 600 kg ha^{-1} and biochar was applied with a ratio of 0.25%. In contaminated soil 600 mg Pb kg^{-1} was added by using lead nitrate salt and 18 pots were filled by this soil, while 18 pots were filled with normal soil. Inoculated and uninoculated seeds were sown in their respective normal and Pb-contaminated soil. Recommended N, P, and K (120, 90, 60 kg ha^{-1}) doses were used in the form of urea, (DAP) di-ammonium phosphate and SOP (sulfate of potash). Seeds are sown in pots at 4cm depth. Recommended agronomic practices (weeding, thinning, application of fertilizers, and irrigation) were implemented where and when required. Good quality irrigation water is used to irrigate pots that fulfill the criteria of fitness as proposed by Ayers and Westcot (Ayers *et al*., 1985). Data about physiological parameters were taken at physiological maturity and data regarding growth attributes (length and weight of shoot and root) and nutritional status were taken at harvest.

Table 1. Physio-chemical attributes of soil, biochar and compost used in pot trial

Characteristics	Unit	Values
Compost		
pH	---	6.34±0.070
Carbon	%	27.62±0.18
Organic matter	%	46.0±0.33
Total Nitrogen	%	1.16±0.006
Total Phosphorus	%	2.15±0.053
Total Potassium	%	1.06±0.005
Biochar		
pH	---	8.13±0.042
EC	(dS m^{-1})	1.59±0.047
Carbon	%	39.57±0.26
Total Nitrogen	%	0.21±0.005
Total Phosphorus	%	0.34±0.007
Total Potassium	%	1.15±0.054
Soil		
Clay	%	15.9±0.026
Sand	%	45.48±0.098
Silt	%	39.5±0.026
Textural Class	---	Loam
pH_s	---	8.5±0.030
EC_e	(dS m^{-1})	1.5±0.019
Saturation Percentage	%	41.3±0.30
Nitrogen	%	0.023±0.0004
Extractable phosphorus	mg kg^{-1}	5.7±0.026
Extractable potassium	mg kg^{-1}	105.3±0.54
Organic Matter	%	0.61±0.0041
Lead (Pb)	mg kg^{-1}	ND

**ND=Not detected*

2.4. *Antioxidant enzyme estimation*

Fresh leave samples (0.5g) were homogenized in ice-cooled mortar and placed in ice with 4 mL phosphate buffer [prepared in 1 L distilled water by dissolving $Na_2HPO.12H_2O$ (16.385g) + $NaH_2PO_4.2H_2O$ (0.663g)] at pH 7.8. Centrifuged the mixture at 4 °C at 10000*g* for 20 min. In the Eppendorf tube, the supernatant solution was collected, and the enzymatic activity was analyzed. The reaction solution was prepared for various enzyme activities such as POD (peroxidase) measured at 436 nm, APX (ascorbate peroxidase) measured at 290 nm, SOD (superoxide dismutase) measured at 560 nm, CAT (catalase) measured at 240 nm for color development and absorbance reading was measured by using a spectrophotometer. A blank solution was also prepared without enzyme extract, run at the start of each enzyme measurement, and enzyme activity was calculated by using the formula described in various publications (Mayer *et al*., 1966; Beauchamp *et al*., 1971; Asada, 1992; Bach *et al*., 2013).

2.5. *Growth and biochemical analysis*

At physiological maturity, SPAD value was noted, and samples of maize leaves were collected and examined for relative water content using the standard procedure described by Mayak *et al*. (2004). At plant maturity, the maize plants were harvested, and data regarding parameters like root, shoot length, and dry biomass were calculated. From each pot, root and shoot samples were air-dried, oven-dried, and ground into powder separately. Digest the root, shoot samples by adopting the procedure of wet digestion described by Wolf (1982). Nutrient contents in maize root, shoot was examined using three replications of each treatment. For the determination of nitrogen in root and shoot, the Kjeldhal method was adopted (Ryan *et al*., 2001). Measurement of phosphorus was done using the yellow color vanadomolybdophosphoric acid method (Jackson, 1973). A flame photometer is used for potassium determination from digested samples (Ryan *et al*., 2001). An atomic absorption spectrophotometer was used to find the lead contents in root and shoot digested samples. The BCF (biological concentration factor) and TF (translocation factor) are proposed in the equations,

$$BCF = Cshoot / Csoil \qquad (1)$$

Cshoot = metals contents in shoot (mg kg^{-1}) and Csoil = metals contents in soil (mg kg^{-1}), respectively (Cui *et al*. 2007).

$$TF = Cshoot / Croot \qquad (2)$$

Cshoot = metals contents in the shoot (mg kg^{-1}) and Croot = metals contents in the root (mg kg^{-1}), respectively (Yoon *et al*. 2006).

2.6. Statistical analysis

The data regarding different parameters was analyzed by two-way analysis of variance (ANOVA), by using CRD design under two-way factorial arrangement with three replications in each treatment, and means of treatment were compared by LSD test at 5% probability level (Steel, 2007).

3. Results

3.1. Growth attributes

The results confirmed that the use of biochar, compost, and *Bacillus* sp. (N18) in normal soil and lead-contaminated soil improves plant growth over control (Tables 2 and 3). In normal soil and lead-contaminated soil, the maximum increase in growth parameters in terms of root length, shoot length, and fresh and dry biomass was observed in treatment T4, where Biochar + *Bacillus* sp. (N18) was applied that was 25.9, 35.1, 25.2, 35.1, 18.7 and 39% in normal soil, while in contaminated soil 50.9, 82.6, 43.1, 73.3, 36.6 and 46.7% as compared to their respective control. While the subsequent improvement was observed in treatment T5 where Compost + *Bacillus* sp. (N18) was applied showed 27.6, 19.7, 13.4, 19.5, 28.7, 30.2% in normal soil and 79, 45.2, 28.7, 37.3, 40, 65.9% in Pb contaminated soil. Sole application of biochar (T2) also performs a significant result as compared with the control which was 63.9, 39.1, 21.8, 27.2, 33.8, and 59.9% in contaminated soil.

3.2. N, P, K contents in root and straw

Inoculation of *Bacillus* sp. (N18) and soil application with biochar and compost significantly improve the N, P, and K contents in the root and straw of maize plants in normal and lead-contaminated soil over control (Tables 4 and 5). Combine use of biochar + *Bacillus* sp. (N18), maximum increase the N, P, and K contents in root and straw that were 45.3, 72.2, 47.9% and 35.6, 42.2, 41.4%, respectively, as compared to normal soil control, while in contaminated soil N, P, K contents in roots and straw was 52.8, 117.8, 74.8% and 61.8, 68.8, 58%, respectively, compared to control. Sole application of biochar, compost, and *Bacillus* sp. (N18) also shows prominent results both in normal and contaminated soil over their respective controls. Sole application of biochar shows 21.5, 38.7, 22.1% N, P, K in roots and straw 26.1, 22.2 and 25.4% in normal soil, while in contaminated soil 33.1, 65.7, 40.5% in root and 48, 46.1 and 44.5% in straw over their respective controls.

Table 2. Impact of sole and combined application of lead-tolerant *Bacillus* sp. (N18), biochar, and compost on shoot length, shoot fresh and dry weight of maize, n=3

Treatments	Shoot length (cm)		Shoot fresh weight (g plant^{-1})		Shoot dry weight (g plant^{-1})	
	Normal Soil	Contaminated Soil	Normal Soil	Contaminated Soil	Normal Soil	Contaminated Soil
Control	135.3 g	93.7 k	311.5 h	258.5 j	30.7 e	20.7 g
N18	152.7 e	118.7 j	321.0 g	300.0 i	33.3 d	26.2 f
Biochar	157.7 c	130.3 h	346.4 d	328.8 f	36.5 b	33.1 d
Compost	155.3 d	123.7 i	339.1 e	320.3 g	34.0 d	31.1 e
N18 + Biochar	170.3 a	141.3 f	390.0 a	369.8 b	41.5 a	35.9 bc
N18 + Compost	162.0 b	136.0 g	372.1 b	354.9 c	40.0 a	34.3 cd
LSD($p \leq 0.05$)		2.24		4.24		1.78

Table 3. Impact of sole and combined application of lead-tolerant *Bacillus* sp. (N18), biochar, and compost on root length, root fresh, and dry weight of maize, n=3

Treatments	Root length (cm)		Root fresh weight (g plant^{-1})		Root dry weight (g plant^{-1})	
	Normal Soil	Contaminated Soil	Normal Soil	Contaminated Soil	Normal Soil	Contaminated Soil
Control	63.5 g	43.6 i	71.2 de	58.0 g	8.4 gh	6.5 j
N18	66.8 f	57.7 h	72.9 cd	68.4 f	9.4 de	7.5 i
Biochar	78.7 bc	71.4 de	80.4 b	70.6 d-f	10.3 c	8.7 fg
Compost	73.4 d	69.1 e	78.3 b	70.1 ef	9.8 d	8.1 h

N18 + Biochar	85.8 a	79.5 bc	84.5 a	79.2 b	11.6 a	9.5 d
N18 + Compost	81.0 b	78.0 c	80.7 b	74.6 c	10.8 b	9.1 ef
LSD($p\leq 0.05$)		2.36		2.50		0.41

Table 4. Impact of sole and combine application of lead-tolerant *Bacillus* sp. (N18), biochar, and compost on N, P, K contents in roots of maize, n=3

Treatments	**Nitrogen in roots (%)**		**Phosphorus in roots (%)**		**Potassium in roots (%)**	
	Normal Soil	**Contaminated Soil**	**Normal Soil**	**Contaminated Soil**	**Normal Soil**	**Contaminated Soil**
Control	1.21 ef	0.95 g	0.77 g	0.56 h	0.93 fg	0.74 h
N18	1.29 c-e	1.13 f	0.86 f	0.57 h	1.03 ef	0.91 g
Biochar	1.47 b	1.26 d-f	1.06 d	0.93 ef	1.14 cd	1.04 de
Compost	1.36 b-d	1.19 ef	0.96 e	0.85 f	1.09 de	0.99 e-g
N18 + Biochar	1.75 a	1.45 b	1.32 a	1.23 bc	1.38 a	1.29 ab
N18 + Compost	1.64 a	1.42 bc	1.28 ab	1.16 c	1.22 bc	1.14 cd
LSD($p\leq 0.05$)		0.14		0.08		0.11

Table 5. Impact of sole and combine application of lead-tolerant *Bacillus* sp. (N18), biochar, and compost on N, P, K contents in straw of maize, n=3

Treatments	**Nitrogen in straw (%)**		**Phosphorus in straw (%)**		**Potassium in straw (%)**	
	Normal Soil	**Contaminated Soil**	**Normal Soil**	**Contaminated Soil**	**Normal Soil**	**Contaminated Soil**
Control	1.08 g	0.85 h	0.62 f	0.43 h	1.20 f	0.94 g
N18	1.29 de	1.20 f	0.68 e	0.55 g	1.31 e	1.19 f
Biochar	1.36 bc	1.25 e	0.75 c	0.62 f	1.51 c	1.36 d
Compost	1.30 de	1.26 e	0.72 d	0.60 d	1.36 d	1.28 e
N18 + Biochar	1.46 a	1.37 bc	0.88 a	0.72 d	1.70 a	1.49 c
N18 + Compost	1.39 b	1.32 cd	0.81 b	0.68 e	1.60 b	1.39 d
LSD($p\leq 0.05$)		0.06		0.02		0.05

3.3. Physiological attributes of maize

Soil amended with biochar and compost along with the inoculation of *Bacillus* sp. (N18) in normal and lead-contaminated soil notably enhanced the chlorophyll SPAD value and relative water contents in maize plant as compared to control (Table 6). As compared to the control, treatment T4, where biochar+ *Bacillus* sp. (N18) was applied, performed maximum enhancement in SPAD value and relative-water contents, which were 23.1, 24.9% in normal soil and 65.7, 52.4% in contaminated soil, respectively, over their respective control. While the subsequent increase was in treatment T5 where Compost + *Bacillus* sp. (N18) applied that was 16.7, 17.9% in normal soil and in contaminated soil 51.7, 44.6%, respectively, over control.

Table 6. Impact of sole and combine application of lead-tolerant *Bacillus* sp. (N18), biochar, and compost on SPAD value and relative water contents of maize, n=3

Treatments	**SPAD value**		**Relative water contents (%)**	
	Normal Soil	**Contaminated Soil**	**Normal Soil**	**Contaminated Soil**
Control	41.0 de	28.5 f	53.7 g	41.9 i
N18	43.3 cd	38.5 e	56.4 f	49.5 h
Biochar	47.0 b	44.8 bc	61.5 cd	57.5 ef
Compost	45.4 bc	43.5 cd	59.3 de	52.6 g
N18 + Biochar	50.4 a	47.2 ab	67.1 a	63.9 b
N18 + Compost	47.8 ab	43.2 cd	63.3 bc	60.6 d
LSD ($p\leq 0.05$)		3.24		2.32

3.4. Lead contents in root and straw

As presented in Figure 1 (A and B), the use of compost, biochar, and inoculation of *Bacillus* sp. (N18) in contaminated soil significantly decreases the uptake of lead contents in roots and straw. In contrast to normal soil, lead is not detected in the plant body. While in contaminated soil maximum decrease was observed in treatment T4 where biochar + *Bacillus* sp. (N18) was applied, that was 76.8% in root and 85% in straw over control. The subsequent decrease was observed in treatment T5, where compost + *Bacillus* sp. (N18) was applied, which showed 73.6% in root and 81.6% in straw. The sole application of biochar, compost, and bacterial strains also shows prominent results but less from their combined application than the control.

3.5. Antioxidant enzyme activity under sole and combined use of biochar, compost, and Bacillus sp. (N18)

Application of *Bacillus* sp. (N18), biochar, and compost showed an improvement in antioxidant activity in maize plants under normal soil and contaminated soil conditions as compared with control Figure 2 (A, B, C, and D). Compared to normal and contaminated soil, inoculation of *Bacillus* sp. (N18), biochar, and compost maximum increases the antioxidant enzymatic activities in contaminated soil over control. Highest improvement in SOD, POD, CAT, and APX was observed in the use of *Bacillus* sp. (N18) +biochar that was 51.6, 42.5, 35, and 45.4%, respectively, in contaminated soil, while in normal soil, 28.2, 20.6, 19.8, 32.6% respectively, over control. All the treatments showed significant performance in normal soil as well as lead-contaminated soil, but the sole application of biochar, compost, and bacterial strain exhibited fewer results. Sole application of biochar improved the SOD, POD, CAT, and APX activity that was 33, 26.6, 21, and 24.9%, respectively, in contaminated soil and 19.4, 14.4, 11.6, 23.3%, respectively, under normal soil as compared with their respective control. A minimum increase was observed in treatment where the sole application of *Bacillus* sp. (N18) was done over control.

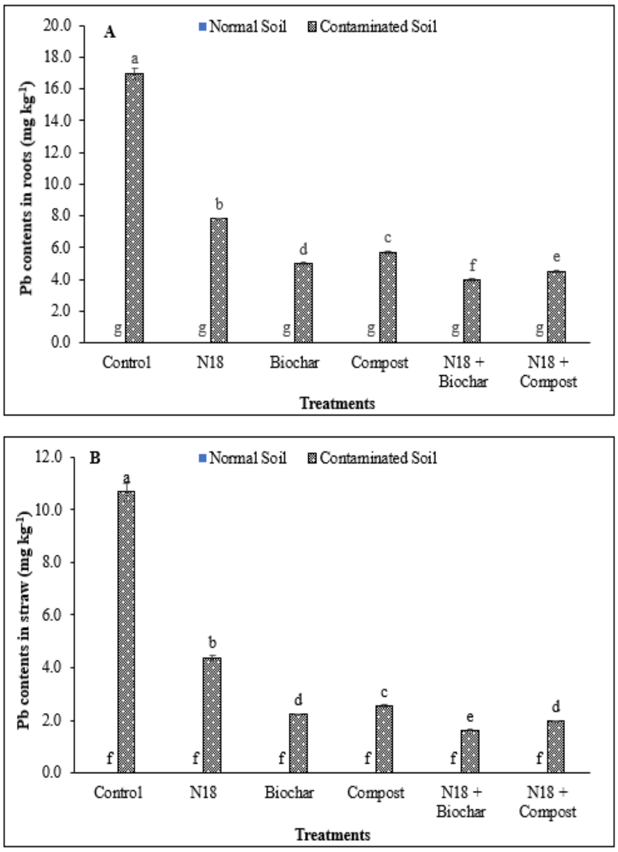

Figure 1. Impact of sole and combine application of lead-tolerant Bacillus sp. (N18), biochar, and compost on lead contents in roots (A) and straw of maize (B). Bars showing different letters that are significant statistically with one another at p≤0.05 (n =3)

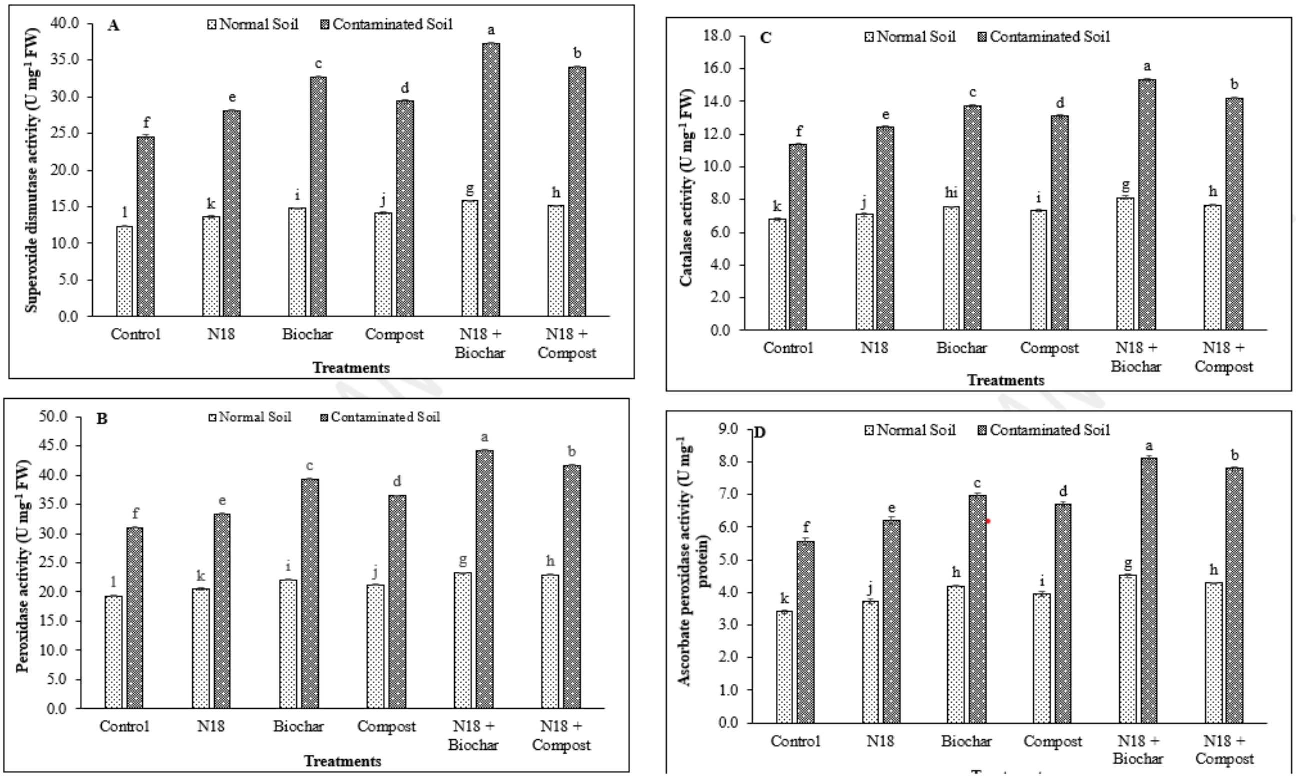

Figure 2. Impact of sole and combine application of lead-tolerant *Bacillus* sp. (N18), biochar, and compost on SOD (A), POD (B), CAT (C), and APX (D) activity in maize. Bars showing different letters that are significant statistically with one another at p≤0.05 (n =3)

3.6. *Lead contents in soil after harvest*

The impact of lead-tolerant bacterial strain *Bacillus* sp. (N18), along with compost and biochar, significantly decreases the lead contents in the soil after harvest, is presented in Figure 3. A significant decrease was seen in T4 treatment where *Bacillus* sp. (N18) and biochar were applied, which was 72.8% in contaminated soil over control, while in normal soil, lead was not detected. Subsequent results were observed in treatment T5, where *Bacillus* sp. (N18) + compost was used, which showed a 71.1% decrease in lead contents as compared to contaminated control where no amendment was used. While in sole application of biochar perform significant results over control that was 67.6% decrease in Pb contents in soil. The other treatments where the sole application of *Bacillus* sp. (N18), and compost were applied also exhibited good results as compared with the control.

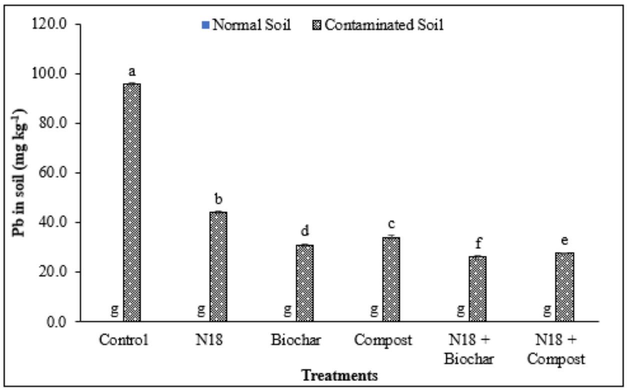

Figure 3. Impact of sole and combine application of lead-tolerant Bacillus sp. (N18), biochar, and compost on lead contents in soil after harvest of maize. Bars showing different letters that are significant statistically with one another at p≤0.05 (n =3)

3.7. *Translocation and bioaccumulation factor*

The use of organic amendments and lead-tolerant bacterial strain showed a negative correlation between lead concentration and translocation factor (Figure 4-A). A maximum decrease in lead translocation factor was observed when biochar + *Bacillus* sp. (N18) was applied to lead-contaminated soil. The treatment T4 showed a maximum decrease of 35.2% over control. At the same time, treatment T5, where compost + *Bacillus* sp. (N18) was applied, showed a 30.2% decrease. All the treatments showed better performance under lead contamination.

Present study results depicted that the bioaccumulation factor was also decreased by the application of *Bacillus* sp. (N18), compost, and biochar, as shown in Figure 4-B. Treatment, where biochar + *Bacillus* sp. (N18) was applied in contaminated soil, decreased the bioaccumulation process by 44.7%. A further decrease was observed in treatment T5, where the application of compost + *Bacillus* sp. (N18) was done, which was 36.2% compared to the control. A minimum reduction in bioaccumulation factor was calculated in treatment T1, where the sole application of *Bacillus* sp. (N18) was done, was 11%.

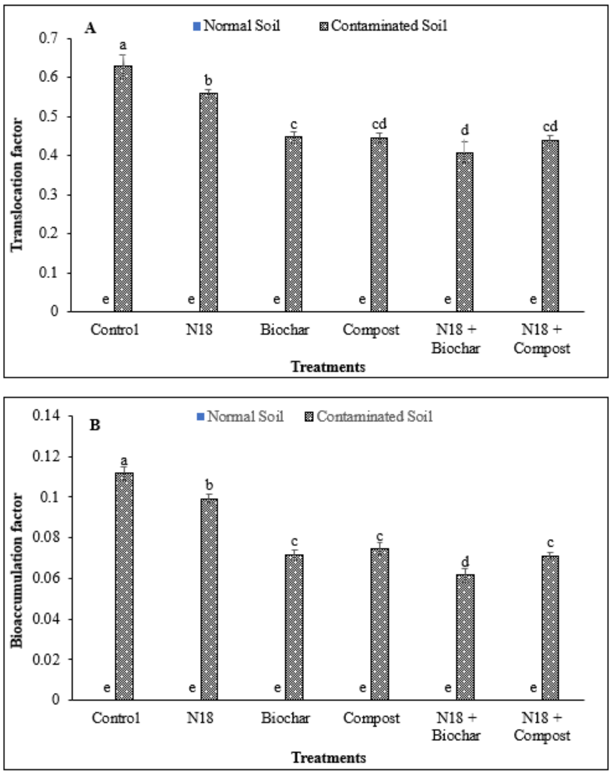

Figure 4. Impact of sole and combine application of lead tolerant Bacillus sp. (N18), biochar, and compost on translocation (A) and bioaccumulation (B) factor of maize. Bars showing different letters that are significant statistically with one another at p≤0.05 (n =3)

4. Discussion

Heavy metal contamination of the environment has arisen as a main threat that was due to natural sources and anthropogenic interference with the environment (Zahid *et al*., 2024). For ideal growth and production of plants, it is necessary to adopt sustainable techniques to overcome the toxic effects of contaminants (Rehan *et al*., 2023). Heavy metals, like arsenic, lead, mercury, and cadmium, can contaminate the soil and have significant impacts on the environment and human health (Rahman and Singh, 2019; Aimen *et al*., 2024). Soil enriched with organic matter and diverse types of microbial populations plays a significant part in the elimination of metal pollutants (Abdu *et al*., 2017). These amendments decrease the mobility and bioavailability of heavy metals through processes such as immobilization, complexation, and adsorption (Li *et al*., 2021). Furthermore, soil amended with compost and biochar can help to remediate the polluted soil in integration with PGPR (Lebrun *et al*., 2019; Ayub *et al*., 2024). Healthier soil is important not only for sustainable and healthy food production but also protects the environment and well-being of humans in the condition of metal pollution and deficient nutrient challenges in soil (Lal *et al*., 2021).

In the current study, compost and biochar were applied as organic amendments along with pre-isolated, characterized, and identified lead-tolerant *Bacillus* sp. (N18) to check their effect on maize plant growth, physiology, nutritional status, and lead uptake in the plant body. Based on our results, growth attributes i.e., shoot length, root length, and dry and fresh weight were significantly influenced by the use of amendments. Previous studies documented that a prominent effect was observed by the application of biochar-PGPR integration improves the development and growth of metal-stressed crop plants (Abbas *et al*., 2017; Liu *et al*., 2024). However, inoculation of lead-tolerant *Bacillus* strains also accelerates the significant improvement in plant growth as compared to control. Such useful impacts of bacteria are due to their impact on the mobility of heavy metals and plant availability through the production of organic acids, chelating agents, alteration in redox reaction, and nutrient solubilization (Abou-Shanab *et al*., 2019; Raag Harshavardhan *et al*., 2022). Our research outputs are compared with the studies of Saleem *et al*. (2018), who stated that mitigating the toxic effects of lead by the use of lead-resistant bacteria increases sunflower productivity in lead-contaminated soil. Improvement in plant growth by the inoculation of lead-tolerant *Bacillus* strain under metal stress conditions is due to their capability to solubilize potassium, phosphorus and zinc (Anwar *et al*., 2024; Noreen *et al*., 2024), production of siderophore (Rajkumar *et al*., 2010), phytohormones (Bilal *et al*., 2019; Dar *et al*., 2022), aminocyclopropane 1-carboxylate deaminase (Gamalero and Glick, 2015; Zhang *et al*., 2023) and increase the resistant against metals toxicity (Nanda *et al*., 2019).

The use of compost and biochar in soil showed significant improvement in pH, EC, and soil organic carbon due to their diverse properties. Soil organic carbon contents improve through compost application by increasing the stable organic carbon resulting from the decomposing of organic matter (Wei *et al*., 2021; Yang *et al*., 2021). Biochar also indirectly improves the level of soil organic carbon by improving the microbial activity in the soil, which can increase the decomposition process of native organic matter (Ding *et al*., 2023). The amendment of compost in soil also increases the survival of plants in toxic or contaminated environments due to its enrichments with plant essential nutrients like N, P, and K (Gim´enez *et al*., 2021). The findings of Ming and Allen (2018) are also parallel with the finding that in both plant shoots and roots, nitrogen and phosphorus improved significantly. Similar results were also described by other scientists, who found that the use of biochar also alters the pH and EC activity in soil (Puga *et al*., 2015; Wu *et al*., 2024). The increase in EC and pH of the soil is also due to higher pH and the occurrence of organic carbon in biochar (AL-Huqail *et al*., 2023). Naturally, biochar has an alkaline nature that helps in buffering the acidic nature of toxic metals in contaminated soil (Lu *et al*., 2017). After application, during the process of pyrolysis, basic cations such as Mg, Ca, Na, and K changed into their oxidase, carbonates, and hydroxides that adhere on the surface of biochar and act like a liming agent to increase the alkalinity of the soil. Bioavailability and mobility of toxic heavy metals result in the development of various metals precipitate, e.g., $CdCO_3$, $Pb_5(PO_4)_3OH$, $Cu(OH)_2$ and $Cd(OH)_2$ in high pH soils (Khan *et al*., 2018; Chen *et al*., 2023). However, lead exposure caused a significant decrease in photosynthetic activity like chlorophyll contents and RWC (relative water contents), as seen previously by Shabaan *et al*. (2021). Under metal-stressed conditions, alterations in pigment contents have a direct influence on plant health and the production of photosynthates. Additionally, the lead-related decrease in plant SPAD value is associated with a reduction in the capturing ability of leaves (Zhou *et al*., 2020). Toxic metals decrease the biosynthesis of chlorophyll by deactivating the associated enzyme activity and damaging its proper functioning (Altaf *et al*., 2023). The decrease in relative water contents under lead contamination is due to the lead-induced stomatal closing, which decreases the uptake of water, as earlier described by Venkatachalam *et al*. (2017).

For crop plants, lead is the non-essential element, yet its soil accumulation, primarily accredited to human activity, creates a substantial hazard to agricultural soils. In soil, typically, lead is present in various forms, surrounding free ions of metals or complexes with inorganic and organic substances. Different factors influence lead toxicity, containing concentration and chemical form of various species (Fatemi *et al*., 2021).

5. Conclusion

The application of biochar, compost, and the inoculation of lead-tolerant *Bacillus* sp. (N18) significantly improve the plant's growth, physiology, and nutrient content. *Bacillus* sp. (N18) + biochar significantly improves the growth and antioxidant activity in lead-contaminated soil and have the potential to remediate the contaminated soil by 72.8%. Plant samples taken from contaminated soil, after analysis confirmed that these amendments have the potential to reduce/remove the Pb toxicity in plant body. On the other hand, inoculation of *Bacillus* sp. (N18) and use of biochar, compost not only bioremediate the contaminated soil but also improve the nutritional status in soil/plant. Further these amendments tested in field conditions to check their potential and also to evaluate their impact on soil physio-chemical properties and finally recommended to the farming community.

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Data availability

Data presented in current research work are available on request.

Conflicts of interest

The authors declare no conflict of interest.

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