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Co-applied AMF and biogas digestate biochar in Pb-polluted soil: Efficient strategy for minimizing Pb mobility in soil-plant system and improving dietary value of oats

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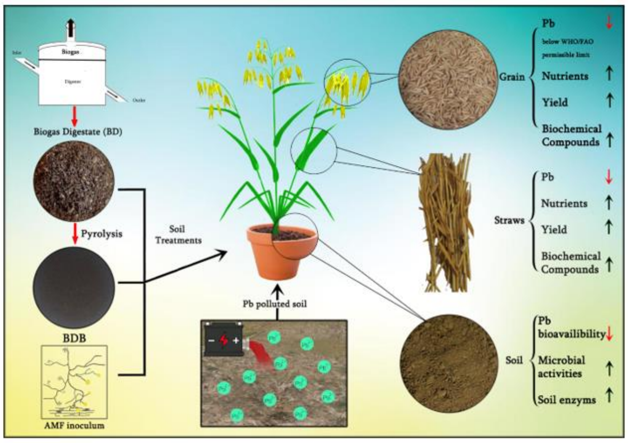
Graphical abstract



Abstract

Biochar (BCR) coupled with suitable microorganisms can reduce lead (Pb) mobility in soil-plant system. Biogas plant digestate (BD), an abundant, inexpensive, and year-round available waste, can yield low-cost and nutrient-rich biochar (BDB). This pot experiment involves sole BD, BDB, and arbuscular mycorrhizal fungi (AMF) soil treatments and combining AMF with BD and BDB for Pb immobilization in Pb-polluted soil. Treatment effects on Pb uptake in oat plants, plant growth, and yield were assessed. Moreover, enzymatic activities, AMF root colonization, easily extractable glomalin (EEG), microbial biomass carbon (MBC), and microbial numbers in soil were also measured. BDB+AMF showed the highest soil Pb immobilization index (62.1%), leading to maximum Pb reductions in oats grain (80%), straw (66%), roots (45%), and its soil available fraction (62%), with grain Pb concentrations (3.27 mg kg^{-1} DW) below the WHO/FAO critical limit, than control. This treatment also maximally enriched grain and straw with nutrients (N, P, K, Ca, Mg, Fe, Zn, and Mn) than control. Moreover, topmost soil enzymatic activities (>68%), MBC (73%), EEG (165%), AMF root colonization (110%), and microbial numbers (>74%) were achieved with BDB+AMF treatment. Conclusively, BDB+AMF is a cost-effective soil amendment that can remediate Pb-polluted soil and produce safer and nutrient-rich food.

Keywords: Lead, Biogas plant digestate, Immobilization, Nutrients, Colonization

1. Introduction

Due to the poor economic status of several countries, the recycling and repairing of defunct Pb–acid batteries is widespread (Poudel *et al.* 2023). With high effluent treatment costs and ignoring environmental regulations, small-scale enterprises that recycle and repair these batteries discharge untreated effluents into the nearby water bodies and land, resulting in Pb pollution of soil and water (Boorboori *et al.* 2022). Resultantly, a higher accumulation of Pb in agricultural fields has been reported, leading to Pb entry into the food chain (Adeyemi *et al.* 2021; Kumar *et al.* 2022). Before releasing Pb-containing effluents into water bodies, Internet of Things (IoT) sensors and an HG-RNN model can be used to ensure safe water quality. Herein, the IoT sensor can detect pollutants. At the same time, the Hierarchical Gated Recurrent Neural Network (HG-RNN) model can optimize the treatment, thereby protecting aquatic systems from Pb pollution (Maruthai *et al.* 2025). Interestingly, as an alternative to growing food crops in Pb-polluted soils, another viable option for obtaining pollution-free food is a hydroponic system with nutrient film technology, powered by the IoT and automation, which can enhance plant productivity and sustainability compared to traditional agricultural practices (Venkatraman and Surendran 2023). However, for Pb-polluted soils, cost-effective and eco-friendly remediation approaches are the most effective ways to prevent Pb

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entry into the food chain (Afzal *et al.* 2024; Boorboori *et al.* 2022). In parallel, the increasing emphasis on sustainable resource management and eco-innovative practices across sectors has elevated the role of financial strategies and coordinated development models in addressing environmental degradation (Ma and Appolloni 2025; Ma *et al.* 2024; Ma *et al.* 2025a). In agriculture, cost-effective bio-based amendments such as BDB offer practical solutions for remediating Pb-contaminated soils. These environmentally friendly approaches reflect a growing alignment between ecological conservation and regional economic development goals, as highlighted by recent studies on coordinated ecological-economic models (Wang *et al.* 2025).

Previously, phytoremediation and chemical fixation have been evaluated for the remediation of soils contaminated with heavy metals (HMs). However, phytoremediation has several drawbacks, including being a slow process that depends on the life cycle of the selected phytoremediation crop, low HMs removal efficiency due to the limited extent and depth of root growth, being labour-intensive, and requiring continuous monitoring (Shen *et al.* 2022). Unfortunately, high concentrations of HMs in soil can also lead to poor plant establishment for phytoremediation purposes, hindering the achievement of the full potential of this approach (Mankė *et al.* 2024). To accelerate the phytoremediation process, HMs bioavailability is increased by several chemical agents, which also cause secondary pollution of groundwater and surface water through leaching and runoff (Tauqeer *et al.* 2024). After the harvest of phytoremediation crops, the HMs-contaminated biomass also presents severe disposal issues (Shen *et al.* 2022). Whereas, the in situ chemical fixation remediation technique involves the addition of chemical substances like lime, limestone powder, various apatites, and phosphate fertilizer to the soil, which reduces the solubility, mobility, and phytoavailability of HMs (Xu *et al.* 2024). Unfortunately, the use of single amendments partially immobilize HMs, posing a risk of HMs dissolution due to changes in environmental conditions (Xu *et al.* 2021). Furthermore, these chemical binders pose a risk to the survival and functionalities of soil microorganisms, compromising soil quality and incurring higher costs (Xu *et al.* 2024).

Biochar (BCR) is a carbon (C) and nutrient-rich product obtained after the pyrolysis of various bioresidues. It has been successfully used to minimize Pb uptake in different crops after Pb immobilization in the soil via raising soil pH, precipitation, ion exchange, and adsorption reactions (Siles *et al.* 2022; Pandey *et al.* 2022). Interestingly, BCR also boosts plant growth and yield by improving soil fertility, enzymology, and water holding capacity (WHC) (Afzal *et al.* 2024; Ayaz *et al.* 2022). Despite these advantages, the steady demand for vast amounts of BCR for its commercial success remains a question due to the inconsistent availability of cheap feedstock around the year (Hu *et al.* 2021). Secondly, to fulfil the increased demand for BCR for ecological restoration and plant growth enhancement, using poor-quality feedstock and reduced pyrolysis time yields low-quality BCR. Lastly, BCR cost greater than producing charcoal (100%), like algae BCR (1500%), exotic crops BCR (800%), and nano-biochars (1100%), are not commercially effective for achieving various environmental advantages (Maroušek *et al.* 2023).

Biogas production through anaerobic digestion of bio–residues is cheap and has covered the energy shortage in several nations (Dutta *et al.* 2021; Mickan *et al.* 2022). The biogas plant digestate (BD) is a waste which is produced in huge quantities, is rich in nutrients, and can be used as organic fertilizer. However, it contains various pathogenic bacteria (*Escherichia coli* and *Salmonella* sp.), negatively affecting soil and plant attributes and limiting its safe disposal (Baştabak and Koçar 2020; Tsai *et al.* 2018). Converting BD into digestate-derived biochar (BDB) can overcome problems, such as 1) cheap feedstock availability all year around, 2) nutrient richness of BDB, and 3) disposal issues (Mickan *et al.* 2022; Dutta *et al.* 2021). The BDB is superior to BD due to its nutrient richness, larger surface area, surface charges, and abundant functional groups, which depict its suitability for crop nutrition and the remediation of HMs-polluted soils (Ayaz *et al.* 2022; Tsai *et al.* 2018; Wang *et al.* 2022a).

A probiotic microorganism, arbuscular mycorrhizal fungi (AMF), coexist with plant roots through symbiosis (Boorboori *et al.* 2022). Nowadays, different AMF inocula containing multiple AMF species are cheap and available in powder, granular, tablet, and liquid forms (Yang *et al.* 2023) for their usage in forestry, horticulture, agriculture, and soil rehabilitation (Bisht and Garg 2022; Ramírez-Zamora *et al.* 2022). AMF improve plant growth and yield through enhancing water use efficiency and mineral acquisition, and reduce Pb uptake by the plants by producing glomalin, phytochelatins (PCs), extracellular polymeric substances (EPS) and trapping Pb into fungal parts (Li *et al.* 2023; Farhad *et al.* 2024; Gu *et al.* 2025).

The interaction between BCR and AMF is quite appealing for the sustainability of soil environment and favoring plant growth by improving soil quality, promoting beneficial microorganisms, enhancing water retention in the soil, minimizing HM mobility in the soil-plant system and improving plant growth through nutrient provision (Jia *et al.* 2024; Vejvodová *et al.* 2020). BCR+AMF reduces the mobility of HMs in soil and their plant uptake through several processes, thereby lowering the dissolution risk of HMs in the soil and leaching (Li *et al.* 2023; Farhad *et al.* 2024; Gu *et al.* 2025; Zhao *et al. 2024*). Herewith, AMF also play an essential role in immobilizing HMs in their vesicles, spores, mycelium and hyphae (Gujre *et al.* 2021; Zhao *et al.* 2024). In the BCR+AMF association, BCR supports AMF perpetuation in the soil via improving spore germination and hyphal branching and significantly protects the AMF from different grazers (Gujre *et al.* 2021; Jia *et al.* 2024). Moreover, this combination also enhances the rhizosphere microenvironment, creating a favorable niche for soil indigenous microorganisms and promoting their activities (Gujre *et al.* 2021). In this combined treatment, AMF actively interacts with plant growth-promoting rhizobacteria (PGPR), thereby enhancing soil nitrogen fixation and increasing plant growth and yield (Khaliq *et al.* 2022). In a Pb polluted soil, AMF+BCR was regarded as best compared to AMF and BCR treatments alone for reducing Pb bioavailability in soil by 30.94% and maize shoot Pb concentration by 32.94%, compared to unamended soil (Jia *et al.* 2024). In another pot experiment, the combined treatment of dry olive residue BCR and AMF reduced Pb concentrations in roots (47%), shoots (16%), and grain (58%) of *Triticum aestivum* grown on Pb-polluted soil (Vejvodová *et al.* 2020). Besides, amending Cd polluted soil with BCR and AMF decreased water-extractable Cd by 24% while reduced Cd accumulation in shoots by 26% and its translocation in rice grain (Zhao *et al.* 2024).

Oats, being high in protein, lipids, minerals, fat, carbohydrate, thiamine, riboflavin, pyridoxine, and fiber contents, are significant in cereal crops due to their economic, medicinal, and dietary importance for livestock and humans (Chawla *et al.* 2022; Shahidi *et al.* 2021). Interestingly, oat straw is softer than other cereals, nutrient-rich, and is widely used as an animal ration (Chawla *et al.* 2022). However, the presence of Pb in soil reduces growth, yield, mineral nutrition, and biochemical compounds, as well as high Pb concentrations in various edible plant portions of cereals, which results in malnutrition and Pb toxicity in animals and humans (Khan *et al.* 2020; Poudel *et al.* 2023; Wang *et al.* 2022b).

The literature lacks information regarding the use of BDB with AMF inoculum for the restoration of Pb-polluted soils, reducing Pb uptake in oat plants, improving the mineral nutrition of oat grain and straw, and enhancing soil health. Our hypothesis is that co-applying AMF with either BD or BDB can have additive effects on reducing Pb concentrations in the edible portions of oat plants, thereby enhancing their dietary value and improving soil health. Thus, we conducted a pot study with the objectives 1) to evaluate the capacity of BD, BDB, and AMF as solitary soil treatment and integrate AMF with BD and BDB for Pb fixation in soil and its restricted accumulation in edible plant tissues, 2) effects of soil additives on grain and straw dietary value and 3) additives effects on soil health.

2. Materials and methods

2.1. Soil acquisition and its analysis

The soil was gathered from cropland getting irrigation with canal water, having raw effluents originating from Pb–acid battery refurbishing enterprises. The upper layer (0–20 cm) of soil was collected and carefully delivered to the laboratory. Later, the soil samples were blended to achieve a homogenized sample, sieved (2-mm) to remove foreign bodies, and air-dried. Further, the physicochemical traits of the soil were assessed, as presented in **Table 1**, according to the standard procedures (Zubair *et al.* 2021).

Table 1. Soil properties after its analysis.

Characteristics	Units	Values
Texture	–	Clay loam
Sand	g kg^{-1}	390
Silt	g kg^{-1}	290
Clay	g kg^{-1}	320
Soil pH	–	6.60
Electrical conductivity (EC)	dSm^{-1}	1.99
Cation exchange capacity (CEC)	cmol$_c$ kg^{-1}	12.9
Organic matter	g kg^{-1}	6.80
CaCO$_3$	g kg^{-1}	37.0
Available P	mg kg^{-1}	8.50
Exchangeable K	mg kg^{-1}	103
N	g kg^{-1}	0.17
Ca	g kg^{-1}	1.24
Fe	mg kg^{-1}	109
Zn	mg kg^{-1}	13.1
Mg	mg kg^{-1}	1490
Mn	mg kg^{-1}	7.4
Cu	mg kg^{-1}	45
Bioavailable Pb	mg kg^{-1}	2.90
Total Pb	mg kg^{-1}	639

2.2. AMF consortium

A commercial AMF consortium, "Endo Roots Soluble", was purchased from https://www.bioglobal.com.tr/, containing nine species of AMF (*Glomus intraradices*, *Glomus aggregatum*, *Glomus mosseae*, *Glomus clarum*, *Glomus monosporum*, *Glomus deserticola*, *Glomus brasilianum*, *Glomus etunicatum*, and *Gigaspora margarita*), having concentration of 1 × 10^4 CFU g^{-1} of inoculum.

2.3. Digestate and its biochar

The BD was obtained from a local methane biogas facility in Faisalabad, Pakistan. This facility was using buffalo manure near a livestock farm having 1237 heads, producing about 800 kWh day^{-1}. After getting the BD, it was carefully transferred to the laboratory. To prevent odour, the wet BD was oven–dried for multiple days at 60°C to acquire the proper state for soil amendment. Further, half of the BD was converted into BDB. For this purpose, the BD was pyrolyzed in a local charcoal retort at

600°C to produce BDB. Later, BDB was cooled at room temperature and ground (2 mm). To examine the surface morphology of the digestate-derived biochar (BDB), scanning electron microscopy (SEM) was performed using a ZEISS GeminiSEM 300 (Germany). The specific surface area and porosity characteristics of the sample were evaluated using a Micromeritics TriStar II Plus 2.02 instrument based on the Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) approaches, respectively, under standardized operating conditions. The surface functional groups of BDB were analyzed using an FTIR spectrometer (Nicolet, Thermo Scientific). Samples were scanned in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} using the KBr pellet method. The BD exhibited properties such as pH = 8.6, EC = 1.22 dS m^{-1}, C = 42.7%, H = 5.91%, O = 37.2%, N = 2.91%, P = 1.02%, K = 2.77%, Ca = 2.14%, Mg = 1.14%, S = 0.37%, Fe = 784.6 mg kg^{-1}, Zn 217.1 mg kg^{-1}, Mn = 194.2 mg kg^{-1}, and Cu = 43.4 mg kg^{-1}. Likewise, BDB exhibited properties as pH = 9.8, BET surface area = 52.37 m^{2} g^{-1}, EC = 1.49 dS m^{-1}, CEC = 32.1 cmolc kg^{-1}, C = 51.4%, H = 4.86%, O = 23.4%, average pore diameter 19.25 nm, cumulative pore volume 0.045 cm^{3} g^{-1}, N = 2.14%, P = 1.13%, K = 3.53%, Ca = 2.27%, Mg = 1.33%, S = 0.49%, Fe = 1013.1 mg kg^{-1}, Zn 232.2 mg kg^{-1}, Mn = 213.4 mg kg^{-1}, and Cu = 47.3 mg kg^{-1}.

2.4. *Pot experiment and economic analysis of soil remediation*

Six soil treatments were prepared in separate plastic vats, for three pots per treatment, each containing 15 kg of soil. These treatments were signified as control, BD, BDB, AMF, BD+AMF, and BDB+AMF. Soils where BD or BDB were to be mixed received 5% (w/w of soil) of these soil amendments. For the treatments receiving AMF inoculum, each corresponding vat received 0.57 g of inoculum per kg of soil. This dose of AMF is based on the manufacturer's recommendation and has been used in our previous study, where it was found to be effective for Pb immobilization in the soil (Farhad *et al.* 2024). In contrast, the control treatment did not receive any additives. The soil of each plastic vat was uniformly mixed, manually watered (WHC 65%), and incubated (dark place, 6 weeks, room temperature). After incubation, three replicates of each treatment were prepared by filling three plastic planters (length = 30 cm, diameter = 24.5 cm) with 15 kg of soil. After filling, all 18 planters were placed in the greenhouse (light ≈ 10–12 h, temperature ≈ 25 °C, and humidity ≈ 50%) of Government College University Faisalabad, Pakistan, in a completely randomized order. The plastic planters were irrigated until suitable moisture was gained for planting oat seedlings. Three healthy seedlings of oats (3 cm long, grown in perlite) were planted in each planter. A recommended dose of a balanced plant fertilizer [Osmocote classic (14–14–14)] was applied in each planter. The distilled water was used to irrigate plants while considering the atmospheric conditions. Plants grew for 143 days until the whole plants and ears became solid yellow, indicating plant maturity.

The production cost of the BDB (energy consumption, labour, and equipment amortization) in Pakistan ranged from PKR 20–30 kg^{-1}, which is approximately USD (\$) 0.07–0.10 kg^{-1}. The application rate of BDB was 5%, representing 50 g of BDB kg^{-1} soil. At the upper bound of production cost (\$0.10 kg^{-1}), this corresponds to a BDB input cost of \$0.005 kg^{-1} of soil. Likewise, the AMF inoculum was applied at a rate of 0.57 g kg^{-1} of soil, costing \$12.0 per 250 g, or \$0.048 g^{-1}. With this cost and dose, the AMF cost kg^{-1} of soil was \$0.02736. There was no rent for the greenhouse, as it is provided free of charge to university faculty and research students. The cost of each plastic planter (length = 30 cm, diameter = 24.5 cm), which was locally fabricated and capable of retaining 15 kg of soil, was \$0.40. Therefore, the planter cost to hold each kg of soil was \$0.026. Whereas, the costs of three oat seeds (\$0.01 pot^{-1}), fertilizer (75 g pot^{-1} = \$0.35) and irrigation (\$0.19 pot^{-1}) collectively were \$0.55 pot^{-1}, containing 15 kg of soil. With this cost, oat seeds, fertilizer and irrigation were only \$0.036 kg^{-1} of soil.

Therefore, the combined cost for both amendments and experimental setup is:

- Cost of BDB: 50 g × \$0.10 = \$0.005
- Cost of AMF**:** 0.57 g × \$0.048 **=** \$0.02736
- Cost of planter kg^{-1} of soil **=** \$0.026
- Cost of oat seeds, fertilizer and irrigation kg^{-1} of soil **=** \$0.036
- Total cost kg^{-1} of soil: \$0.094 (9.4 cents)

Therefore, the price for remediating one kg of Pb-polluted soil was only 9.4 cents.

2.5. *Crop harvesting and sample collection*

A tapeline was used to measure plant height and ear length. Plant above–ground biomass was harvested carefully with a fine sickle. The number of tillers per plant was determined by counting. Further, the grain were collected from each pot, and the remaining aerial biomass of plants was denoted as straw. As for the collection of roots, the soil from each pot was carefully removed. The grain, straw, and roots were oven–dried (Memmert, Beschickung-loading, model 100–800, Schwabach, Germany) for 24 h at 70°C to measure their dry weight (DW). Later, the oven-dried samples were crushed in a mill, sieved, and stored for further analysis.

2.6. *Analysis*

2.6.1. *Soil*

2.6.1.1. *Soil pH, Pb bioavailability and its immobilization index in soil*

The soil pH was measured with pH meter (WTW 7110, Weilheim, Germany). Further, for quantifying the labile soil Pb fraction, the soil was extracted with 5 mM diethylenetriaminepentaacetic acid (DTPA) extractant (soil-DTPA solution = 1:2) (Lindsay and Norvell 1978) and then measured on ICP–MS (PerkinElmer's NexION® 2000). Likewise, to calculate the Pb immobilization index (IMDX–Pb) in soil, Equation 1 was used (Naeem *et al.* 2021).

$$\mathbf{IMDX-Pb}(\%)=\frac{\text{Pb extracted}(\text{control treatment})-\text{Pb extracted}(\text{desired treatment})}{\text{Pb extracted}(\text{control treatment})}\times 100 \qquad (1)$$

2.6.1.2. AMF root colonization, hyphal density, spore density, and easily extractable glomalin

For estimation of AMF root colonization and spore density, the standard methods were adopted, already described in a previous publication (Farhad *et al.* 2024). The grid-line intersection method was used to calculate the hyphal length density after staining the hyphae with a trypan blue solution (0.05%, w/v) (Jakobsen *et al.* 1992). The easily extractable glomalin (EEG) was extracted from soil according to the Wu *et al.* (2015) method. Briefly, a soil sample (0.5 g) was mixed with citrate buffer (4 mL, 20 mM, pH = 7), extracted (103 kPa), heated (121°C, 30 min), and later centrifuged (10,000× g min^{-1}, 5 min). The EEG in the supernatant was measured by the Bradford method (1976).

2.6.1.3. Microbial numbers and microbial biomass carbon

For counting the microbial numbers and microbial biomass carbon (MBC) in the soil matrix, the recommended methods were adopted (Iqbal *et al.* 2023).

2.6.1.4. Soil enzymology

The wet soil was used to estimate the activities of chitinase, protease, catalase, urease, β-glucosidase, and peroxidase by following various recommended protocols described in earlier research (Zubair *et al.* 2021).

2.6.2. Plants

2.6.2.1. Pb concentrations in plant roots, straw, and grain

The concentrations of Pb in straw, roots, and grain were measured after digesting them via the open flask digestion method (Jones and Case 1990). Briefly, samples (1 g) of each of them were digested in a blend of $HClO_4$ and HNO_3 (2:1, v/v). Following, the ICP–MS was employed to measure Pb in these digests.

2.6.2.2. Nutrients and biochemical compounds in oat grain and straw

The ICP–MS was used to check the Mg, Fe, Mn, Zn, and Ca concentrations in the grain and straw digests. The phenol–sulfuric acid method was utilized to assess carbohydrate contents in the grain and straw (Smith *et al.* 1964). Further, for the quantification of N, P, and K concentrations and the contents of protein, fat and fiber in grain and straw, the protocols can be seen in previous research (Naeem *et al.* 2021).

2.7. Quality control and quality control in analysis

To ensure the quality of analysis, certified reference materials, i.e., CTA-OTL-1 (plant material) and DCI 7004 (soil material), as well as blank samples (simple solutions with no plant or soil samples), were used. Pb recovery from these reference materials (both soil and plant materials) was 91-95%. Before digesting the samples and extracting Pb, all laboratory wares were initially dipped in dilute nitric acid and then washed several times with deionized water. Moreover, each chemical used in this experiment was of analytical grade. All instruments were precise and calibrated before each analysis.

2.8. Statistical analysis

The data are represented as the mean of triplicates from each treatment with standard errors. One-way ANOVA (through *Statistics 8.1*, Analytical Software, Tallahassee, FL, USA, Copyright 2005) and later least significant difference (LSD) test ($P < 0.05$) were carried out to equate significant differences between treatment means of every treatment (Steel *et al.* 1997) and were labelled by lower case alphabet letters. Principal component analysis (PCA) was conducted to identify patterns and relationships among soil properties, enzymatic activities, plant biomass, and Pb accumulation across the treatments. Pearson correlation analysis was performed to quantify associations between variables and to assess the impact of soil and biological parameters on Pb mobility and plant performance. These statistical analyses were carried out using SPSS 26.0 and OriginPro 2024.

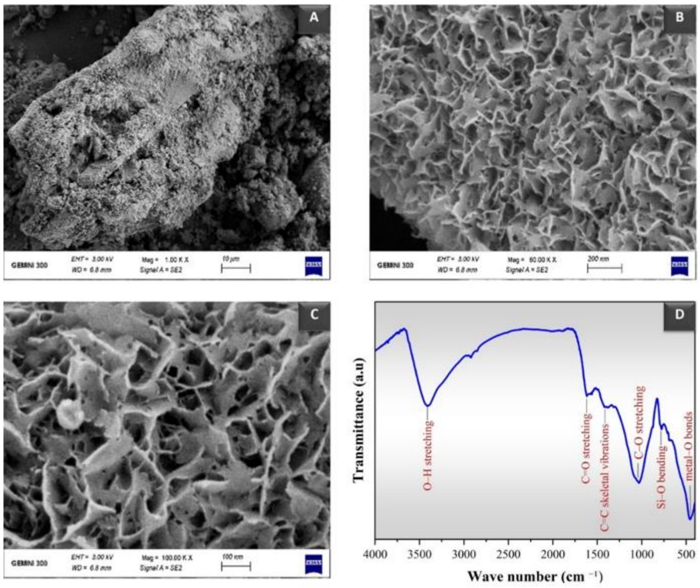

Figure 1. SEM micrographs of digestate-derived biochar (BDB). (A) Low magnification (1,000×), (B) higher magnification (6,000×), (C) Ultra-high magnification (100,000×), and (D) FTIR spectrum of BDB, showing key functional groups.

3. Results

3.1. SEM and FTIR analysis

The micromorphological features of digestate-derived biochar (BDB) were characterized using field-emission scanning electron microscopy (FE-SEM) at successive magnifications of 1,000×, 6,000×, and 100,000× (**Figure 1A-C**). At 1,000×, the BDB structure exhibited irregular macropores with diameters ranging from ~10 to 80 µm, likely resulting from the volatilization of organic fractions during pyrolysis at 600 °C. These large pore spaces contribute to aeration, increased water-holding capacity, and microbial ingress. At 6,000× magnification, the surface revealed a complex network of micro-pores and fissures with widths of approximately 1–5 µm and depths exceeding 10 µm, increasing the specific surface area (~228.17 m^2 g^{-1}, as previously measured). These features enhance the potential for root-fungal interface development, supporting colonization by AMF extraradical hyphae and soil bacterial consortia. At ultra-high magnification (100,000×), nanoscale surface irregularities became apparent, including ridge-like carbon lamellae and submicron particulate clusters (~200–500 nm). These nanostructures likely function as reactive sorption sites for Pb^{2+} ions via complexation, ion exchange, and surface precipitation mechanisms. The high surface charge density and morphological heterogeneity observed at this resolution affirm the suitability of BDB as both a Pb immobilizer and microbial habitat in Pb-contaminated soil.

The FTIR spectrum of BDB exhibited several distinct peaks corresponding to various surface functional groups (**Figure 1D**). A broad absorption band at 3409 cm^{-1} is attributed to O–H stretching vibrations, indicating the presence of hydroxyl groups. The peaks at 1619 cm^{-1} and 1420 cm^{-1} correspond to C=O stretching and C=C skeletal vibrations of aromatic rings, respectively, indicating partial retention of aromatic carbon structures after pyrolysis. A prominent peak at 1034 cm^{-1} is associated with C–O stretching vibrations, indicating the presence of oxygen-containing functional groups on the BDB surface. The absorption at 778 cm^{-1} is attributed to Si–O bending vibrations, likely originating from silica components in the digestate. The band at 453 cm^{-1} corresponds to metal–O bonds, indicating the presence of mineral components derived from the digestate. These functional groups indicate a chemically active BDB surface that can interact with HMs and enhance nutrient retention, thereby contributing to its effectiveness in soil remediation.

3.2. AMF-associated parameters, EEG, MBC, and microbial numbers

AMF root colonization, contents of EEG and MBC, as well as numbers of fungi, bacteria, and actinomycetes, ranged from 44.4 to 93.4%, 0.76–2.01 µg g^{-1} soil, 261.4–453.3 mg C kg^{-1} soil, 14.9–25.8 CFU × 10^6 g^{-1} soil, 34.6–115.8 CFU × 10^6 g^{-1} soil and 14.1–33.2 CFU × 10^5 g^{-1} soil, correspondingly. Whereas, AMF hyphal length density and spore density in the soil were from 2.99 to 8.19 m g^{-1} soil and 124 to 381.3 number 100 g^{-1} soil, respectively (**Figure 2**). Compared to the control, AMF root colonization, numbers of bacteria, MBC content and hyphal length density were significantly improved with each treatment. Moreover, EEG content and the numbers of fungi, actinomycetes and spore density were also enhanced in rest treatments, except BD, than control. The BDB+AMF brought about the most remarkable improvements in the percentage of root colonization, contents of EEG and MBC, numbers of fungi, bacteria, and actinomycetes, hyphal length density, and spore density, by 110, 165, 73, 74, 235, 135, 173, and 207%, compared to the control.

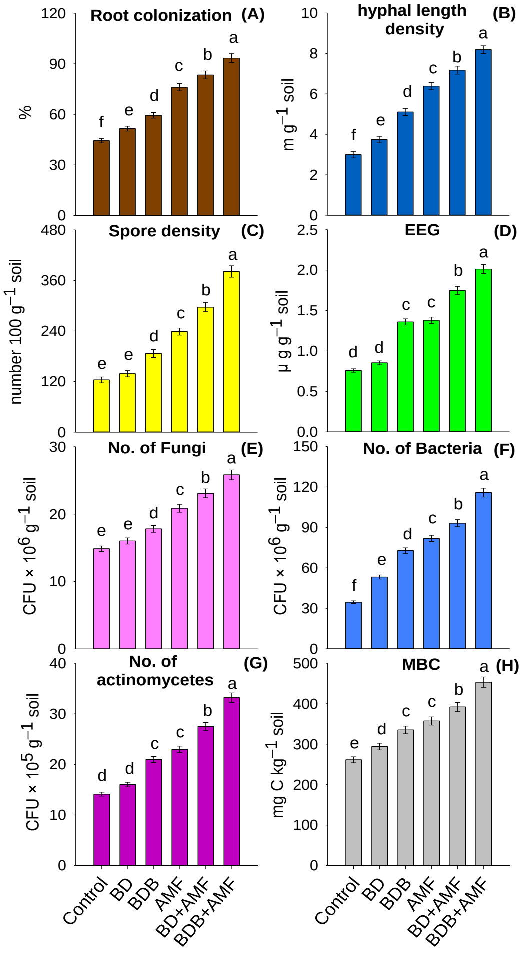

Figure 2. Effects of biogas digestate (BD), its biochar (BDB), AMF, and joint application of BD and BDB with AMF on the AMF root colonization (A), hyphal length density (B), spore density (C), easily extractable glomalin (EEG) (D), numbers of fungi (E), bacteria (F), actinomycetes (G) and microbial biomass carbon (MBC) (H) in soil. The bars represent the mean of triplicate of each treatment, and the error bars indicate the standard error (± SE). The different alphabets above the error bars differ significantly ($P < 0.05$) from one another.

3.3. Status of Pb in plant parts and its soil bioavailability

The Pb concentrations in grain, straw, and roots ranged from 3.27 to 16.3, 54.5–157.1, 180.2–327.8 mg kg^{-1} DW, correspondingly, while DTPA-extractable Pb from 1.16 to 3.06 mg kg^{-1} soil. Similarly, the soil pH values varied between 6.60 and 7.71, whereas IMDX–Pb ranged from 6.89 to 62.1% (**Figure 3**). Compared to the control, every treatment markedly decreased Pb accumulation in plant parts and DTPA extract. However, the maximum decline of 80, 66, 45, and 62% in Pb concentrations of grain, straw, and roots and DTPA-extract, respectively, were noted with BDB+AMF treatment. Moreover, the highest noteworthy increase in soil pH by 0.9 and 1.1 units was found in BDB and BDB+AMF treatments, respectively, than control. Interestingly, the order of IMDX−Pb in post-harvest soil was as follows: AMF (6.89%) < BD (20.7%) < BDB (31.0%) < BD+AMF (41.4%) < BDB+AMF (62.1%).

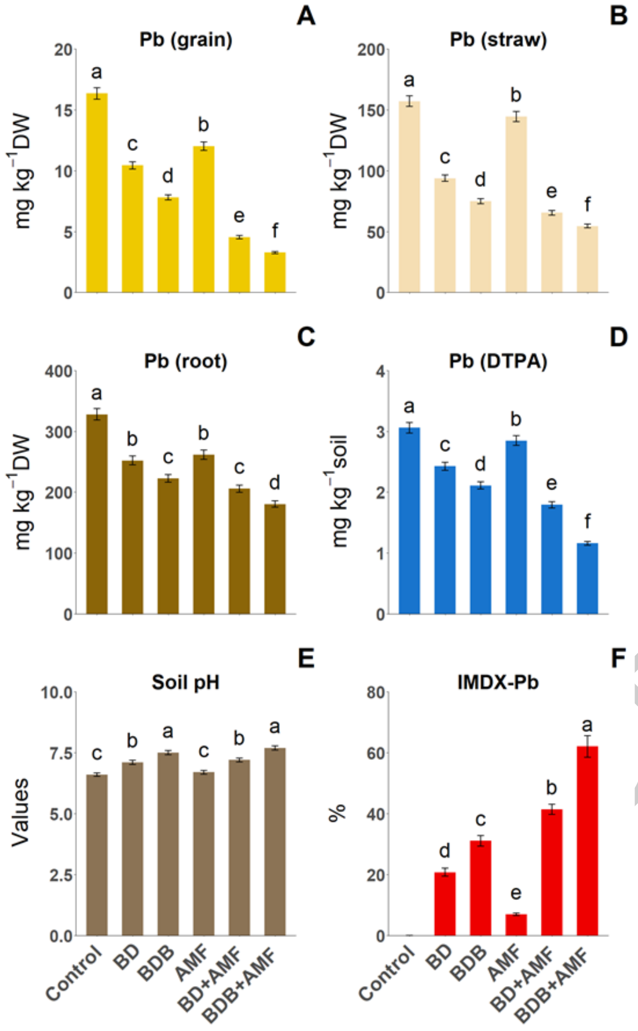

Figure 3. Effects of biogas digestate (BD), its biochar (BDB), AMF, and joint application of BD and BDB with AMF on Pb concentrations in oats grain (A), straw (B), roots (C) and DTPA extract (D), soil pH (E) and Pb immobilization index in soil (IMDX-Pb) (F). The bars represent the mean of triplicate of each treatment, and the error bars indicate the standard error (± SE). The different alphabets above the error bars differ significantly (P < 0.05) from one another.

3.4. Plant growth and yield

Data of plant height, straw DW, root DW, grain DW, no. of tillers per plant, and ear length ranged as: 92.9 to 142.2 cm, 7.61−11.7, 1.01−1.65, 6.36−11.1 mg pot^{-1}, 1.67−4.33 number and 11.7−18.1 cm, respectively (**Figure 4**). Except for AMF, other treatments significantly increased plant height, straw DW, grain DW, and no. of tillers per plant than control. In contrast, root DW showed significant increases in all treatments over control. Soil addition of BDB+AMF resulted in maximum enhancement of plant height, straw DW, grain DW, and ear length by 53, 54, 74, and 47%, correspondingly, than unamended control. Interestingly, the topmost improvements in the no. of tillers per plant by 47, 32 and 21% were seen with BDB, BD+AMF, and BDB+AMF treatments, respectively, while by 57 and 65% in root DW with BD+AMF and BDB+AMF treatments, correspondingly, than control.

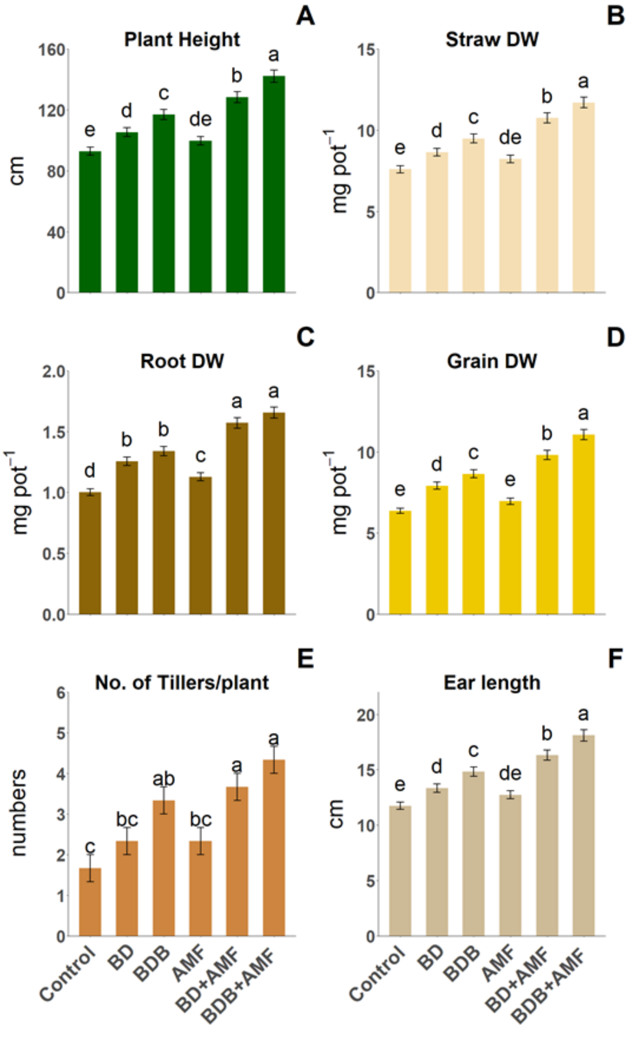

Figure 4. Effects of biogas digestate (BD), its biochar (BDB), AMF, and joint application of BD and BDB with AMF on plant height (A), straw DW (B), root DW (C), grain DW (D), number of tillers per plant (E) and ear length (F) of oats. The bars represent the mean of triplicate of each treatment, and the error bars indicate the standard error (± SE). The different alphabets above the error bars differ significantly (P < 0.05) from one another.

3.5. Grain and straw status for nutrients and biochemical compounds

The protein, fat, fiber, and carbohydrate contents in grain ranged from 9.81 to 13.8, 3.27−5.06, 7.78−10.9, and 50.9−69.4%, while from 3.01 to 4.85, 1.50−2.27, 28.1−47.2, and 4.02−6.98% in straw, respectively (Table 2). Every treatment resulted in significant ($P < 0.05$) improvements in these compounds in both plant parts, than control. The topmost contents of protein, fat, and fiber were noted in the BDB+AMF treatment, depicting 41, 55, and 40, respectively, compared to control, in grain. Whereas, 62, 51, 68, and 74% improvements in straw protein, fat, fiber and carbohydrate were noted with BDB+AMF treatment than control. Improvements of 36%

and 31% compared to the control were noted in grain carbohydrate content with BDB+AMF and BD+AMF, respectively. The N, P, K, Ca, and Mg concentrations were from 0.64 to 0.97, 0.51–0.83, 0.70–1.05, 0.34–0.57, and 0.22–0.43 g kg^{-1} in straw, while from 2.01 to 3.47, 0.97–2.11, 2.06–4.41, 0.69–1.48, and 0.46–1.01 g kg^{-1} in grain. While concentrations of Fe, Zn, and Mn in straw ranged from 41.4 to 75.7, 24.4–48.3, and 21.5–41.2 mg kg^{-1}, while from 62.8 to 114.9, 41.5–73.3, and 30.9–63.8 in grain, respectively (**Figure 5**). With the exception of N and Fe in grain and Mn in straw with AMF, other treatments brought significant improvements in the concentrations of all minerals in these plant parts. BDB+AMF brought the highest enhancements in N, P, K, Ca, Mg, Fe, Zn, and Mn up to 51, 63, 50, 66, 91, 82, 77, and 91% in straw, while 73, 117, 114, 112, 115, 83, 97 and 106% in grain, accordingly.

Table 2: Effects of biogas digestate (BD), its biochar (BDB), AMF, and joint application of BD and BDB with AMF on protein, fat, fiber and carbohydrate contents in oats grain and straw. The means of triplicate of each treatment with standard error (±SE), shown in each column, bearing dissimilar alphabets differ from one another significantly (P < 0.05).

Treatments	**Protein**		**Fat**		**Fiber**		**Carbohydrate**	
	Grain	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**
				mg kg−1 DW				
Control	9.81±0.22**e**	3.01±0.05**d**	3.26±0.09**e**	1.51±0.03**e**	7.77±0.21**e**	28.0±0.79**e**	50.8±1.15**e**	4.02±0.12**e**
BD	11.4±0.26**cd**	3.79±0.11**c**	4.01±0.11**cd**	1.92±0.04**c**	9.06±0.25**cd**	33.1±0.93**d**	59.9±1.42**cd**	4.97±0.15**cd**
BDB	12.0±0.28**bc**	4.22±0.12**b**	4.22±0.12**bc**	2.06±0.04**b**	9.69±0.27**bc**	36.3±1.02**c**	62.9±1.49**bc**	5.39±0.16**c**
AMF	10.9±0.25**d**	3.48±0.09**c**	3.69±0.11**d**	1.78±0.03**d**	8.75±0.25**d**	31.8±0.89**d**	56.1±1.31**d**	4.55±0.14**d**
BD+AMF	12.7±0.29**b**	4.42±0.12**b**	4.53±0.13**b**	2.14±0.04**b**	10.0±0.28**b**	41.7±1.17**b**	66.9±1.61**ab**	6.13±0.19**b**
BDB+AMF	13.8±0.33**a**	4.84±0.14**a**	5.06±0.14**a**	2.27±0.05**a**	10.8±0.31**a**	47.2±1.33**a**	69.3±1.67**a**	6.98±0.21**a**

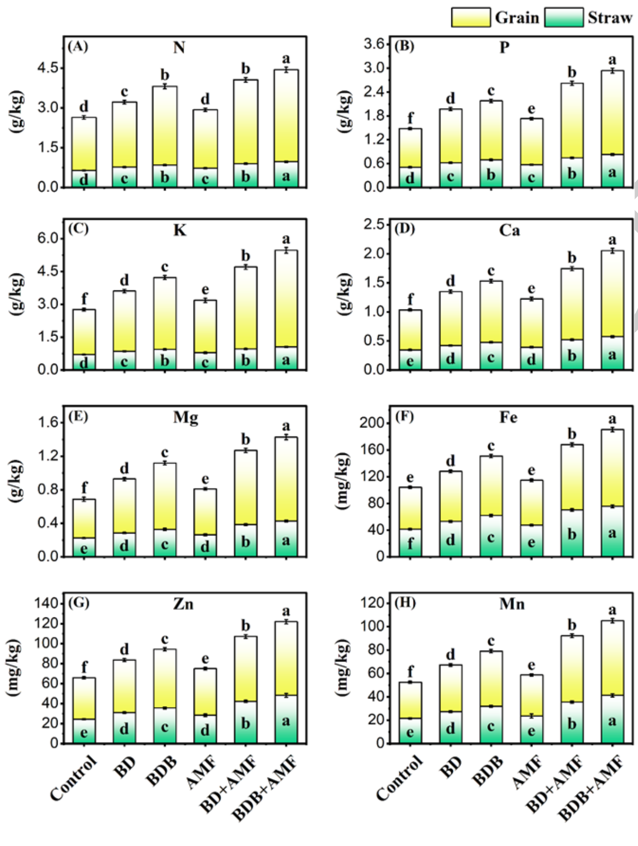

Figure 5. Effects of biogas digestate (BD), its biochar (BDB), AMF, and joint application of BD and BDB with AMF on N (A), P (B), K (C), Ca (D), Mg (E), Fe (F), Zn (G) and Mn (H) concentration in oat grain and straw. The bars represent the mean of triplicate of each treatment, and the error bars indicate the standard error (± SE). The different alphabets above the error bars differ significantly (P < 0.05) from one another.

3.6. Soil Enzymology

The measured activities of soil enzymes, i.e., urease, protease, β-glucosidase, catalase, chitinase, and peroxidase, were in the following ranges: 1.05−2.31 μg N-N (H$_4$ kg^{-1} h^{-1}), 26.9−48.3 mg kg^{-1} 24 h^{-1}, 0.56−1.40 μg p-NP g^{-1} 24 h^{-1}, 32.3−54.1 vol. of 0.1 M KMnO$_4$ g^{-1} of soil, 3.90−8.52 mg *p*-NP kg^{-1} soil h^{-1} and 2.11−4.64 mol g^{-1} h^{-1}, respectively (**Figure 6**). Surprisingly, compared to the control, each treatment led to significant improvements in soil enzymatic activities. In the case of BDB+AMF treatment, the highest enhancements in urease, protease, β-glucosidase, catalase, chitinase, and peroxidase activities by 119, 80, 151, 68, 118, and 121%, respectively, compared to control, were noted.

3.7. Pearson correlation analysis and principal component analysis

Pearson correlation analysis was conducted to investigate the relationships between soil properties, enzyme activities, Pb accumulation, and plant growth traits (**Figure 7**). DTPA-extractable Pb was significantly and negatively correlated with soil pH (r = –0.92, p < 0.01), microbial biomass carbon (MBC, r = –0.86, p < 0.01), and enzymatic activities such as urease (r = –0.86, p < 0.01), catalase (r = –0.75, p < 0.01), and dehydrogenase (r = –0.70, p < 0.05). This indicates that increased biological activity and soil health are associated with reduced Pb bioavailability. Shoot and grain Pb concentrations also exhibited strong negative correlations with plant biomass (r = –0.85 to –0.90, p < 0.01), suggesting that Pb stress directly impairs growth. In contrast, shoot dry weight (SDW), root dry weight (RDW), and grain dry weight (GDW) showed significant positive correlations with MBC and antioxidant enzymes, highlighting the role of microbial and enzymatic enhancement in alleviating Pb toxicity. Soil pH was positively associated with both biological activity and plant biomass, and inversely related to Pb translocation. These findings collectively demonstrate that soil microbial and enzymatic responses are strongly linked to reduced Pb mobility and improved plant performance, affirming the effectiveness of the applied amendments in remediating Pb-contaminated soils.

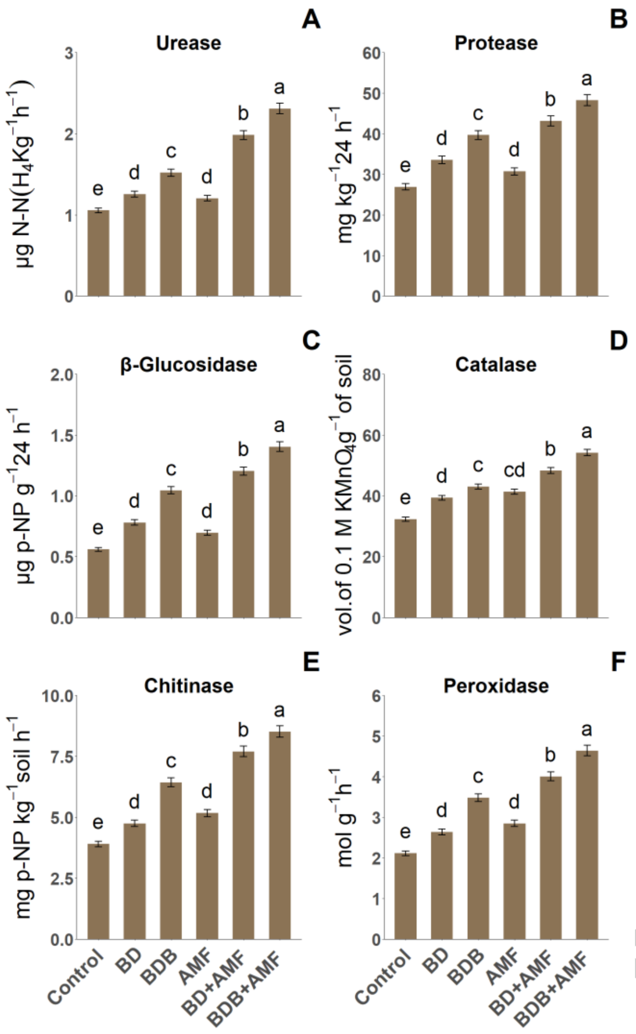

Figure 6. Effects of biogas digestate (BD), its biochar (BDB), AMF, and joint application of BD and BDB with AMF on urease (A), protease (B), β-glucosidase (C), catalase (D), chitinase (E) and peroxidase (F) in soil. The bars represent the mean of triplicate of each treatment, and the error bars indicate the standard error (± SE). The different alphabets above the error bars differ significantly ($P < 0.05$) from one another.

Figure 7. Correlation matrix showing relationships among soil biochemical properties, Pb accumulation in plant tissues, and plant growth parameters. Positive correlations are shown in red, negative correlations in blue, with the intensity of the colour reflecting the strength of the correlation (Pearson's r).

Principal component analysis (PCA) was performed to assess the relationships among soil properties, enzyme activities, biomass traits, and Pb accumulation under different treatments (**Figure 8**). The first two principal components (PC1 and PC2) explained 94.6% and 3.3% of the total variance, respectively, accounting for a cumulative 97.9% of the variation among the measured parameters. PC1 was primarily driven by positive loadings of soil microbial biomass carbon (MBC), catalase (CAT), chitinase, and plant growth parameters (shoot dry weight, root dry weight, and grain dry weight), while DTPA-extractable Pb, shoot Pb, and grain Pb were negatively associated with PC1. This indicates that treatments enhancing microbial activity and plant biomass were negatively correlated with Pb accumulation. PC2 contributed less variation but separated parameters such as soil pH and protease activity. Treatments positioned on the positive side of PC1 were associated with improved soil biological health and reduced Pb bioavailability, whereas those on the negative side were characterized by high DTPA-Pb concentrations and Pb translocation to shoots and grains. The clear separation of variables in the biplot highlights the contrasting effects of amendments on soil–plant interactions and the effectiveness of treatments in our study in mitigating Pb stress and promoting plant performance.

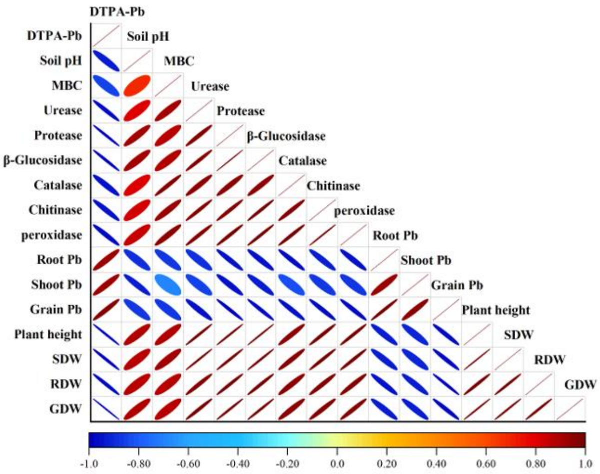

Figure 8. PCA biplot showing the relationships among soil biochemical properties, Pb accumulation, and plant growth parameters. Arrows represent variable loadings, and dots indicate treatment scores.

4. Discussion

4.1. AMF-associated parameters, EEG, MBC, and microbial numbers

Excessive Pb concentration in soil has detrimental effects on soil microbial survival and activities, including declining microbial abundance, reduced AMF root colonization, and decreased glomalin secretion by AMF (Siles *et al.* 2022). Contrarily, BCR supports AMF root colonization, as well as the contents of glomalin secreted by AMF and soil MBC content in Pb-polluted soil (Khan *et al.* 2020). Additionally, it enhanced microbial diversity in soil with Cd and Zn pollution (Shi *et al.* 2023). Inoculation of Pb-contaminated soil with AMF improved the ability of AMF to colonize roots, expanded the diversity of beneficial microbes (*Rhodobacter*, *Archangium*, and *Longimicrobium*), and soil glomalin content (Siles *et al.* 2022; Li *et al.* 2023; Adeyemi *et al.* 2021). In our case, BDB amplified AMF root colonization, the contents of EEG and MBC, and microbial numbers as well as hyphal length density, and spore density (**Figure 2**) because 1) BDB improved soil C content (Tsai *et al.* 2018), which was utilized by microbes including AMF, leading to their higher numbers, activities, and perpetuation in soil (Shi *et al.* 2023), and 2) a large surface area and porosity of BDB (Dutta *et al.* 2021) provided habitat to soil microbes and AMF and protected them against predation and desiccation (Hosseini *et al.* 2021;

Poveda *et al.* 2021). Moreover, BDB immobilized Pb in soil and alleviated the stress on microbes and AMF induced by Pb, leading to enhancements in their growth, perpetuation and activities (Haider *et al.* 2022). Interestingly, BCR improved soil conditions for AMF spores to germinate, promoted the development of AMF mycelium, the ability to colonize roots, and secrete EEG (Zhao *et al.* 2024; Khan *et al.* 2020). Furthermore, AMF inoculation raised glomalin and MBC contents and microbial numbers by 1) solubilizing nutrients from soil and BCR (Zhao *et al.* 2024; Bisht and Garg 2022) and 2) secreting EEG containing C, N, P, K, Ca, Mg, Fe, Zn, and Cu and organic compounds required for boosting microbial activities, diversity, and structure (Singh *et al.* 2022). AMF accounts for 20 to 30% of soil microbial biomass (Sun *et al.* 2022). Moreover, AMF improved the aggregate stability of the soil, reduced wind and water erosion, and enhanced the infiltration of water and air, thereby boosting microbial growth and activities (Ai *et al.* 2022).

4.2. Status of Pb in plant parts and its soil bioavailability

The higher bioavailability of Pb in soil led to increased Pb accumulation in the edible portions of crops (Pandey *et al.* 2022). Applying BCR efficiently reduced soil Pb bioavailability and its concentrations in the aerial parts of *Prosopis laevigata* (Ramírez-Zamora *et al.* 2022) and in the roots and shoots of *Zea mays* L. (Afzal *et al.* 2024). Interestingly, *Funneliformis mosseae* inoculation in Pb-polluted soil declined Pb uptake (57.6%) in soybean (Adeyemi *et al.* 2021). The lowest Pb concentrations in oat grain, straw, root and DTPA-extract, while the highest values of soil pH and IMDX-Pb were noted with BDB+AMF (**Figure 3**), with Pb concentration in grain (3.27 mg kg^{-1} DW) below the permissible level of WHO/FAO (2007) with this treatment (**Figure 3**). With BDB+AMF, numerous mechanisms caused reduced Pb accumulation in oat roots, straw, and grain. When BD was pyrolyzed to BDB, basic cations (Mg, K, Na, and Ca) of BD were converted to their oxides, carbonates, and hydroxides (Tsai *et al.* 2018), which raised soil pH upon their dissolution in the soil and precipitated Pb as hydroxyl-bound species of Pb, i.e., $PbOH^+$, which reduced Pb bioavailability in the soil (Haider *et al.* 2022). Interestingly, BDB had a spongy structure, a larger surface area, surface charges, aromatic functional groups, and negative sites, which reduced Pb bioavailability in the soil through immobilization, thereby reducing its plant uptake (Wang *et al.* 2022a; Dutta *et al.* 2021). Not only that, AMF also has additive roles in reducing Pb phytoavailability in soil and its uptake in oats. During colonizing plant roots, AMF reduced Pb entry into the roots by 1) acting as a physical barrier and 2) sequestering Pb into vesicles, spores, and mycelia (both intra and extraradical), which also minimized Pb concentrations in the parts of oat plants (Farhad *et al.* 2024). AMF secrete EEG and EPS, which bind Pb in the soil, thus reducing its plant uptake (Singh *et al.* 2022; Farhad *et al.* 2024). A higher production of PCs is reported in AMF-colonized roots, which chelate Pb and reduce its mobility to plant aerial parts (Gu *et al.* 2025).

4.3. Plant growth and yield

The growth, biomass and yield of cereals are reduced by Pb toxicity (Wang *et al.* 2022b; Pandey *et al.* 2022). Conversely, improved growth, yield and 1000-grain weight of barley were reported with soil conditioning with BCR (Hosseini *et al.* 2021). BCR addition in Pb-polluted soil augmented plant height, fresh and dry weights of roots and shoots of sedum (Shi *et al.* 2023) and mesquite (Ramírez-Zamora *et al.* 2022). Interestingly, inoculating Pb-polluted soil with AMF improved root and shoot biomass of Whipstick Wattle (Li *et al.* 2023) and rosemary (Alinejad *et al.* 2024). Various mechanisms of BDB+AMF improved plant height, straw DW, root DW, grain DW, number of tillers per plant and ear length of oats (**Figure 4**). Being porous, nutrient-rich and having a large surface area (Ayaz *et al.* 2022; Dutta *et al.* 2021; Tsai *et al.* 2018), BDB improved soil WHC, porosity, nutritional status, and plant water relations, which promoted plant growth (Kumar *et al.* 2024; Ayaz *et al.* 2022). Besides this, BDB improved oats growth and yield by reducing nutrient leaching and enhancing organic matter content in soil (Ramírez-Zamora *et al.* 2022; Dutta *et al.* 2021). BDB immobilized Pb and reduced Pb-induced toxicity to oats plants, thereby increasing plant growth and yield (Ayaz *et al.* 2022). Moreover, AMF solubilized nutrients from the soil matrix, improving the ability of roots to absorb these nutrients along with water, thereby enhancing plant growth and yield. This concept of integrating cost-effective and localized strategies to address environmental challenges aligns with broader innovation pathways, including digital finance, ecological restoration, and sustainable agricultural mechanization, which promote environmental recovery in resource-constrained settings (Wang and Ma 2024; Ma *et al.* 2025b; Ma *et al.* 2025c).

AMF also supported plant growth and yield by secreting glomalin, which not only provides essential nutrients to plants but also encourages soil microbes to secrete growth hormones, such as indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid deaminase (ACC deaminase). These growth hormones also promoted growth, yield and biomass of oats (Singh *et al.* 2022; Liu *et al.* 2022). Upsurging levels of plant endogenous growth hormones, reducing Pb toxicity, Pb-induced oxidative stress, and nematode attack are other key roles of AMF by which the growth, biomass, and yield of oats were improved (Gu *et al.* 2025; Ai *et al.* 2022; Pires *et al.* 2022).

4.4. Grain and straw status for nutrients and biochemical compounds

Reduced nutrient uptake and synthesis of biochemical compounds in various plant species are noted with Pb stress (Adeyemi *et al.* 2021; Boorboori *et al.* 2022). BCR incorporation in Pb-contaminated soil raised protein, fiber, and fat contents, while N, P, K, Ca, Mg, Mn, Fe, and Zn concentrations in spinach (Turan 2020). Soil inoculation with *Diversispora eburnea* uplifted P, K, Ca, Mg, Mn, Fe and Zn contents in maize (Sun *et al.* 2022). The rise in contents of nutrients and biochemical compounds (protein, fat, fiber and carbohydrate) of oat grain and straw with BDB+AMF is due to the versatile mechanisms of these amendments (Table 2, **Figure 5**). BDB was nutrient-rich, supplying essential nutrients to plants and thereby increasing the nutrient content of grain and straw, which is positively linked to enhanced synthesis of biochemical compounds (Tsai *et al.* 2018; Ayaz *et al.* 2022). Moreover, BDB improved soil moisture content, porosity, and health. Such ambient soil conditions enabled the plants to exhibit better growth and improved metabolism, ultimately enhancing the synthesis of biochemical compounds in them (Dutta *et al.* 2021; Turan 2020). BCR improves nutrient availability to plants as it minimizes 1) nutrient leaching from soil (Ramírez-Zamora *et al.* 2022) and 2) enhances the numbers of soil microbes to solubilize nutrients (Kumar *et al.* 2024). Moreover, BDB enhanced the synthesis of biochemical compounds in oat plants by mitigating Pb-induced oxidative stress, which they faced in the Pb-polluted soil (Afzal *et al.* 2024; Haider *et al.* 2022). Furthermore, AMF raised nutrient uptake by oats through 1) expanding root adsorptive area through tweaking its architecture (shape, branches, and volume) (Sun *et al.* 2022; Zhou *et al.* 2022; Gu *et al.* 2025), 2) minimizing nutrient leaching from the soil, and 3) fetching nutrients from distant zones (Yang *et al.* 2023). In BDB+AMF, the highest EG content was observed (**Figure 2**). Glomalin contains significant concentrations of C, N, P, Fe, Cu, Zn, Mg, Ca, S, and K. These nutrients were readily available to oat plants throughout their entire growth phase, resulting in enhanced nutritional status of both straw and grain (Singh *et al.* 2022). Interestingly, this rise in nutrient uptake by oats enhanced the ability of plants to synthesize various biochemical compounds (Bisht and Garg 2022; Pirsarandi *et al.* 2022).

4.5. Soil Enzymology

Soil health is crucial for improved plant growth, yield, and quality of the harvestable part. Compared to traditional soil health monitoring methods, the recurrent neural networks (RNN)-IoT methodology is efficient, accurate, and minimizes ecological impacts by judiciously using water and fertilizer (Selvanarayanan *et al.* 2024). Soil health is closely linked to nutrient cycling, which is driven by the enzymes secreted by soil microbes (Siles *et al.* 2022). BCR addition enhanced urease, dehydrogenase, and ß-glucosidase activities in Pb-polluted soil (Pandey *et al.* 2022), while urease, phosphatase, and protease activities in the soil with Cd and Zn pollution (Shi *et al.* 2023). Moreover, phosphatase, invertase, glucosidase, and protease activities were improved in Cd-polluted soil upon inoculation with AMF mixture (*Rhizoglomus intraradices*, *Claroideoglomus etunicatum*, *Claroideoglomus claroideum* and *Funneliformis mosseae*) (Bisht and Garg 2022). The highest activities of urease, protease, β-glucosidase, catalase, chitinase and peroxidase with BDB+AMF are due to several mechanisms (**Figure 6**). BCR addition in soil improves microbial secretion of enzymes by 1) alleviating Pb toxicity to microbes (Zhao *et al.* 2024) and 2) improving the overall soil environment (Shi *et al.* 2023; Kumar *et al.* 2024). The nutrient richness of BDB (Ayaz *et al.* 2022; Dutta *et al.* 2021) and porosity (Dutta *et al.* 2021) also raised microbial enzyme secretion by providing nutrition and shelter to them (Kumar *et al.* 2024). Surface adsorption of extracellular enzymes and/or substrates or inhibiting the reaction site of enzymes in a way that affects their affinity for substrates with BCR also positively impacts soil enzymatic activities (Siles *et al.* 2022). Interestingly, AMF boost activities and enzyme secretion by microbes by providing them with 1) glomalin, a rich source of nutrition, and 2) alleviating HMs toxicity (Singh *et al.* 2022). Apart from the secretion of several extracellular enzymes on its own, AMF improve the contents and composition of root exudates, thus positively affecting microbial enzyme secretion (Singh *et al.* 2022; Zhou *et al.* 2022).

5. Conclusions

Lead can enter the food chain from the release of untreated effluents from Pb acid battery repairing workshops. The BD is a waste of biogas plants available the whole year. It is cheap and rich in nutrients, but has severe ecological issues at its disposal. Moreover, AMF inoculum is inexpensive, boosts plant growth and mineral nutrition, and reduces Pb uptake in plants. Thus, preparing BDB from BD is practical for such waste material to use as a source of plant nutrition and remediation of Pb-polluted soils. Charring of BD to prepare BDB increases its surface area and mineral nutrients, thus making it a suitable candidate for enhancing plant growth and reducing Pb mobility in soil. In the current pot experiment, BDB+AMF showed premium results in reducing Pb accumulation in grain (below the WHO/FAO limit [5 mg kg^{-1} of DW]), straw, and roots. Other outcomes of this treatment are 1) enhanced mineral nutrition and biochemical worth of grain and straw, and 2) improved plant growth and yield. Regarding soil health, this treatment also boosted the enzyme activities and microbial numbers in the soil. Conclusively, applying BDB with AMF has the suitability to remediate Pb pollution of soil but also boost the nutritive value of oat grain and straw. Due to the richness of BD with nutrients and its availability throughout the year, the conversion of BD into BDB can be very cost-effective and overcome the shortage issue of feedstock to prepare BCR for agricultural and environmental usage. However, certain limitations, such as the availability of inexpensive equipment to produce BDB at low costs, the properties of BDB, the AMF inoculum with different types of AMF strains, and local climatic conditions, including precipitation, humidity, and temperature, may necessitate further management to utilize this amendment by farmers effectively. Since the immobilization technique does not remove Pb from the soil but firmly fixes it in the soil matrix, long-term monitoring of the amended soils is also crucial, which requires expertise from skilled persons or scientists. Compared with existing research outcomes like AMF+BCR effects on wheat for reducing Pb concentrations in grain (58%), shoots (16%) and roots (47%), while reduction of maize shoot Pb (32.94%) and soil bioavailable Pb (30.94%), our findings are superior in terms of Pb reductions in soil bioavailable fraction (62%), roots (45%), shoots (66%) and grain (80%), than control. Thus, our results highlight the significance of BDB+AMF as a superior soil amendment. Future directions could involve the use of advanced modelling tools to monitor Pb immobilization in soils with BDB+AMF, having variable pollution levels, and their effects on soil health and Pb distribution in different edible parts of food crops in pot experiments. Later, the efficacy of this amendment should also be observed in contaminated agricultural fields, where soil, microbial communities, and climatic conditions vary significantly from pots. Government research institutes should support the farming communities by providing consultancy services and supplying them with BDB and AMF at cheaper rates to remediate soil polluted with Pb.

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Declaration of competing interest

Authors declare no competing interests.

Availability of data and material

The data of this manuscript is present in this manuscript and can be made available on a reasonable request.

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