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POTENTIAL OF RHIZOSPHERIC FUNGUS *ASPERGILLUS TERREUS* IN MITIGATING LEAD TOXICITY IN WHEAT SEEDLINGS

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Abstract

Lead toxicity is a significant kind of contaminants that adversely affects crop productivity and threatens environmental sustainability. In this study, we isolated the rhizofungus *Aspergillus terreus* (*A. terreus*) from *Cannabis sativa* L. to investigate its potential for plant growth promotion and mitigation of the adverse effects of Pb toxicity on *Triticum aestivum* L. The experiment was conducted in a completely randomized design with three replicates per treatment. The treatments consisted of a control and *A. terreus* group, as well as groups exposed to 100, 200, and 300ppm lead, and *A. terreus* in combination with 100, 200, and 300ppm lead. *A. terreus* successfully colonized the roots of wheat seedlings subjected to lead acetate (Pb) stress, significantly promoting plant growth and development. The results demonstrated a dose-dependent decrease in growth and biochemical markers in response to increasing Pb stress, with 300ppm Pb exhibiting the most significant adverse effects. Pb stress caused oxidative damage and stunted development by interfering with the production of chlorophyll, reducing protein content, and decreasing antioxidant activity. Pb-induced stress was considerably reduced the adverse influence after the inoculation of *A. terreus* at all tested concentration (100, 200 and 300ppm Pb + *A. terreus*). This improvement was accomplished through the promotion of root and shoot development, stabilization of photosynthetic pigments, inducement of proline level, enhancement of antioxidant defences, flavonoids, and maintenance of sugar and protein levels. The results indicate that the *A. terreus* serve as environmentally sustainable solutions for reducing heavy metal stress and improving plant resilience. More studies at the molecular level and large-scale field tests are needed to confirm these results and enhance microbial treatments for sustainable farming in polluted soils.

Key words: Heavy metal stress; Lead (Pb) toxicity; *Aspergillus terreus*; *Triticum aestivum* L.; Biochemical analysis; Plant microbe interaction

Introduction

Heavy metals are generally detrimental at low concentrations, characterized by specific gravities exceeding 5 g cm^{-3} or atomic masses greater than 20 (Rascio and Navari-Izzo, 2011). For examples lead (Pb), arsenic (As), silver (Ag), and cadmium (Cd). Lead, chromium, arsenic, cadmium, and mercury present considerable hazards owing to their widespread use, environmental distribution, and the toxicity linked to specific mixed or elemental forms (Tositti *et al.*, 2014). Heavy metal stress represents a critical concern in various terrestrial ecosystems worldwide. Currently, the heavy metals accumulation resulting from extensive industrialization adversely affects crop yield and soil quality (Shahid *et al.*, 2015). Soil texture degradation, alterations in pH, the accumulation of various elements, and the presence of heavy metals can adversely affect multiple physiological and molecular processes in plants, ultimately leading to a reduction in plant growth (Hassan *et al.*, 2017). Developmental pathways and essential biological functions rely on heavy metals such as Ni, Zn, Cu, Mo, Mn, and Co, whereas the excessive concentrations of these metals can significantly diminish crop productivity. This effect is also observed with four other highly toxic heavy metals: As, Pb, Cd, mercury (Hg), chromium (Cr), aluminum (Al), and beryllium (Be) (Pierart *et al.*, 2015). These detrimental substances lead to metabolic issues and morphological abnormalities resulting low plant productivity (Amari *et al.*, 2017).

Plants have the capacity to absorb heavy metals present in soil solutions or those released by root exudates (Blaylock and Huang, 2000). Certain heavy metals are necessary for plant growth and maintenance; however, high concentrations of some heavy metals can harm plants. Furthermore, the capacity of plants to accumulate essential metals facilitates their uptake of non-essential elements (Djingova & Kuleff, 2000). Pb is naturally present in the environment; however, human activities such as mining, manufacturing, and the combustion of fossil fuels contribute substantial amounts of this element. Pb has various applications in industrial, agricultural, and household contexts (Canady *et al.*, 1997), whereas it is the second most toxic metal following arsenic and adversely impacts living organisms (Atsdr, 1997). All morphological, physiological, and biochemical systems are significantly affected when Pb levels exceed the critical

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threshold (Kushwaha *et al.*, 2018). The pollution of soil leads to several adverse effects on plants, such as reduced nutrient uptake, alterations in plant-water relations, and the generation of reactive oxygen species (ROS), which diminish photosynthesis and induce cell death, ultimately causing a substantial decline in crop yield (Hadi *et al.*, 2015). Altering the activity of peroxidase and polyphenol oxidases results in toxicity that delays the development of radicals by increasing protein and carbohydrate levels, thereby affecting the roots' ability to oxidize (Uzu *et al.*, 2009).

Pb is known to inhibit germination in various crops, including rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) (Zhang *et al.*, 2018). It also inhibits enzymes like amylase and protease, which are vital for seed germination (Sengar *et al.*, 2008). Furthermore, less food is mobilized under Pb toxicity, which reduces the generation of radicals, interferes with cellular osmoregulation, and breaks down proteolytic activities, all of which have an impact on seedling germination and development (Cokkizgin & Cokkizgin, 2010). Pb has detrimental effects on transpiration, cell division, root development, and chlorophyll synthesis, it slows down seed germination and is linked to poor seedling growth. Pb-induces decreased cell division, resulting from an increased interphase stage of mitosis, is the primary reason of reduced plant development (Patra *et al.*, 2004). When subjected to elevated Pb concentrations, physical nut cuttings and seedlings (*Jatropha curcas* L.) were unable to grow and develop properly (Shu *et al.*, 2012). Pb exposure significantly reduced sprouting, growth, seedling development in *Triticum aestivum* L. (*T. aestivum*) (Dey, 2007), which might be associated with impaired photosynthesis, disrupted water relations, and altered nutrient metabolism (Alsokari & Aldesuquy, 2011).

Most interactions between microorganisms and plants occur in the rhizosphere, the soil region directly influenced by the roots due to their proximity to plant organ. These interactions may be mutualistic or non-mutualistic (Philippot *et al.*, 2013; Yaseen *et al.*, 2018). Root activities affect the soil microbial population by modifying the physicochemical properties of the adjacent soil. Rhizosphere microorganisms reside both within and on the surfaces of plant roots, utilizing substrates derived from the roots in the surrounding soil (Philippot *et al.*, 2013). The rhizosphere zone serves as a highly active interface between ecosystems, characterized as a microclimate of soil that surrounds and is influenced by plant roots (Saha *et al.*, 2008). The breakdown of various components of soil organic matter by soil microorganisms results in the production of diverse secondary metabolites and biomolecules, facilitated by the activity of distinct enzymes (Tortella *et al.*, 2008). These substances and biomolecules are recognized for their capacity being assimilated by plants, functioning as biofertilizers during the cycle process, and may indirectly enhance soil diversity, metabolism, stability, and health. A diverse array of microbes colonizes plants, establishing symbiotic relationships (Manoel da Silva *et al.*, 2015), whereas the mechanisms that designate these microorganisms as plant growth promoters (Scervino *et al.*, 2009; Yaseen *et al.*, 2020). *T. aestivum* is an important global cereal crop recognized for its high nutritional value, which includes vitamins, amino acids, and essential minerals. Pb contamination negatively impacts wheat germination, growth, and photosynthesis, interfering with metabolic pathways and resulting in reduced crop yields. In this study, we investigate the potential of rhizospheric fungi (*A. terreus*) in enhancing the resilience of *T. aestivum* to Pb-induced stress, with an emphasis on morphological, physiological and biochemical responses.

Material and Methods

Laboratory experiment: Rhizosphere soil samples form *Cannabis sativa* L. underwent serial dilution and were subsequently inoculated onto PDA media. Following a 72-hour incubation period at 28°C, fungal colonies were isolated with sterile inoculation loops, subsequently transferred to PDA media, and incubated at 27°C for an additional 72 hours (Murali *et al.*, 2012). Fungal strains were preserved at 4°C. The PDA media was prepared by dissolving 39g in 1000mL of distilled water, followed by autoclaving and supplementation with streptomycin to inhibit bacterial contamination. Fungal cultures were applied to solidified plates for growth assessment. Czapek medium, consisting of 10g glucose, 10g peptone, 0.5g KCl, 0.5g $MgSO_4$, and 0.01g $FeSO_4$ in 1000mL distilled water, was autoclaved, inoculated with pure fungal strains, and incubated in a shaker at 120 rpm and 30°C for seven days (Bibi *et al.*, 2018). Fungal biomass and filtrates were isolated and preserved at 4°C. Seedlings in the two-leaf stage underwent treatment with fungal culture filtrates. After a duration of seven days, measurements of root-shoot length, root count, leaf length, as well as fresh and dry weights were conducted and compared to control groups (Hamayun *et al.*, 2020).

Field experiment: *T. aestivum* seeds were collected from the Cereal Crop Research Institute (CCRI) in Nowshehra. Selected seeds that have been subjected to surface sterilization for 30 seconds with 1% perchloric acid, followed by a one-minute treatment with 70% ethanol. The plants were grown in clay pots with soil that had been autoclaved. The experiment used soil samples taken from Bacha Khan University Charsadda. Before seeds were sown, the soil was prepared by wetting it to field capacity and letting it equilibrate for a week under normal environmental circumstances. Ten seeds were in each pot and have three replicates for each treatment. After sprouting, 05 seedlings of uniform size were selected, and the pots were arranged in a Randomised Complete Block Design (RCBD). Selection of uniform seedlings allows controlling genotypes variation which may be a confounding variable. *T. aestivum* seedlings were collected following the three-leaf stage, and their biochemical and morphological characteristics were analyzed (Ikram *et al.*, 2019).

Photosynthetic contents: The concentrations of carotenoids, chlorophyll a, chlorophyll b, and total chlorophyll were evaluated in the leaves of *T. aestivum*. Following the grinding of 0.1g of fresh leaf in 2 ml of 80% acetone using a pestle and mortar for 5 minutes, the mixture was centrifuged at 1000 rpm. A final homogenate volume of 7 ml was obtained using acetone. Optical density measurements were conducted at wavelengths of 480, 645, 663, and 510 nm (Sestak *et al.*, 1971).

Proteins content: One gram of fresh *T. aestivum* leaves was homogenized in 1 ml of phosphate buffer using a pestle and mortar. The culture filtrate and leaf extracts underwent centrifugation for 10 minutes at 3,000 revolutions per minute. Distilled water was added to 0.1 ml of the culture filtrate and leaf extract supernatant to produce a 1 ml solution. Nil. 1 ml of Reagent C was added and agitated for 10 minutes. Subsequently, 1 ml of Reagent D was added and allowed to rest at room temperature for 30 minutes. The Folin reagent functioned as a blank for optical density measurement at 650 nm, according to (Lowry *et al.*, 1951).

Sugar contents: According to Lubna *et al*., (2018), 0.5g of fresh *T. aestivum* was crushed. leaves in 80% methanol to extract sugars from the culture filtrate. 9.5 ml of distilled water was added to the solution after 0.5 ml of it had been extracted, and the combination was centrifuged for 10 minutes at 3000 rpm. About 0.5 ml of leaf extract and fungal filtrate After collecting the supernatant, 1 ml of 80% phenol was added. After that, the mixture was incubated for ten minutes. Five ml of concentrated H2SO4 were then added, and the mixture was incubated for 4 more hours. The optical density was measured at 420 nanometers (Van Handel, 1985).

Flavonoids contents: Flavonoid content were measured using the colorimetric technique plant. The mixture was vortexed and left to stand at room temperature for half an hour after 0.5 ml of fungal culture filtrate, 0.1 ml of 1M potassium acetate, 0.1 ml of 10% aluminum chloride, and 4.8 ml of 80% methanol were added. Methanol was used as a blank to determine the optical density at 415 nm (Van Handel, 1985).

Phenol contents: The total phenolics in the culture filtrate were quantified using the colorimetric technique. The reaction mixture was prepared by combining 0.5 ml of fungal culture filtrate, 0.5 ml of Folin-Ciocalteu reagent, and 2 ml of 20% Na_2CO_3 in a test tube, which was then heated for 1-2 minutes at 40°C in a water bath. Following cooling, the absorbance was recorded at 650 nm (Lubna *et al.*, 2018).

Statistical analysis

Statistical analysis utilized R software to assess the impact of Pb concentrations and microbial inoculation on the growth and biochemical parameters of *T. aestivum* seedlings. One-way ANOVA was employed to evaluate individual and interaction effects, accompanied by Tukey's HSD test for pairwise comparisons ($p<0.05$). PCA identified significant factors contributing to variability, while Pearson correlation and regression analyses quantified the relationships and microbial mitigation of Pb stress. Graphs featuring error bars and PCA biplots, created with ggplot2, illustrated the significant effects and interactions, providing clear insights into Pb toxicity and the protective roles of microbial bioinoculants.

Results

Isolation and purification of rhizospheric fungi: Fungi from the rhizosphere of *Cannabis sativa* L. were isolated using the serial dilution technique. Soil samples were cultured on PDA plates and incubated for seven days, yielding a variety of fungal colonies. Pure fungal strains (Fig. 1), designated as *A. terreus* and HBP, were acquired via successive sub-culturing on fresh PDA plates.

Phylogenetic tree illustrating the evolutionary relationships among fungal strains: The phylogenetic tree depicts the evolutionary relationships among various fungal strains, specifically *A. terreus* strain MSEF75, *Aspergillus nomiae*, *Neurospora tetrasperma*, *Penicillium chrysogenum*, and *Trichoderma koningiopsis* (Fig. 2). The tree was constructed using genetic sequence analysis, with hierarchical branching indicating genetic similarity and evolutionary divergence among the strains. *A. terreus* MSEF75 exhibited strong phylogenetic ties to *Aspergillus* species, including isolate 241 of Aspergillus sp. and strains KG 21 and F1 of *A. nomiae*. This implies that the genus is monophyletic. Different branches in other genera, such as *Penicillium chrysogenum* and *Neurospora tetrasperma*, showed that they diverged from *Aspergillus* in their evolutionary history. *Trichoderma koningiopsis's* phylogenetic divergence from the other strains was highlighted by its inclusion as an outgroup. By elucidating the genetic diversity and evolutionary histories of different fungal strains, the tree offers a basis for comprehending their ecological and functional functions in diverse contexts. Applications like fungal taxonomy, biotechnology, and bioremediation depend on this knowledge.

Fig. 1. A purified rhizospheric strain isolated from the soil.

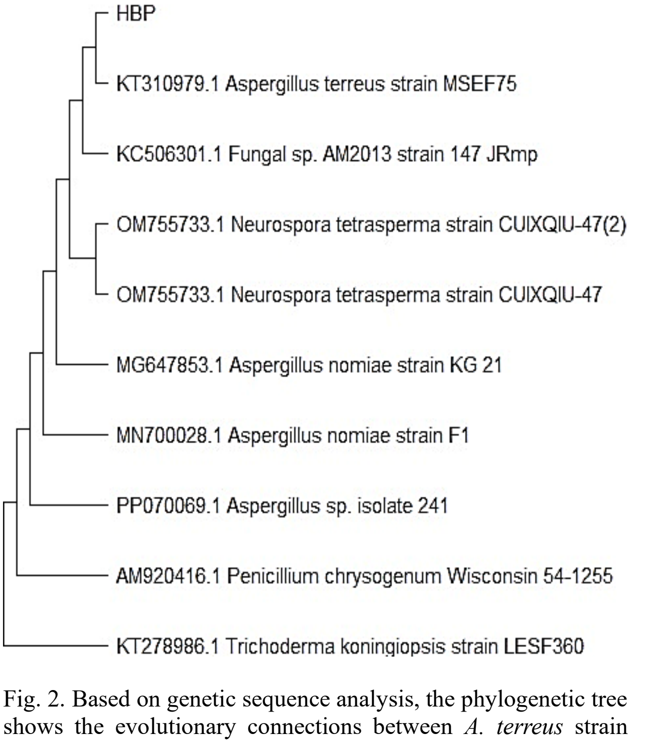

Fig. 2. Based on genetic sequence analysis, the phylogenetic tree shows the evolutionary connections between *A. terreus* strain MSEF75, other fungal strains, and related species.

Fungal strain isolation and culture on Czapek medium: After seven days of shaking, fungal biomass was observed in Czapek media. Pure colonies were successfully obtained and cultivated from three distinct fungal strains, identified as HBP, HGP, and HBDP, in PDA medium. Each isolate was subsequently cultivated on Czapek-Dox medium supplemented with varying concentrations of Pb (100, 200, 300ppm Pb + *A. terreus*) for preliminary screening to assess their resistance to heavy metal stress. The HBP isolate, identified as *A. terreus*, exhibited the highest resistance to Pb among the strains. The biomass production (g/L) of *A. terreus* (AT) at different Pb concentrations was as follows: 62.16 g/L at Pb-100 + AT, 50.14 g/L at Pb-200 + AT, 54.02 g/L at Pb-300 + AT, and 56.4 g/L in the control (AT alone). These results underscore the strain's potential for Pb bioremediation by confirming its resistance to Pb stress and demonstrating relatively good biomass retention even at elevated metal concentrations.

Morphological parameters: Fig. 3 illustrates the impact of varying Pb concentrations (100, 200, and 300ppm) and combination with *A. terreus* on root number and length, shoot length, number of leaves of *T. aestivum*. The control and *A. terreus* + 200ppm (Pb) treatments exhibited maximum root number and longer root lengths relative to other treatments, whereas the Pb treatments, particularly at 300ppm, demonstrated an adverse effects on root number and length. However, *A. terreus* inoculation, mitigated the adverse effects of Pb, illustrating the advantageous role of *A. terreus* in enhancing root development under stress conditions. Shoot length exhibited a dose-dependent response. These results shows that *A. terreus* treatments partially restored shoot length; however, elevated Pb concentrations (300ppm) significantly impeded shoot development, underscoring their role in mitigating Pb toxicity (Fig. 3).

The 100, and 200ppm (Pb), with combination of *A. terreus* treatments exhibited minimal variation in leaf numbers compared to control, consistently demonstrating increased leaf counts. *A. terreus* + 300ppm (Pb) exhibited a slight decrease, suggesting that higher Pb concentrations exert an inhibitory effect on leaf development. The control, *A. terreus*, and *A. terreus* + 100ppm (Pb) treatment exhibited the greatest leaf length, whereas 200, 300ppm (Pb) and *A. terreus* with combination of 300 ppm (Pb) significantly mitigated the reduction in leaf length caused by Pb stress (Fig. 3). These findings indicate that Pb inhibits root and shoot development, leaf number, and leaf length in a dose-dependent manner, with significant reductions observed at elevated Pb concentrations (300 ppm) (Fig. 3).

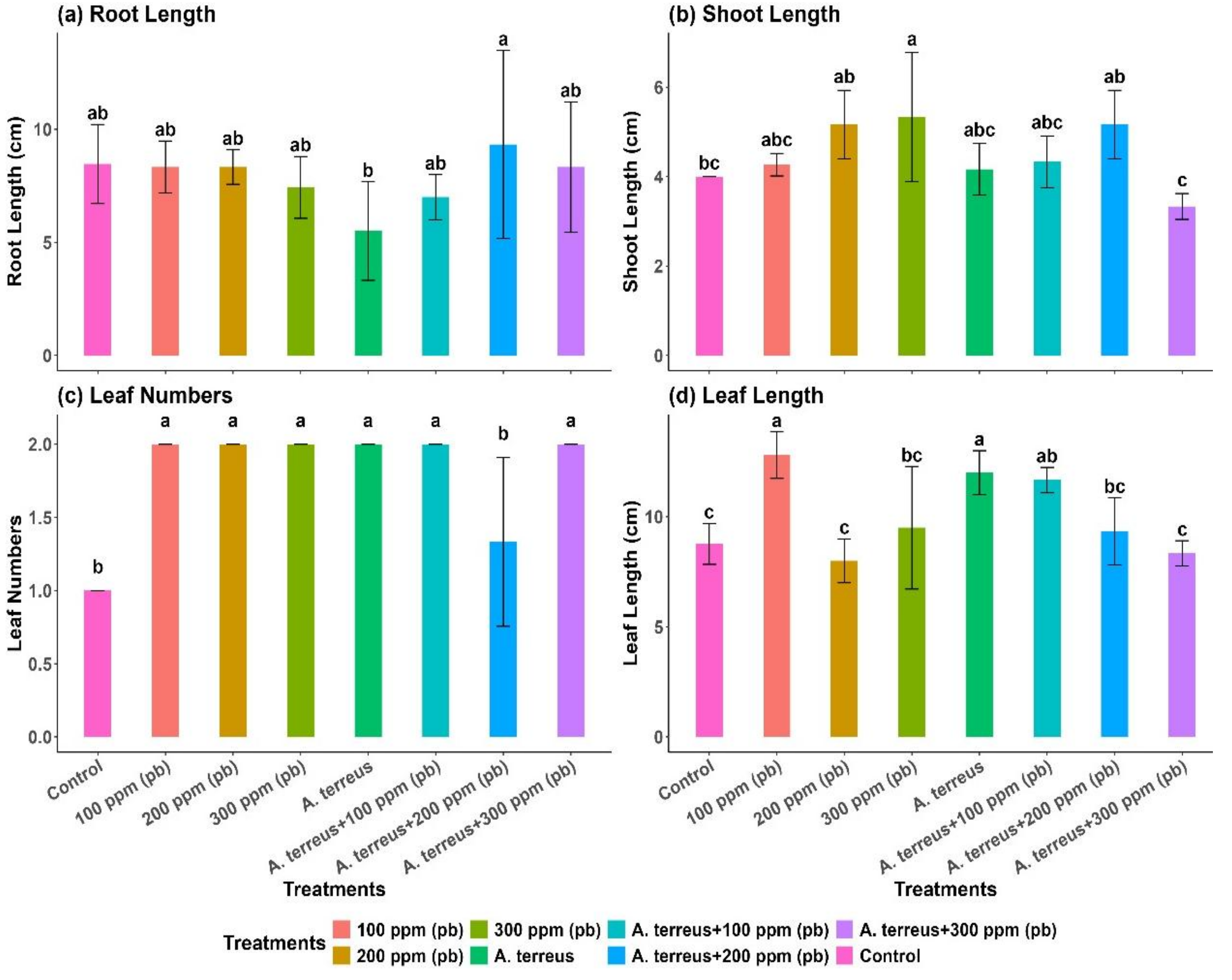

Fig. 3. Impact of Pb and *A. terreus* strain on *Triticum aestivum* L. seedlings' (a) root length, (b) shoot length, (c) leaf numbers, and (d) leaf length.

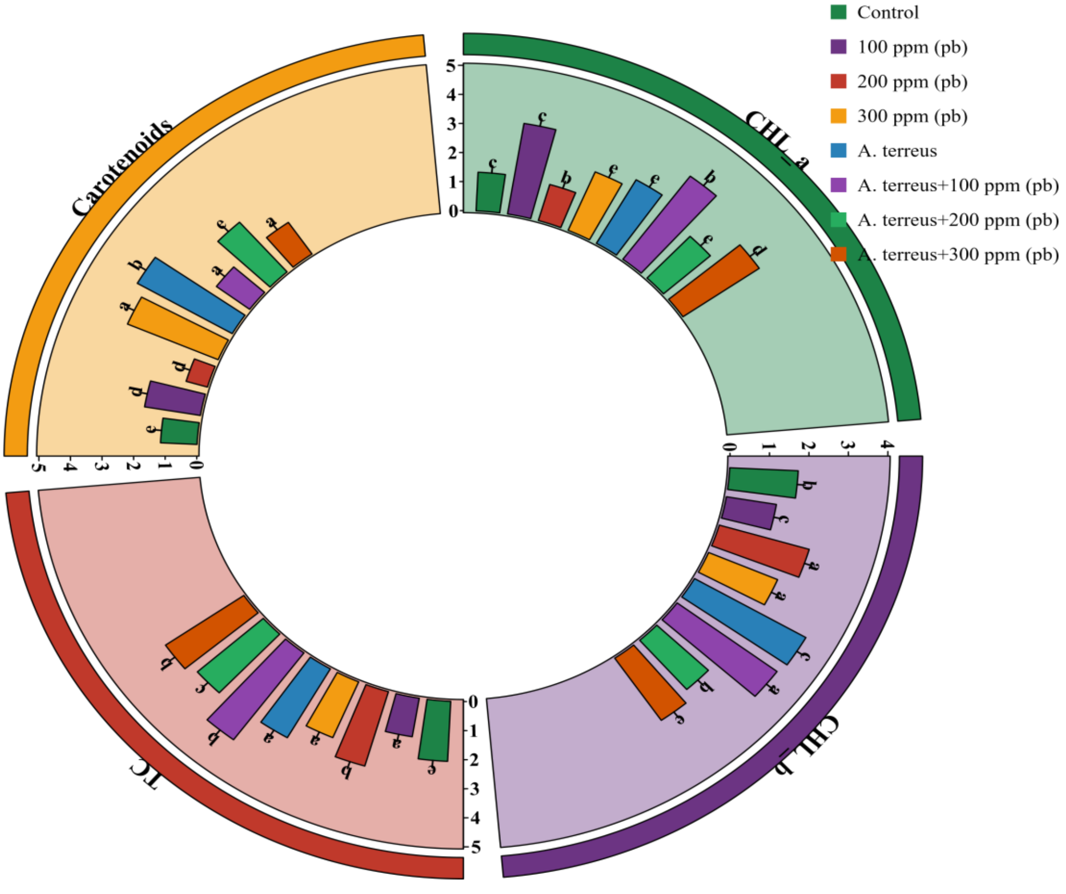

Fig. 4. The impact of Pb and *A. terreus* strain on (a) Chlorophyll a (b) Chlorophyll b, (c) Total Chlorophyll, and (d) Carotenoid content in *T. aestivum* seedlings.

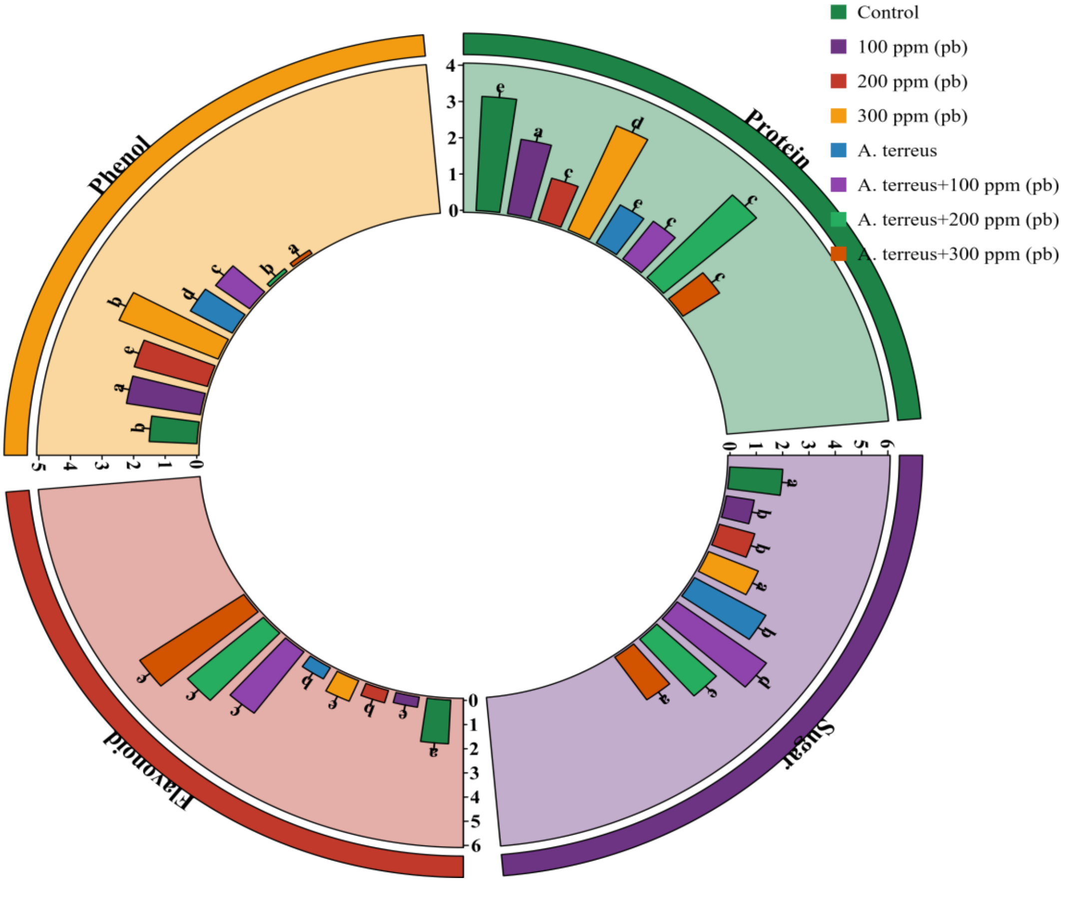

Fig. 5. Illustrates the impact of Pb and *A. terreus* strain on the contents of (a) phenol, (b) protein, (c) flavonoid, and (d) sugar in *Triticum aestivum* L. seedlings.

Determination of photosynthetic pigments: The Fig. 4 illustrates the impact of varying concentrations of Pb and fungus *A. terreus* on chlorophyll a, b, total chlorophyll and carotenoid pigments of *T. aestivum* seedlings. These measurements serve as critical indicators of photosynthetic efficiency and the comprehensive stress response in plants subjected to Pb. The control and *A. terreus* + 100ppm (Pb) treatments exhibited the highest levels of chlorophyll a (Chl a), suggesting optimal conditions for photosynthesis. Chl. a levels were decreased significantly with increasing Pb concentrations, especially at 300 ppm, indicating that Pb inhibited chlorophyll synthesis. Related to control group, the Chl a levels were relatively stable after *A. terreus* treatments, indicating the protective function of *A. terreus* inoculants in mitigating Pb-induced stress. Compared to treatments involving only Pb, which exhibited a significant reduction in chlorophyll b content, the *A. terreus* and *A. terreus* + 100ppm (Pb) treatments demonstrated the highest levels of chlorophyll b. Pb stress disrupts chlorophyll synthesis pathways, leading to reduced pigment stability and diminished photosynthetic efficiency. *A. terreus* with 100ppm (Pb) and *A. terreus* + 200ppm (Pb) demonstrated the highest recovery, indicating the *A. terreus* strain's role in mitigating Pb-induced damage. Total chlorophyll exhibited a significant decrease with increasing Pb concentrations, whereas the lowest in the treatment with 300 ppm of Pb, while the highest levels were recorded in the *A. terreus* + 100ppm (Pb) treatment.

The analysis of carotenoid content exhibited a dose-dependent response, with the *A. terreus* group and combination of 200ppm (Pb) groups demonstrated the highest concentrations of carotenoids, whereas treatment with 300 ppm (Pb) revealed the lowest levels (Fig. 4). The *A. terreus* treatments with Pb stress was effective in maintaining certain carotenoid levels, underscoring the significance of these microbes in mitigating oxidative stress (Fig. 4). These results demonstrate the efficacy of microbial bioinoculants as a sustainable approach to improving photosynthetic pigment stability and overall plant health under heavy metal stress conditions.

Determination of phenol, protein, flavonoid and sugar content: Fig. 5 shows the effects of various treatments of Pb (100, 200, and 300 ppm) and with combination of fungus (*A. terreus*) on phenol, protein, flavonoid, and sugar levels in wheat seedlings. Pb exposure resulted in a dose-dependent effect in phenol levels, with the 300ppm (Pb) treatment showing the highest values, whereas with inoculation of A. *terreus* with 100 and 200ppm (Pb) treatments, alleviated the reduction of phenol (Fig. 5).

The protein content showed a similar trend, with the control and *A. terreus* + 200ppm (Pb) treatments having the highest quantities. Protein levels were considerably lowered by Pb stress, especially at 200 ppm (Pb). Moderate protein levels were observed at treatments 100ppm (Pb) and *A. terreus* at 100ppm (Pb) showed a protective effect. This suggests that even in the face of heavy metal stress, *A. terreus* inoculation enhances protein synthesis and enzymatic stability (Fig. 5). A significant increase in flavonoid content was observed with elevated Pb + *A. terreus* inoculation, known for its antioxidant qualities (Fig. 5). The control and *A. terreus* groups had the higher quantities of flavonoids, whereas the treatment with 300 ppm (Pb) showed the lower levels. The treatments with microbes (*A. terreus* + 100 ppm (Pb) and *A. terreus* + 200 ppm (Pb)) helped keep flavonoid levels relatively stable compared to treatments with higher Pb levels, indicating that the microbes boosted the plant's ability to defend against oxidative stress.

Sugar content, essential for osmotic control and energy metabolism was decreased in proportion to rising Pb concentrations. Sugar levels were significantly reduced by Pb treatments, especially at 200 and 300 ppm. At lower Pb concentrations (*A. terreus* +100 and 200ppm (pb)), the *A. terreus* inoculation maintained sugar content, suggesting the microbial strain's role in plant metabolic activities under heavy metal stress (Fig. 5). These results showed that the Pb effect had a dose-dependent impact on the contents of sugar, protein, phenol, and flavonoids level, whereas the inoculation with the *A. terreus* and its combinations, especially at Pb, exhibited significant protective effects by reducing biochemical disruptions and enhancing metabolic stability.

Discussion

Contamination by heavy metals is very hazardous and its influence may result in a decline of the crop yield and production in affected heavy metals areas. In the developing countries, direct disposal of industrial pollutants, which are usually heavy metals, in water sources and use of contaminated water for irrigation, worsens the cultivable land pollution. Bioremediation is an effective technique for the remediation of soil contaminated with heavy metals, making it suitable for uses in agriculture. Even though it is widely known that rhizofunhus have useful effects on plants under normal and stress conditions (Shaukat *et al*., 2025), research on their use as a bio-agent for remediation and reduction of metal stress on plants is limited (Natasha *et al*., 2022). This research assesses the level of toxicity of Pb on *T. aestivum* seedling and reviews the bioremediation interaction of the isolates of *A. terreus* against various concentrations of Pb. This was accomplished by first isolating rhizofungi from *Cannabis sativa* plant samples gathered from a location close to an industrial region in the Chardadda District. The rhizofungal isolates were screened twice to determine their heavy metal resistance and plant growth-promoting (PGP) capabilities. Among the isolated strains, the screening assay were selected for the potential rhizofungus against the Pb stress and was identified as *A. terreus* through homology of the ITS region of their rDNA.

Our results showed a dose-dependent inhibitory impact from Pb exposure, with substantial reductions in root and shoot elongation, leaf number, and leaf length observed at high doses (300ppm Pb). Pb toxicity-induced oxidative stress, hormonal abnormalities, and disturbances in nutritional absorption are probably the causes of these consequences (Sharma & Dubey, 2020; Ahmad *et al*., 2022). Microbial inoculation helped protect the plants by reducing damage caused by Pb, especially when using the *A. terreus* strain alone or with 100 ppm or 200 ppm of Pb. This was accomplished by maintaining the general well-being of the plant, controlling the leaf growth, and promoting the growth of the roots and shoots. Similar to earlier research, the *A.*

terreus strain helped increase food and water consumption, reduced oxidative stress, and significantly lowered the availability of Pb (Gupta *et al*., 2021; Khan *et al*., 2022; Ahmad *et al*., 2024). Biochemical analysis, such as Chl. "a" and Chl. "b" contents, critical for light energy capture and conversion in photosynthesis, were significantly diminished at increased Pb levels, presumably due to Pb interference with chlorophyll biosynthesis and structural damage to chloroplast membranes (Sharma *et al*., 2021; Shahid *et al*., 2024). Total chlorophyll (TC) content, which indicates cumulative chlorophyll levels was significantly decreased under Pb stress, suggesting impaired photosynthetic machinery and diminished light-harvesting capacity (Ahmad *et al*., 2022; Asad *et al*., 2021). On the other hand, adding *A. terreus* and its combination with Pb, helped keep higher levels of Chl a, Chl b, and TC compared to control group and individual treatments of Pb. *A. terreus* promoted of intake of more nutrients, reduced the availability of Pb, and kept chlorophyll levels stable during stressful conditions. Similarly, the carotenoid content, essential for photoprotection and ROS scavenging, exhibited a dose-dependent decrease as Pb concentrations increased. Increased Pb levels interfere with carotenoid biosynthesis and heighten oxidative stress, resulting in diminished carotenoid content (Kumar *et al*., 2022; Shahid *et al*., 2023). *A. terreus* treatments, successfully reduced the carotenoid levels and improved the plant's ability to fight against oxidative stress. The results show that Pb pollution harms the levels of chlorophyll and carotenoids in wheat seedlings, which reduces their ability to perform photosynthesis and stay strong. Adding *A. terreus* strain and its mixes at lower Pb levels, greatly reduced the harmful effects by lowering Pb toxicity, boosting the plant's defense systems, and keeping the photosynthetic pigments stable (Sharma *et al*., 2021; Ahmad *et al*., 2022, and Gupta *et al*., 2022).

Pb exposure caused a reduction in protein and sugar levels, whereas the flavonoid and phenol induced, with the highest negative impact was seen at 300 ppm (Pb). At higher Pb concentrations, the phenol level, essential for defense mechanisms and antioxidative responses, significantly increased. High levels of Pb interfere with phenolic metabolism and increase phenol buildup, making it easier for the plant to withstand oxidative stress. Phenol levels were significantly raised by microbial treatments (*A. terreus*), indicating the role of the microbial strain in enhancing the antioxidative defense system (Ahmad *et al*., 2022; Begum *et al*., 2021). As Pb concentrations rose, protein content, a crucial indicator of metabolic activity and cellular function, showed a declining trend. Pb disrupts the functions of enzymes and the processes involved in protein synthesis when it attaches itself to sulfhydryl groups in proteins (Sharma *et al*., 2021). The *A. terreus* strain was able to lower protein loss by taking in more nitrogen and making less Pb available, showing that it's important to keep proteins stable in cells. Significant increases in flavonoid content, which is known for its antioxidant qualities and role in scavenging reactive oxygen species (ROS), were the outcome of increased Pb concentrations. Their increase at 300 ppm (Pb) indicates significant oxidative stress. Flavonoids offer defense against oxidative caused by heavy metal stress (Kumar *et al*., 2022). The ability of the microbial strain to lessen oxidative damage and stabilize cellular processes was demonstrated by the *A. terreus* and its combinations, successfully retained flavonoid content. Pb concentrations decrease the sugar content, which is essential for osmotic control and energy metabolism, thereby affecting the amount of energy available for cellular processes (Khan *et al*., 2022). The phenolic contents, protein, flavonoid, and sugar in wheat seedlings were all considerably impacted by lead exposure, with the effects being more noticeable at higher lead levels (Sharma *et al*., 2021; Ahmad *et al*., 2022; Gupta *et al*., 2022).

The scree plot and circular PCA plot (Fig. 6) illustrate the variance and interrelationships among plant growth parameters across various treatments, including differing Pb concentrations (100, 200, and 300ppm). The scree plot indicates that the first two principal components (PC1: 38.9%, PC2: 35.1%) capture a substantial portion of the variance, thereby validating their application in dimensionality reduction. The circular PCA plot illustrates the contributions of specific growth parameters, indicating that shoot length is significantly linked to PC1 and root length to PC2, thereby suggesting these factors are the main contributors to variability. Leaf length and leaf number exhibit moderate contributions, highlighting their significance in multivariate responses. The Cos^2 gradient highlights the significant representation of shoot length and root length, likely affected by Pb-induced stress. Elevated lead concentrations are known to hinder root elongation due to oxidative stress, while shoot growth may initially be less impacted as a result of resource allocation strategies. The results show that when plants face heavy metal stress, they tend to focus their resources on growing shoots rather than roots, which is an important point for future research on how plants respond to stress. The *A. terreus* ability to reduce heavy metal toxicity showing that, under stressed circumstances, resource allocation mechanisms give priority to shoot growth over root growth (Théry *et al*., 2018).

The biplot of principal component analysis (PCA) illustrates the relationships between various treatments and their biochemical variables, condensing complex data into two primary components (Fig. 7). The first principal component (Dim1) accounts for 44.3% of the variance, while the second component (Dim2) explains 21.5%, together representing 65.8% of the total variability. Treatments are represented as points, with proximity to variable vectors signifying strong associations. *A. terreus* at 300 ppm (Pb) demonstrates a significant correlation with flavonoid levels, whereas 300 ppm (Pb) is associated with carotenoid levels, indicating elevated concentrations of these compounds. The control group, located near the origin, exhibited a balanced profile with minimal deviation. The arrows represent variables such as Chl "a", "b", and carotenoids, with their lengths indicating the extent of their contributions to the components. Treatments involving *A. terreus*, particularly at elevated doses, demonstrate notable effects on specific variables, indicating potential synergistic benefits. This PCA analysis shows how treatments affect plant biochemical properties, providing useful information for improving experimental designs (Jolliffe & Cadima, 2016; Lever *et al*., 2017).

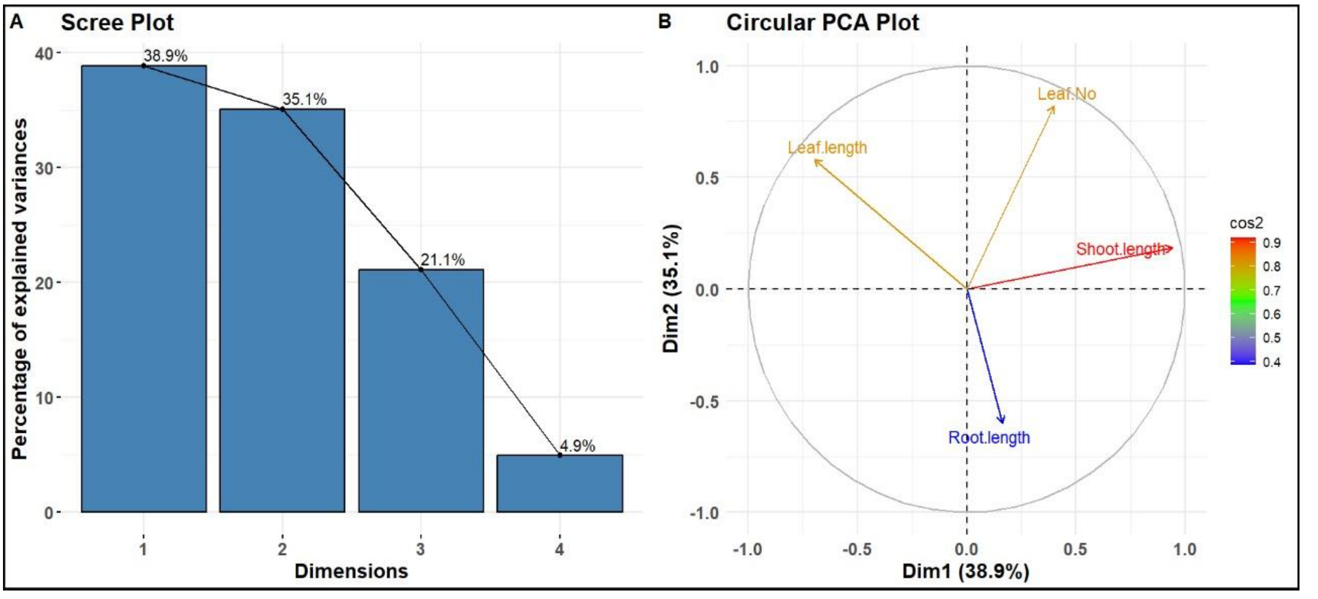

Fig. 6. The scree plot illustrates the explained variance attributed to principal components, highlighting PC1 (38.9%) and PC2 (35.1%) as significant contributors. A circular PCA biplot demonstrates the relationship between growth parameters (root length, shoot length, leaf length, and leaf number) and principal components in *Triticum aestivum* L. seedlings subjected to Pb and *A. terreus* treatments.

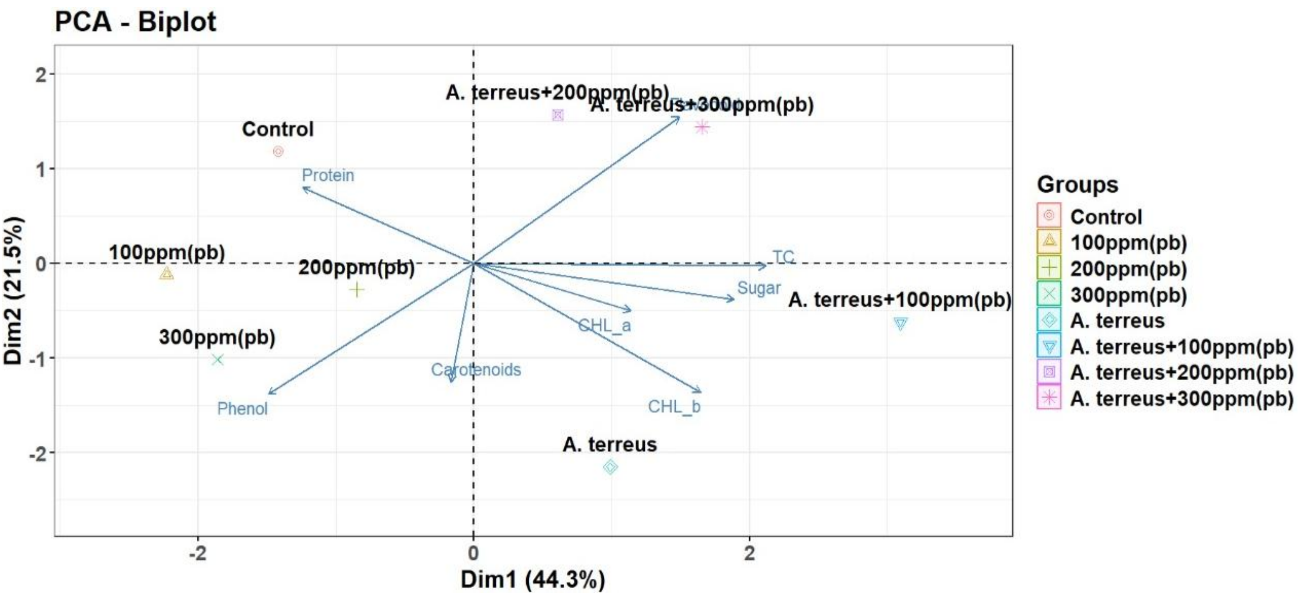

Fig. 7. The PCA biplot illustrates the impact of Pb concentrations and *A. terreus* inoculation on the biochemical parameters of *Triticum aestivum* L. seedlings. Dim1 (44.3%) and Dim2 (21.5%) account for the majority of variance, indicating the effects of treatment on chlorophyll, carotenoids, protein, sugar, flavonoids, and phenol.

Conclusions

This study demonstrates that increased Pb concentrations (100, 200, and 300 ppm) significantly inhibit the growth, physiological processes, and biochemical parameters of *T. aestivum*. This includes root length, shoot length, leaf number, leaf length, Chl. "a", Chl. "b", total chlorophyll (TC), carotenoids, phenol, protein, flavonoids, and sugar content. Pb toxicity disrupts essential physiological and biochemical processes, resulting in reduced plant biomass, heightened oxidative stress, and compromised photosynthetic efficiency. The inoculation of *A. terreus*, along with its combinations of Pb (*e.g*., *A. terreus* + 100ppm (Pb), *A. terreus* + 200ppm Pb), significantly mitigated Pb-induced damage. This was achieved through the enhancement of antioxidant activity, stabilization of chlorophyll and carotenoids, and elevation of phenolic, protein, flavonoid, and sugar levels. PCA analysis indicated that shoot length and root length are the main growth parameters affecting overall variability. The study highlighted the ability of microbial inoculation to modify PCA loadings and enhancing plant resilience under Pb stress. The results of this study demonstrate the effectiveness of microbial bioinoculants, specifically the *A. terreus*, as sustainable approaches for mitigating heavy metal stress and improving plant health in Pb-contaminated environments. Future research should focus on field-scale validation and molecular-level studies to improve the use of microbial inoculants in sustainable agriculture.

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