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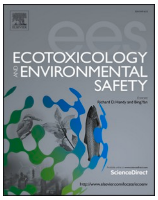

Synergistic effects of allantoin and *Achyranthes japonica*-biochar profoundly alleviate lead toxicity during barley growth

Ghulam Murtaza [a,b], Najmaldin Ezaldin Hassan [c], Muhammad Usman [d], Gang Deng [a,*], Zeeshan Ahmed [e,f,g], Javed Iqbal [h], Mohamed S. Elshikh [i], Humaira Rizwana [i], Basharat Ali [j], Rashid Iqbal [k,l,**], Maximilian Lackner [m,*]

[a] *School of Agriculture, Yunnan University, Kunming, Yunnan 650504, China*
[b] *School of Ecology and Environmental Sciences, Yunnan University, Biocontrol Engineering Research Center of Crop Diseases & Pests, Yunnan Province, Kunming 650500, China*
[c] *College of Engineering, Civil and Environment Department, University of Zakho, Kurdistan, Iraq*
[d] *School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China*
[e] *Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, Xinjiang 830011, China*
[f] *Xinjiang Institute of Ecology and Geography, Cele National Station of Observation and Research for Desert-Grassland Ecosystems, Chinese Academy of Sciences, Xinjiang 848300, China*
[g] *College of Life Science, Shenyang Normal University, Shenyang 110034, China*
[h] *Department of Botany, Bacha Khan University, Charsadda, Khyber Pakhtunkhwa 24420, Pakistan*
[i] *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*
[j] *Department of Agricultural Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahimyar Khan, Punjab 64200, Pakistan*
[k] *Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan*
[l] *Department of Life Sciences, Western Caspian University, Baku, Azerbaijan*
[m] *Department of Industrial Engineering, University of Applied Sciences Technikum Wien, Hoechstaedtplatz 6, Vienna 1200, Austria*

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ABSTRACT

Lead (Pb), a toxic metal, causes severe health hazards to both humans and plants due to environmental pollution. Biochar addition has been efficiently utilized to enhance growth of plants as well as yield in the presence of Pb-induced stress. The present research introduces a novel use of biochar obtained from the weed *Achyranthes japonica* to enhance the growth of plants in Pb-contaminated soil. An experiment was performed with 7 treatments: Control, Pb^{2+} ($10\ mg\ kg^{-1}$) only, biochar (4 %) only, allantoin ($4\ g\ kg^{-1}$) only, biochar combined with Pb^{2+}, allantoin combined with biochar, as well as a combination of allantoin and biochar with Pb^{2+}. Lead toxicity alone markedly diminished plant growth metrics, including root and shoot length, biomass (wet and dry), chlorophyll concentration, and grain production. The application of biochar, allantoin, or their joint administration markedly enhanced the length of shoots (by 50.3 %, 29 %, and 70 %), length of roots (by 69 %, 50 %, and 69 %), and fresh biomass of shoots (by 5 %, 29 %, and 5 %), respectively. This enhancement is ascribed to improved soil characteristics and more efficient absorption of nutrients. The application of biochar, allantoin and their combination improved the tolerance against Pb^{2+} by increasing the total chlorophyll level by 12 %, 16 %, and 17 %, respectively, vs. the control. Likewise, these amendments significantly ($p < 0.05$) improved the activity of antioxidant enzymes, including SOD, POD, and CAT by 49 %, 29 %, and 49 %, respectively. The resistance towards Pb^{2+} was enhanced by biochar, allantoin, and their combined application, with lower Pb^{2+} concentrations in shoots (59.9 %, 40.1 %, and 49.8 %), roots (48.2 %, 24.1 %, and 58.3 %), and grains (60.2 %, 29.7 %, and 40.1 %) compared to solely Pb-stress, respectively. In summary, converting the weed *Achyranthes japonica* into biochar and integrating it with allantoin provides an eco-friendly approach to control its proliferation while efficiently alleviating Pb-induced toxicity in plants.

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[*] Corresponding authors.

[**] Corresponding author at: Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan.

E-mail addresses: murtazabotanist@gmail.com (G. Murtaza), najmaldin.hassan@uoz.edu.krd (N.E. Hassan), usmanphytologist@gmail.com (M. Usman), denggang1986@ynu.edu.cn (G. Deng), zeeshanagronomist@yahoo.com (Z. Ahmed), javed89qau@gmail.com (J. Iqbal), melshikh@ksu.edu.sa (M.S. Elshikh), hrizwana@ksu.edu.sa (H. Rizwana), dr.basharat@kfueit.edu.pk (B. Ali), rashid.iqbal@iub.edu.pk (R. Iqbal), maximilian.lackner@technikum-wien.at (M. Lackner).

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1. Introduction

Lead (Pb^{2+}) toxicity is a worldwide environmental issue that endangers fauna and flora (Gupta et al., 2024) and negatively impacts plant growth and productivity (Bouida et al., 2022). Lead has greater persistence and is not degradable in soil compared to other contaminants (Raimi et al., 2022). Plants can efficiently absorb Pb^{2+} from soil through an active transport route (), which impairs photosynthesis and the activity of antioxidant enzymes, leading to oxidative stress, inhibited growth, and decreased food production (Li et al., 2024). Effective management of Pb^{2+} pollution in agricultural environments needs an in-depth knowledge of its distribution and significance (Fatemi et al., 2023; Mai et al., 2024). Different approaches have been established to mitigate soil Pb^{2+} contamination, encompassing phytoremediation, immobilization, electrokinetic remediation, and the application of soil supplements (Pangaribuan et al., 2023; Long et al., 2024). Soil amendments like biochar offer significant environmental and agronomic advantages, and their application is well-acknowledged as an effective method for the remediation of soil (Sindesi et al., 2023; Shoudho et al., 2024). Biochar is ecologically beneficial and significantly reduces the toxicity of Pb^{2+} in soil due to its exceptional adsorption properties (Amalina et al., 2023; Wei et al., 2024).

Biochar acts as a negatively charged, C-enriched material characterized by a highly porous and large specific surface area (SSA), which facilitates the reduction of toxic metal availability in soil (Su et al., 2024). Biochar is garnering considerable interest worldwide as a soil additive because of its ecological safety [when no polycyclic aromatic hydrocarbons, PHA, or other toxins are adsorbed to it] and beneficial impacts. Gusiatin and Rouhani (2023) proved that biochar can bind pollutants, capture carbon, and decrease greenhouse gases (GHGs), thus aiding in climate change management. Furthermore, biochar has demonstrated the ability to alleviate Pb^{2+} toxicity and drought stress in barley by decreasing the accumulation of Pb^{2+} and promoting the growth of plants (Mansoor et al., 2021; Liu et al., 2022; Mehrabi et al., 2024).

Achyranthes japonica, known as the Oriental chaff flower, is a perennial species under the Amaranthaceae family (Garrie, 2017). It can typically be found along riverbeds and the roadside. This plant, which is classified as an invasive species, induces ecological harm, detrimental effects on humans and plants, and adversely affects farmland (Jang et al., 2021). The production of biochar from *Achyranthes japonica* not only mitigates its proliferation but also provides a sustainable method for utilizing its waste material. *Achyranthes japonica*-biochar may serve as an effective strategy for mitigating abiotic stresses (He et al., 2017; Victoria et al., 2023) by improving soil nutrient status and antioxidant activity, hence increasing agricultural yield (Bang et al., 2012). The application of biochar with nitrogen-based fertilizer was found to significantly enhance soil productivity and mitigate toxicity induced by heavy metals (Alugoju and Tencomnao, 2023).

The combination of biochar and nitrogen fertilizers, particularly allantoin (CAS no. 97–59–6), can synergistically enhance soil health and plant growth (Kaur et al., 2021). Thus, biochar improves soil structure, microorganism activity and water retention, while allantoin provides easily accessible nitrogen to soil. In combination, they were found to enhance nutrient absorption, diminish the bioavailability of toxic metals, and promote overall plant vitality (Ravichandran, 2024). Amendments such as bio-fertilizers and biochar enhance the reduction of heavy metal bioavailability in soil, favouring the formation of less available compounds (Yadav and Ramakrishna, 2023). The use of biochar in barley (*Hordeum vulgare*) affects growth via modulating glucose, fatty acid, and amino acid metabolism (Ghouili et al., 2023). It enhances tolerance to environmental stresses by releasing substantial quantities of proline from root system (Sadak et al., 2024).

Barley ranks as the 4th largest grain crop worldwide, following wheat, rice, and maize. Barley is frequently utilized in stews, soups, breads, and health-related items (Badea and Wijekoon, 2021). Pb^{2+} is transported to barely grains by different transporters through roots-to-shoots translocation (Tao and Lu, 2022). Considering that barley is a very often consumed and traded cereal worldwide, it is essential to tackle the excessive accumulation of Pb^{2+} in barley grains. Thus, it is essential to find environmentally sustainable and economically viable techniques for mitigating the toxic effects of Pb^{2+} in polluted soils to guarantee the sustainable and effective production of edible crops.

However, no research has explored the synergistic impacts of allantoin and biochar in mitigating Pb^{2+} toxicity in barley. The efficacy of *Achyranthes japonica*-biochar remains completely unexamined, as the majority of reported research concentrates on biochar from traditional materials including animal manure, crop wastes, and wood-based materials. We hypothesized that converting *Achyranthes japonica* into biochar and combining it with allantoin can substantially alleviate its detrimental impacts on the environment by putting this invasive species to use, for mitigating lead contamination in agricultural soil. This work presents a beneficial method that integrates *Achyranthes japonica* biochar with allantoin to effectively mitigate the toxic effects of Pb^{2+} in barely cultivated on Pb-polluted soil. The aims of this study are: a) to determine the efficacy of *Achyranthes japonica*-derived biochar in lowering the bioavailability of Pb in polluted soil, b) to examine the synergistic impact of *Achyranthes japonica*-biochar and allantoin on the barely growth-related parameters and yield in Pb-polluted soil, and 3) to evaluate the value of *Achyranthes japonica*-biochar and allantoin in increasing the biochemical and physiological reactions of barley under Pb-induced stress. This approach has the combined advantage of managing an agricultural weed while implementing a sustainable and eco-friendly strategy for minimizing Pb toxicity in barley, hence enhancing environmental safety, food security, and quality.

2. Methodology

2.1. Soil collection and analysis

This research was performed at the Islamia University of Bahawalpur, Pakistan. The soil was collected from the agriculture field of Islamia University of Bahawalpur, thereafter air-dried at ambient temperature and sieved through a 3 mm mesh. Soils from the pre-planting stage and post-harvest stage were evaluated following the procedure established by Okonkwo et al. (2009). The pH of the soil was assessed through its water suspension with a pH meter (Lutron PH-223) at a water:soil ratio of 3:1 by weight. To ascertain the quantity of soil organic matter (SOM), 2 g of soil was suspended in a solution consisting of concentrated sulfuric acid (20 mL), potassium dichromate (15 mL), and deionized water (350 mL), with the addition of 20 drops of phenolphthalein (Lino et al., 2023). The Olsen method and the salicylic acid technique were deployed to assess the soil concentrations of phosphorus, nitrogen, and potassium (Merwad, 2016). An atomic absorption spectrometer (AA-7800, Shimadzu, Japan) was employed to quantify soil Pb. The ash content and electrical conductivity of biochar were assessed by the stipulations of the European Biochar Certificate (EBC, 2012). Samples were heated in a furnace at 550 °C for 60 minutes. A crucible was employed to ascertain the composition of biochar ash. The crucible with the sample was weighed and positioned in the furnace. After heating, the residual bulk was characterized as an ashy mass (Moreno et al., 2013). The ash content was determined based on the results of ash weights using the following equation (ASTM, 2005)

$$Ash\ content = \frac{W2 - Wc}{W1 - Wc} \times 100, [\%]$$

Wc denotes the mass of an empty crucible in grams; W_1 represents the mass of the crucible with the sample prior to heating in grams; W_2 indicates the mass of the crucible with the ashes post-heating in grams.

For pH measurement, a minimum of 5 mL of air-dried material was placed in a glass container. 25 mL of 0.01 M $CaCl_2$ solution, five times the initial amount, was added to the same bottle. The suspension was agitated for one hour. The pH was directly measured from the suspension using a Mettler Toledo Seven Multi pH meter (EBC, 2012).

Ten grams of the material were added to 100 mL of deionized water and agitated for one hour. The solution was subsequently filtered via filter paper (VWR 413, particle retention 5/13 µm). The electrical conductivity of the filtration water was measured with an InoLab WTW model 740 electrical conductivity meter.

To quantify the total carbon (TC) content of biochar, the coarse particles were crushed using a mortar, and the sample was allowed to air-dry at ambient temperature for 24 hours to eliminate moisture. Then, 20–50 mg of the material was placed into a ceramic sample boat. The sample boat was placed in the TC sample loading zone and subsequently put straight into the TC furnace (Fidel et al., 2017). Table 1 presents the initial physicochemical properties of the biochar and soil.

2.2. *Achyranthes japonica collection and production of biochar*

Achyranthes japonica was gathered from Ahmedpur East in Bahawalpur, Pakistan (29.3981°N, 71.6908°E). Following collection, leaves and stems were separated and the leaves were subsequently washed with tap water twice and processed further. The plant tissue was subsequently pulverized into fine particles and desiccated in a drier at 75 °C for 15 days. The dried feedstock of *Achyranthes japonica* was subjected to pyrolysis in a furnace at 550 °C for 1 hour (İlay et al., 2024). The physicochemical properties of the obtained biochar are presented in Table 1.

2.3. *Experimental setup*

Barley seeds (Neelam Enterprises, Uttar Pradesh, India) were obtained from the agronomy department of IUB. They were subjected to surface sterilization (for preventing contamination) with 10 % hypochloric acid for 2 minutes, followed by 3–5 rinses with tap water. After the washing process, barley seeds were set up in a petri dish, maintained in darkness for three days at ambient temperature to facilitate germination, and subsequently cultivated. Before sowing of seeds and treatments, conventional fertilizer was administered, consisting of phosphorus pentoxide, urea, and potassium oxide at concentrations of 0.24, 0.20, and 0.10 g/kg soil, respectively, to aid in the optimal growth of plants (Shahzaman et al., 2017). Subsequently, each seedling was moved into a separate pot. This research utilized a completely randomized design with three replicates, each consisting of 10 plants, compare Gul et al. (2024). 7 various treatments were employed to evaluate the impacts of allantoin and *Achyranthes japonica*-biochar on barley seedling growth and capacity of lead accumulation. 21 pots were prepared, each containing 3.5 kg of soil. Treatments included (1) control sample (no supplements, CL), (2) Pb^{2+} alone at 10 mg kg^{-1} (as $Pb(NO_3)_2$), (3) 250 g (4 %) biochar, (4) allantoin (AT) at a concentration of 4 g kg^{-1}, (5) 10 mg kg^{-1} Pb^{2+} with 4 % biochar (biochar + Pb^{2+}), (6) 10 mg kg^{-1} Pb^{2+} with 4 g kg^{-1} Allantoin (Pb^{2+} + AT), (7) co-application of allantoin and biochar with Pb^{2+} (Pb^{2+} + AT + BC). The pots were watered every other day using tap water. The greenhouse maintained a temperature of 25–28 °C and relative humidity of 55–75 %, reinforced by sufficient sunlight and ventilation for optimal plant growth. Seventy days post-sowing, a sample of barley was harvested for the examination of biological and physiological characteristics; the remainder was cultivated till maturation, harvested 135 days after planting, and then divided into shoots, roots, and grains for further analysis (Gul et al., 2024).

Table 1
Physicochemical characteristics of biochar and soil.

Physiochemical attributes of biochar		Physiochemical attributes of soil	
pH	9.2	pH	7.7
Ash content level (%)	19.1	EC level (dS*m^{-1})	3.6
EC level (dS*m^{-1})	4.8	TOM (%)	0.60
Total nitrogen (mg*kg^{-1})	1.9	Available phosphorus (mg*kg^{-1})	30
Total Phosphorus (mg*kg^{-1})	0.34	Available Potassium (mg*kg^{-1})	44.2
Total carbon (%)	40	Available Nitrogen (g*kg^{-1})	0.70
Total Potassium (mg*kg^{-1})	3.7	Saturation (%)	41
		Texture	Silt, loam
		Total Pb level (mg*kg^{-1})	24

2.4. *Characteristics of plant growth and phenotypic assessment*

Plant growth characteristics (height, number of tiller nodes, number of seeds, and dry and fresh biomass of both shoots and roots) were measured. The mass was acquired with an analytical balance. Root and shoot lengths as well as grains were quantified, and fresh biomass was measured, before samples were transferred to an oven at 75 °C to achieve desiccated biomass for dry mass determination. The desiccation process was also used to control microbial growth.

2.5. *Chlorophyll levels and flavonoid concentration*

The chlorophyll content examination was conducted using leaves from 70-day-old plants. Matured upper leaves from all treatments were collected by the methodology established by Jia et al. (2019). Collected leaves were directly inserted into experimental tubes filled with 80 % acetone and kept in darkness for 2 days (Gul et al., 2024). Following the removal of the leaves, the chlorophyll was extracted from the tubes, and chlorophyll a, chlorophyll *b*, and total chlorophyll concentrations were quantified with a spectrophotometer at wavelengths of 645 nm and 663 nm. The level of chlorophyll was determined using the following method:

$$Chla = 12.7(A663) - 2.7(A645) \tag{1}$$

$$Chlb = 22.9(A645) - 4.7(A663) \tag{2}$$

$$Total\ Chl = Chla + Chlb \tag{3}$$

A(663) stands for the absorbance at 663 nm, and A(645) for that at 645 nm.

The flavonoid level in fresh leaves was quantified using colorimetry. Leaves extract was diluted in deionized water and combined with a reagent containing 10 % sodium hydroxide, sodium nitrite, and aluminium chloride. Absorbance of the resultant solution was quantified at 510 nm utilizing a spectrophotometer (Shimadzu UV-1800) (Felici et al., 2024).

2.6. *Electrolyte conductivity and oxidative stress exploration*

After 70 days of sowing, to quantify oxidative stress levels, 1.5 g of a freshly collected leaf sample was extracted with 90 % ethanol. The resultant extract was combined with a solution containing 90 % methanol, sulfuric acid (25 mM), xylenol (100 µM), and ammonium iron(II) sulfate (250 µM). The mixture was incubated for 25 minutes, mixed, homogenized with a vortexer, and centrifuged at 12000 rpm for 3 minutes to separate the components by density (Gul et al., 2024). The concentration of hydrogen peroxide was determined by measuring the absorbance at 560 nm. Malondialdehyde (MDA) concentrations were quantified by homogenizing 0.8 g of young leaf tissue in a solution of trichloroacetic acid. Supernatant was acquired by centrifuging the materials at 12000 rpm. The MDA level was quantified with a spectrophotometer (Shimadzu UV-1800) at wavelengths of 532 nm and 600 nm (Chmur and Bajguz, 2023). For the electrolyte leakage (EL) assessment, 0.5 g of plant tissue was put in a test tube containing 20 mL of deionized water and incubated in a water-bath at 35°C for 4 hours. The initial electrical conductivity (EC1) was assessed utilizing a conductivity meter. Samples were subjected to high temperatures for 25 minutes to dissolve plant tissues, thereafter cooled at 25°C, after which conductivity was re-measured, called EC2. The EL was computed as outlined by Dheeravathu et al. (2018) and Gul et al., (2024).

$$EL = EC1/EC2 \times 100 \tag{4}$$

2.7. Photosynthetic and antioxidant activity

Fresh leaf (0.5 g) was macerated with 5 mL of phosphate-buffered saline buffer in an ice bath, containing EDTA (0.3 mM), polyvinylpyrrolidone (2 %), and ascorbate (2 mM). The supernatant was utilized for quantifying the activity of enzymes. Therefore, the sample was centrifuged at 12000 rpm for 15 minutes at 4 °C (Elavarthi and Martin, 2010). 0.1 mL of enzyme extract was combined with 1 mL of a solution containing 0.2 % guaiacol and 0.3 % hydrogen peroxide. The activity of peroxidase (POD) was measured with a spectrophotometer at a wavelength of 470 nm (Lin and Kao, 2001). We mixed enzyme extract (0.1 mL), water (3 mL), and 0.3 % hydrogen peroxide (1 mL), thereafter measuring the activity of CAT at 240 nm. The activity of SOD was assessed by combining hydrogen peroxide (400 µL), Triton (100 µL), NBT (50 µL), as well as riboflavin in a test tube, followed by measuring the absorbance at 560 nm using a spectrophotometer (Shimadzu UV-1800). The enzyme's activity was quantified in units per gram of fresh weight. The activity of ascorbate peroxidase was assessed following the methodology outlined by Lopez et al. (1996). Portable equipment was utilized to measure intercellular CO_2 concentration, net photosynthetic rate, and stomatal conductance (Brodribb, 1996).

2.8. Evaluation of Pb^{2+} concentrations in barley tissue

Desiccated samples of roots and shoots, as well as grains, were pulverized and transferred into 5 mL digestion flasks comprising concentrated nitric acid (Gul et al., 2024), followed by heating at 175 °C for 2 hours for digestion. To enhance statistical validity and minimize experimental error, Pb^{2+} levels were assessed using triple analyses. The lead content was quantified with Atomic Absorption Spectroscopy (AAS 700, Perkin Elmer), as outlined by Rezvani et al. (2015). The translocation factors (TFs) for roots, shoots and grains for Pb^{2+} were determined as follows:

$$Shoot\ TF = Shoot\ Pb\ content/Root\ Pb\ content \tag{5}$$

$$Grain\ TF = Grain\ Pb\ content/Root\ Pb\ content \tag{6}$$

$$Root\ TF = Root\ Pb\ content/Soil\ Pb\ content \tag{7}$$

2.9. Data analysis

The data was evaluated utilizing the software SPSS version 29 (IBM, USA). ANOVA was conducted to assess the impact of Pb^{2+} on growth and physiological characteristics of barley seedlings exposed to various treatments. Multiple comparisons for each treatment were performed employing Tukey's test to assess significance ($p < 0.05$). The findings are shown as means ± standard deviation of 3 replicates. Diverse letters denote groups that exhibit significant differences at $p < 0.05$.

3. Results and discussion

3.1. Combined impact of allantoin and Achyranthes japonica-biochar in promoting plant growth

The synergistic administration of allantoin and biochar has shown great efficacy in alleviating the detrimental impacts of lead stress on barley growth, while simultaneously restricting Pb^{2+} uptake in barley (Fig. 1). The phenotypic study indicates that the combination treatment of allantoin and biochar best promotes barely growth under Pb^{2+} stress (Fig. 1-a). Growth of the plants was significantly greater in the combination treatment compared to other treatments, resulting in the maximum shoot length. With Pb-induced stress, the application of biochar alone and the combined application of biochar and allantoin enhanced the length of the shoot by 50.3 % and 70 %, respectively (Fig. 1-b). The length of the roots was significantly greater in the allantoin-containing and biochar-amended treatments vs. the control. The length of the roots was markedly reduced under Pb-induced stress compared to the control sample. Under Pb-caused stress, allantoin and biochar treatment markedly enhanced the length of the roots (Fig. 1-c). Compared to a control sample, the length of spikes was markedly increased in the allantoin and biochar treatment, but it was greatly decreased by Pb-induced stress (Fig. 1-d). Compared to a control sample, grain numbers per spike increased in the allantoin and biochar amended groups by 30 % and 19.8 %, respectively, but it drastically decreased under Pb-induced stress alone by 49 % (Fig. 1-e). This indicates that allantoin and biochar markedly promote growth in Pb-polluted areas, mitigating the toxicity of Pb^{2+}. Our results align with previous research indicating that the application of biofertilizer and biochar considerably improved the root and shoot length of cotton while mitigating Pb-induced stress (Malik et al., 2022; Baloch et al., 2024). Jia et al. (2024) revealed that biochar addition enhanced barley plant biomass, height, and yields of grains, indicating that biochar promotes the growth of plants, plant development, as well as yield in Pb-polluted regions. Our findings corroborate this result, exhibiting significantly elevated dry and fresh weights in the allantoin, biochar, and combined applications compared to the control group and Pb-stressed plants (refer to Table 2). Under Pb^{2+} stress, allantoin, biochar, and combination treatments markedly enhanced spike, root, and shoot biomass (refer to Table 2). In a maize (*Zea mays)* pot trial utilizing Pb-polluted soil, the fresh shoot and root biomass values were markedly elevated after treatment with biochar and allantoin (El-Tohory et al., 2024), corroborating our results. Using biochar positively affects the root-to-shoot length ratio and may be an advantageous approach to improve resistance to Pb^{2+} stress (Naveed et al., 2020). Elevated levels of Pb^{2+} can significantly impact plants' morphological and physiological traits, including plant biomass and height (Lebrun et al., 2020). Awad et al. (2022) found that Pb-induced stress impairs enzymatic activities and ionic balance, decreasing the levels of chlorophyll in leaves. This decrease is typically linked to reduced plant height and reduced biomass. The present investigation indicated that allantoin and biochar promoted the growth of plants and alleviated Pb^{2+} toxicity in barley (Fig. 1). The enhancement of plant growth attributes and yield characteristics of barley under Pb-stress after allantoin and biochar treatments may be attributed to enhanced soil fertility and availability of nutrients. Another explanation for this enhancement may be the capacity of biochar to stimulate organic mineralization, hence improving plant growth and productivity (Ma et al., 2023). Rizwan et al. (2018) indicated that biochar functions as a buffer, enhancing vital nutrient uptake from the soil, and thus enhancing the performance of plants, which is consistent with our study's findings. The utilization of allantoin and biochar can promote plant health and growth via multiple processes, including soil improvement, microbial activity, water retention, and availability of nutrients (Kaur et al., 2023; Mirheidari et al., 2020). The roots function as protection, and their vitality is greatly affected by soil fertility and structure. Improved soil fertility and structure promote root growth, enhancing retention of nutrients and water. Furthermore, biochar enhances root exudate output, hence facilitating beneficial microbial interactions in soil and improving the accessibility of nutrients (Pathak et al., 2024). Root exudates associate with Pb^{2+} to form complexes, hence enhancing the availability and solubility of Pb^{2+} for absorption (Agarwal et al., 2024). This mechanism may arise via root expansion, leading to higher Pb^{2+} absorption from deeper soil layers, which is then translocated to the top tissues. Consequently, the uptake of Pb^{2+} by roots and its translocation to shoots may constitute a tolerance mechanism

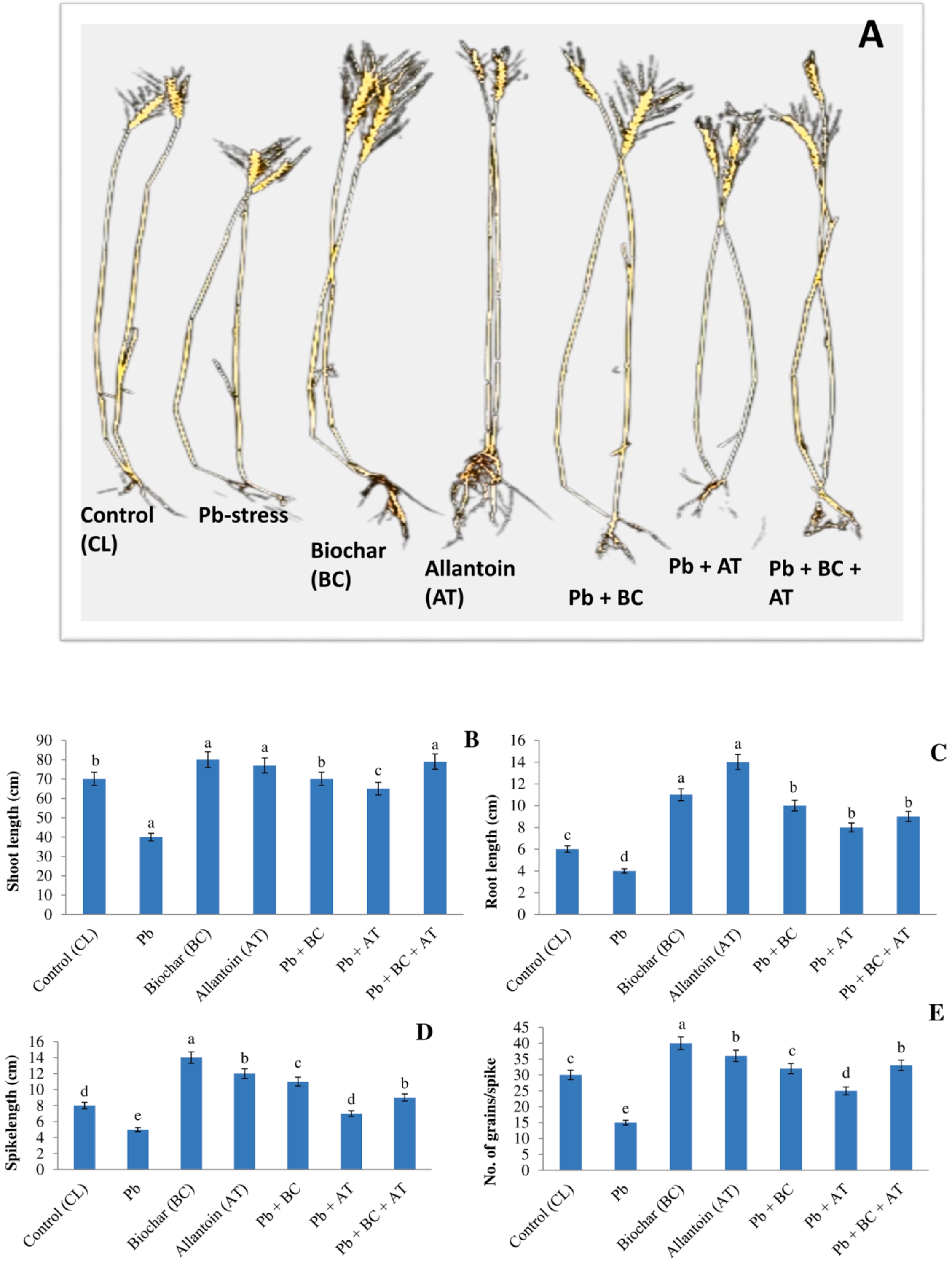

Fig. 1. Effect of biochar, allantoin and their combined use to barely growth-related parameters; (a) phenotypic attributes; (b) length of shoots; (c) length of roots; (d) length of spikes; (e) number of grains per spike; Various letters denote groups that exhibit significant differences at $p < 0.05$. Vertical bars denote the standard deviation (SD) based on 3 replicates. ANOVA and Tukey HSD tests were conducted ($p < 0.05$).

that modulates the root phenotype to alleviate Pb^{2+} toxicity ((Gupta et al., 2024). Hou et al. (2022) reported that creeping-oxeye (*Sphagneticola trilobata,* trailing daisy, wedelia) exhibited deeper root systems, hence improving Pb tolerance through the absorption of Pb^{2+}. Biochar improves the soil structure via increasing aeration and porosity, hence facilitating enhanced water retention and root development. The enhanced soil structure promotes superior root expansion and penetration, permitting plants to acquire increased nutrients and water. Sufficient water and nutrients are essential for crop homeostasis during Pb-induced stress. The combined use of allantoin and biochar may enhance soil conditions and promote root elongation, hence increasing vital nutrient uptake (Qi et al., 2024).

Table 2

dry and fresh biomass of barley under various treatments.

Treatments (g/FW)	Control (CL)	Pb	Biochar (BC)	Allantoin (AT)	Pb + BC	Pb + AT	Pb + BC +AT
Shoot FW	6.40^{b}	4.49^{d}	7.40^{a}	7.30^{a}	6.6^{b}	5.89^{c}	6.99^{a}
Shoot DW	0.59^{b}	0.39^{c}	0.69^{a}	0.70^{a}	0.7^{b}	0.60^{b}	0.8^{a}
Root FW	0.46^{c}	0.30^{d}	0.80^{ab}	0.90^{a}	0.80^{ab}	0.69^{b}	0.79^{a}
Root DW	0.03^{c}	0.03^{d}	0.08^{ab}	0.09^{a}	0.070^{ab}	0.07^{b}	0.080^{a}
Spike FW	0.60^{b}	0.44^{c}	0.89^{a}	0.90^{a}	0.66^{b}	0.70^{b}	0.79^{a}
Spike DW	0.06^{c}	0.046^{d}	0.079^{a}	0.08^{b}	0.057^{b}	0.07^{b}	0.09^{a}

Note: DW= dry weight, FW=fresh weight

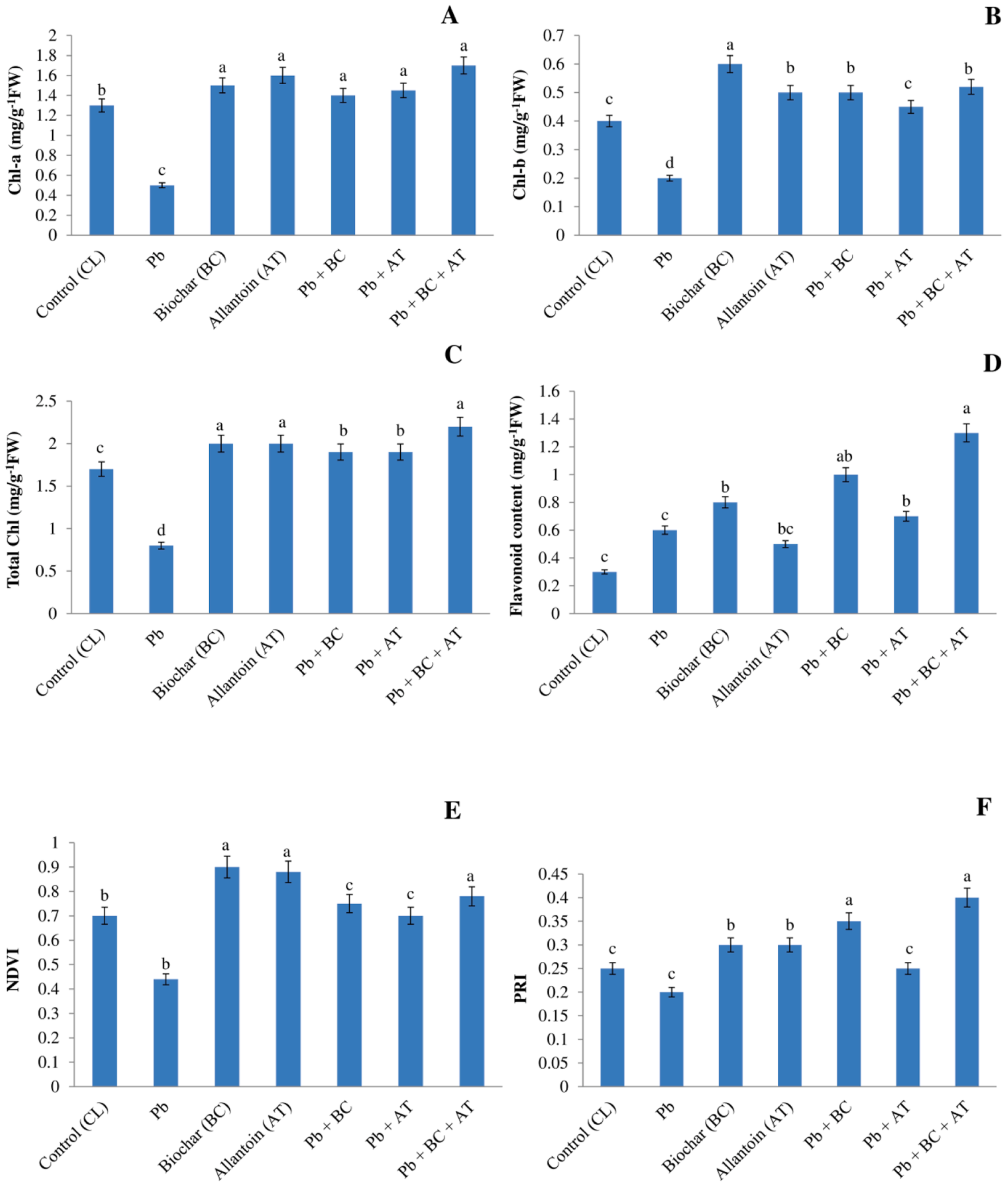

Fig. 2. Application of biochar, allantoin and their combination to examine barely for its content of (a) chlorophyll a, (b) chlorophyll *b*, (c) Total chlorophyll, (d) flavonoids, as well as (e) NDVI and (f) PRI. Various letters denote groups that exhibit significant differences at $p < 0.05$. Vertical bars denote the standard deviation (SD) based on 3 replicates. Tukey HSD and ANOVA tests were conducted ($p < 0.05$).

3.2. Photosynthetic and chlorophyll levels

The application of Pb-induced stress alone markedly decreased the level of chlorophyll compared to other applications. Levels of chlorophyll a, and chlorophyll *b* and total chlorophyll decreased by 39.9, 48.1, and 40.7 %, respectively, under Pb^{2+} stress compared to the control group. The concentration of chlorophyll a, and chlorophyll *b*, and total chlorophyll was greatly increased with biochar treatment by 10, 36, and 17 %, respectively. By contrast, following the allantoin amendment, the increases were 12, 29, and 16 % vs. the control (Fig. 2a-c). Under Pb-induced stress, the levels of chlorophyll a, and chlorophyll *b*, and total chlorophylls were higher with biochar, allantoin and their combined application compared to the control and only Pb-induced stress (Fig. 2a-c). The concentrations of flavonoid metabolites (secondary metabolites) increased (by 58.7, 35, 60, 58, and 96 %, respectively), with biochar, allantoin and their combined application vs. the control (Fig. 2-d). In comparison to the control group, the Photochemical Reflectance Index (PRI) and Normalized Difference Vegetation Index (NDVI) exhibited significant increases after the application of biochar, allantoin and their combination, while they were decreased under application of Pb-induced stress alone (Fig. 2e-f).

Chlorophyll serves as a crucial indication of crop toxicity in response to metal-induced stress (Reddy et al., 2024). Photosynthetic pigment reduction in crops may arise from modifications in the structure of chloroplasts induced by Pb-induced stress (Zhou et al., 2017). Photosynthetic pigments such as chlorophyll a and chlorophyll *b* and total carotenoids, are crucial for absorbing light energy at particular wavelengths required for water molecules photolysis PS II (Figlioli et al., 2019). Pb-induced toxicity can hinder the light-dark responses of photosynthetic mechanisms, thus retarding light reactions (Bessonova et al., 2020). Stomatal guard cells enable gas exchange, resulting in the disruption of the sodium and potassium ion balance/stability and loss of water (Das et al., 2022). The uptake of lead impairs the membrane transport mechanism, lowering stomatal regulation (Kumar and Prasad, 2018). Our results suggest that the porous structure of biochar may facilitate the growth of plants and mitigate oxidative stress during Pb-induced stress, hence enhancing flavonoid and chlorophyll levels (Mazaheri-Tirani et al., 2024). Biochar functions as a soil supplement that improves nutrient availability and retention. It possesses a higher CEC (cation exchange capacity), enabling it to maintain levels of vital nutrients such as N, P, and K, hence enhancing their availability for plants (Turan, 2020). Allantoin is a mediator compound in the purine catabolic process that facilitates N mobilization in crops (Dawood et al., 2020). Allantoin is a significant N source for chlorophyll (Casartelli et al., 2019). Upon application to soil, allantoin undergoes the process of hydrolysis resulting in the formation of nitrate and ammonium which are readily assimilated by crops (Raihan et al., 2023). The enhanced nitrogen supply immediately facilitates the chlorophyll molecule synthesis, hence improving plant vitality and the photosynthesis process (Redillas et al., 2019). Allantoin enhances the proliferation of beneficial N-fixing bacteria, hence increasing the availability of nutrients and facilitating adequate chlorophyll content (Lescano et al., 2016). Dresler et al. (2022) observed that an increased administration rate of trace metals diminished chlorophyll content, whereas the addition of biochar enhanced the essential nutrient availability, hence boosting carotenoid and chlorophyll levels. In line with our trial series, Kamal et al. (2024) showed that biochar enhances nutrient availability, resulting in elevated levels of photosynthetic pigment. Shehzad et al. (2023) indicated that Pb-caused stress significantly diminishes chlorophyll levels and photosynthetic pigment. Biochar treatment enhances chlorophyll pigment in barely (Nasiri et al., 2024). Bagues et al. (2024) proposed that animal manure biochar increases chlorophyll levels and improves the soil nutrient profile, greatly improving levels of nitrogen, potassium, and phosphorus. Parthenium-biochar increases chlorophyll levels in rice growth, mitigating the toxic effects of Pb^{2+} (Wang et al., 2019). Allantoin stimulates plants to produce secondary metabolites, including flavonoids, to alleviate Pb^{2+} toxicity and remediate heavy metals contamination from soil (Khanna et al., 2019). The amount of flavonoid compounds correlates directly with the nitrogen content and fertility of the soil; hence, an increase in the nitrogen level and soil health may improve Pb^{2+} resistance (Han et al., 2024).

3.3. Response of barley leaves to oxidative and antioxidants stress

Pb-induced stress greatly reduces the plant's defence capacity by impairing the activity of antioxidant enzymes. The activity of the antioxidant enzymes SOD, POD, and CAT was greatly reduced under Pb-caused stress compared to the control group, whereas it increased after biochar, allantoin, or their combined application (Fig. 3). Under Pb-induced stress, the activity of CAT was highest in biochar and the combined application, exhibiting a 33.1–49.7 % increment vs. the control (Fig. 3a). In comparison to the control, the activities of SOD, POD, and CAT increased in the biochar and combined addition (Fig. 3b-c). The combination of allantoin and biochar significantly ($p < 0.05$) enhanced the antioxidant enzymatic activity, including SOD, POD, and CAT by 49, 29, and 49 %, respectively, thus alleviating oxidative stress as a protective response. Antioxidant activity is essential for plant defence against metal-induced stresses (Zhang et al., 2024). When crops take up excessive toxic metal levels from polluted soil, they initiate intrinsic defensive processes, such as SOD, POD, and CAT, to counteract ROS (reactive oxygen species) within their cell membranes. The synchronized function of SOD, POD, and CAT reduces lipid peroxidation, therefore maintaining the cell membrane integrity. The surface structure of biochar is favourable for Pb^{2+} interaction, potentially mitigating Pb^{2+} toxicity in crops by functioning as a protective barrier against ROS (Maqbool et al., 2024). The antioxidant enzymatic activity by SOD, POD, and CAT is crucial in mitigating oxidative stress under Pb^{2+} pollution (Rahbari et al., 2021). In rice under Pb-induced stress, the addition of organic amendments significantly enhanced the levels of POD and CAT efficiently mitigating Pb^{2+} toxicity (Hou et al., 2022). The use of biochar with nitrogen fertilizer enhanced the expression rates of genes associated with the antioxidant defence pathway. The overexpression of this gene results in the enhanced generation of enzymatic molecules, thus enhancing the synthesis of enzymes and POD, SOD, and CAT activity. The upregulation is probably attributable to the biochar-stimulated enhancement of soil characteristics, such as microbial activity, availability of nutrients, and pH which in combination foster improved stress tolerance and plant health (Raza et al., 2024). Moreover, allantoin delivers a consistent source of nitrogen, which is crucial for the synthesis of proteins, encompassing the antioxidant enzyme synthesis (Switzer et al., 2020). SOD serves as the primary antioxidant defence system against ROS, converting O_2 into hydrogen peroxide while CAT subsequently transforms hydrogen peroxide into water, thereby alleviating the detrimental effects of hazardous peroxides in crops (Rajput et al., 2021; Redillas et al., 2019). The activity of POD in crops is crucial for the respiratory metabolic process and the transformation of phenolic compounds into quinones. This procedure alleviates the detrimental consequences of oxidative stress generated by heavy metals (Huchzermeyer et al., 2022). The administration of biochar, allantoin, or their combined treatment was found to decrease the accumulation of ROS by enhancing the plant's nutrient status, especially via increased availability of vital micronutrients such as ions of Cu, Mn, and Zn, which serve as crucial catalysts for SOD (Pandey, 2018). Compared to the control group, electrolyte damage in the leaves was greatly increased under Pb-induced stress, but it was reduced by biochar and allantoin application (Fig. 3d). Likewise, MDA and hydrogen peroxide levels were greatly increased by 30.2–95.3 % during Pb-induced stress compared to the control group (Fig. 4e-f). Nonetheless, the levels of MDA and hydrogen peroxide did not exhibit significant differences between the biochar, allantoin, and their combined application compared to the control group (Fig. 4e-f). The increase in MDA and hydrogen peroxide concentrations in barely under Pb-induced stress is attributable to oxidative stress. This happens when

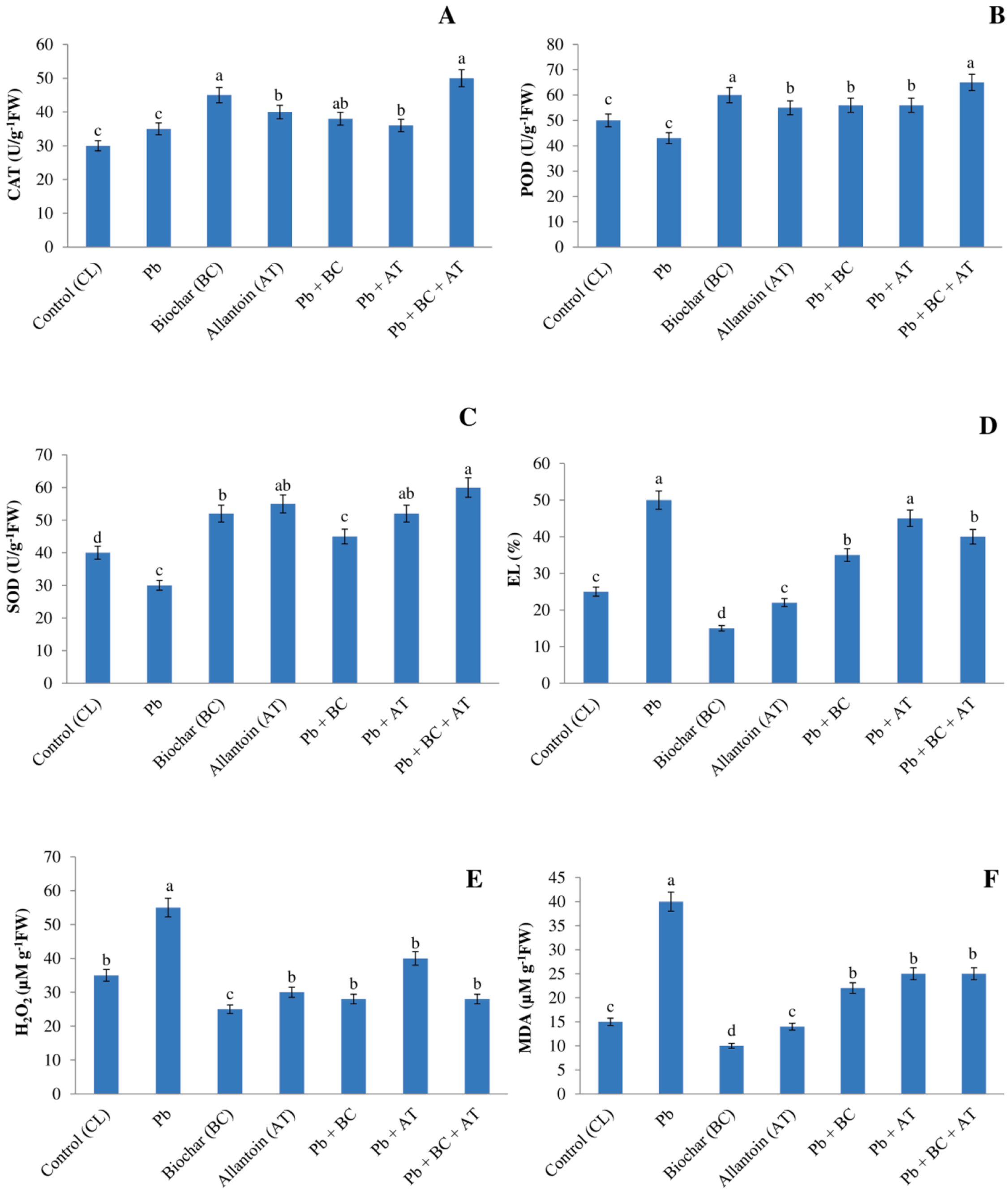

Fig. 3. Application of biochar; allantoin and their combined effects to examine barely for the levels of (a) CAT, (b) POD, (c) SOD, (d) EL, (e) H_2O_2, and (f) MDA. Various letters denote groups that exhibit significant differences at $p < 0.05$. Vertical bars denote the standard deviation (SD) based on 3 replicates. Tukey HSD and ANOVA tests were conducted ($p < 0.05$).

there is an imbalance between the generation of reactive oxygen species and antioxidant mechanisms of plants. The plant membrane integrity may significantly contribute to the decrease of reactive oxygen species (ROS) concentrations and the enhancement of antioxidant enzyme functions (Pandey, 2020). Pb-induced stress alone can elevate levels of MDA in barley leaves, resulting in lipid peroxidation. Elevated concentrations of heavy metals such as Pb^{2+} facilitate the generation of HO^- from O_2 via the Fenton reaction. The elevated MDA levels indicate that metal ions promote free radical generation (Rekhate and Srivastava, 2021). Nonetheless, an application of allantoin or biochar alone is recognized to reduce levels of MDA and mitigate the ROS toxicity in soils polluted with Pb^{2+}. Allantoin or biochar mitigated oxidative stress via improving antioxidant defence mechanisms and decreasing reactive oxygen species generation (Bessonova et al., 2020). Biochar treatment enhances the activity of reactive oxygen species-scavenging antioxidant enzymes, particularly superoxide dismutase and catalase (Krzyszczak et al., 2022). Allantoin may diminish ROS formation and antioxidant activity enhancement by affecting plant physiology and metabolism (Nourimand and Todd, 2016). Our results indicate that allantoin or biochar alleviates oxidative stress through stimulating antioxidant enzymatic defence mechanisms including SOD, POD, and CAT. Allantoin or biochar addition may improve tolerance to Pb-stress in barely by decreasing MDA and EL levels, hence maintaining the permeability of the cell membrane. Biochar addition improves antioxidant defence systems by decreasing levels of ROS, hence mitigating the toxic effects of Pb^{2+} (Amubieya et al., 2024).

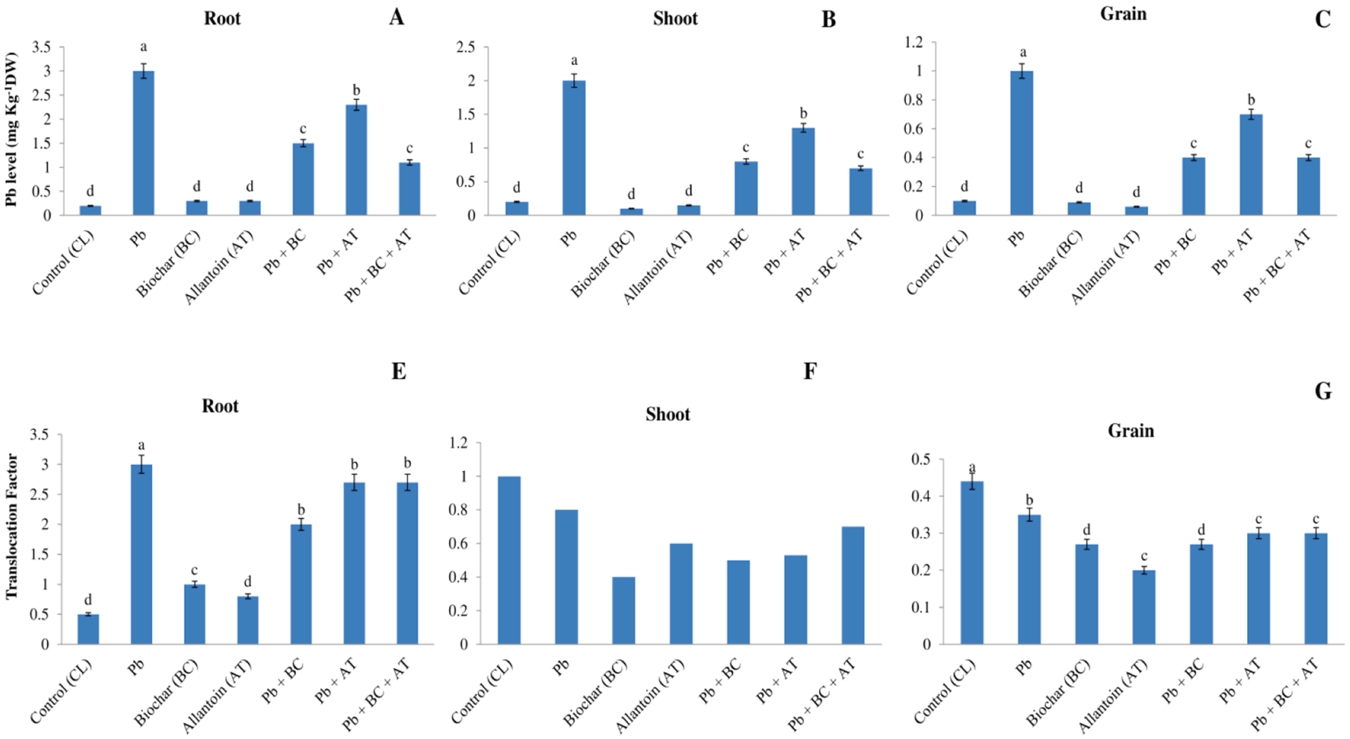

Fig. 4. Application of biochar allantoin and their combination to barely: (a-c) Pb contents in roots, shoots and grains, (d-f) translocation factors for roots, shoots and grains. Various letters denote groups that exhibit significant differences at $p < 0.05$. Vertical bars denote the standard deviation (SD) based on 3 replicates. Tukey HSD and ANOVA tests were conducted ($p < 0.05$).

3.4. Photosynthetic reaction of barley to Pb-induced stress

Gas exchange-related parameters are important for enhancing WUE (water use efficiency) in reaction to environmental stressors. Pb-induced stress adversely impacts photosynthetic characteristics, including net rate of photosynthesis, stomatal conductance, and levels of intercellular CO_2 (Alhammad et al., 2023). Pb-induced stress diminishes stomatal conductance, photosynthetic rate, and levels of intercellular CO_2 by impairing the uptake of nutrients and affecting cellular and metabolic activities in plants (Mfarrej et al., 2024). Stomatal conductance is a critical factor influencing crop output. The velocity of stomatal response to fluctuating environmental factors significantly influences water use and photosynthesis (Asargew et al., 2024). Stomatal conductance is a critical factor influencing photosynthesis, and substantial evidence indicates that altering stomatal conductance can enhance crop performance and production (Liao et al., 2024). Allantoin and biochar improved crop gas exchange parameters under Pb-induced stress. The values of stomatal conductance, photosynthetic rate, and levels of intercellular CO_2 were markedly reduced in Pb-stressed samples compared to the control but were dramatically increased in allantoin and biochar applications (Table 3). Under Pb^{2+} stress, the use of allantoin and biochar and their combination enhanced photosynthetic characteristics (chlorophyll content, net photosynthetic rate, intercellular CO_2 concentration, transpiration rate, and stomatal conductance), mitigating the toxicity of Pb^{2+} (Table 3). Metal-induced stress reduces the plant's hydraulic conductivity, hence impeding stomatal conductivity and photosynthetic pigments (Zafar et al., 2024). This may result from the Pb^{2+} toxicity in barley leaves, which was found to adversely affect gas exchange characteristics and photosynthetic systems (Rahman et al., 2024). Our research indicates that biochar and allantoin enhance the photosynthetic activity of barley under Pb-induced stress. The porous structure of biochar facilitates the retention of more nutrients and water, such as calcium and potassium ions, within the guard cells which are essential for stomatal activity (Xu et al., 2023). By preserving the appropriate ionic equilibrium, biochar aids in optimizing cell functionality, hence improving stomatal conductivity and permitting plants to adapt to adverse environments (Gong et al., 2019; Sinyoung et al., 2024). Biochar facilitates the maintenance of open stomata under Pb-induced stress, hence optimizing the entry of CO_2 into the leaf. Consequently, Pb-induced stress induces stomatal closure, decreasing the availability of carbon dioxide and decreasing levels of intracellular CO_2. Nevertheless, the presence of biochar and allantoin ensures sufficient CO_2 concentrations in the leaf's intercellular spaces, hence optimizing intracellular CO_2 for photosynthesis (Sarraf et al., 2024; Abideen et al., 2023). Legocka et al. (2015) noted that barley transpiration increased under Pb-induced stress. This was ascribed to the capacity of biochar to increase the availability of soil nutrients, specifically potassium and magnesium, hence enhancing stomatal conductance, photosynthetic rate, and intercellular CO_2 levels (Gul et al., 2024).

3.5. Accumulation of Pb^{2+} and translocation in barley under Pb-induced stress

Pb^{2+} accumulated to varying degrees in different plant tissues amid

Table 3
Gas exchange characteristics of barley after different treatments.

Parameters	Control (CL)	Pb	Biochar (BC)	Allantoin (AT)	Pb + BC	Pb + AT	Pb + BC +AT
Intercellular CO_2 (μM CO_2 m^{-2} s^{-1})	12.99^{ab}	8.7^{c}	15.04^{a}	15.01^{a}	13.1^{b}	12.3^{b}	14.41^{ab}
Photosynthetic rate (μM m^{-2} s^{-1})	20^{b}	10^{d}	23^{a}	20.1^{b}	17.03^{b}	14.49^{c}	17.03^{b}
Stomatal conductance (mM H_2O_2 m^{-2} s^{-1})	1.39^{b}	0.69^{d}	2.3^{a}	1.10^{c}	1.50^{b}	1.20^{c}	1.69^{b}

Pb-induced stress: the Pb^{2+} level in the roots, shoots, and grains increased greatly (180 %, 280 %, 90 %, respectively) over the control (Fig. 4a-c). Under Pb-induced stress, biochar, allantoin, and their combination greatly decreased the accumulation of Pb^{2+} in shoots (by 59.9, 40.1, and 49.8 %), roots (by 48.2, 24.1, and 58.3 %), and grains (by 60.2, 29.7, and 40.1 %) respectively, in comparison to Pb^{2+} stress alone (Fig. 4a-c). It was observed that plants employ diverse ways to mitigate the adverse impacts of Pb^{2+} stress, including immobilizing Pb^{2+} by attaching it to cell walls and decreasing the Pb^{2+} concentration in the cells of roots by restricting its uptake. Pb^{2+} forms compounds with metallothioneins and phytochelatins in the cytoplasm, thereby sequestering Pb^{2+} securely into vacuoles (Srivastava, 2016). Biochar can immobilize Pb^{2+} through adsorption, utilizing its large specific surface area which also contains several functional groups (Li et al., 2021). These encompass acidic groups such as phenolic, lactonic, hydroxyl, and carboxylic, as well as basic groups including ketones and pyrones. Furthermore, oxygen-rich groups such as carboxyl and hydroxyl significantly improve the capacity of biochar to collect and stabilize Pb^{2+} (Tan et al., 2019). Numerous heavy metals, such as Pb^{2+}, form compounds with these functional groups, thereby mitigating the toxic effect of metals in plants (Zulfiqar et al., 2019). Minimizing concentrations of Pb^{2+} in edible parts of plants is essential for guaranteeing a safe food supply. Results indicate that while barley absorbs Pb^{2+} from the soil via its roots, the use of biochar, allantoin, and their combination as soil supplements decreases the Pb^{2+} translocation to other parts of plant tissues.

The TFs (translocation factors) exhibited significant variation between the applications, with the highest levels observed in roots. The root's TFs elevated by 24, 23, and 23 % under Pb-induced stress alone, allantoin, and their combined application, respectively vs. other treatments (Fig. 4d). The roots showed greatly increased TFs compared to the grains and shoots under allantoin and combined application (Fig. 4d-f). In comparison to all other treatments, the minimal TF level in shoots was seen in biochar and its combined application (Fig. 4). The TF values in grains were elevated under Pb-induced stress alone, whereas TF was decreased after biochar application (Fig. 4f). This is enabled by the capacity of biochar to immobilize and absorb Pb^{2+} in soil, due to its exceptionally porous structure and large SSA. Consequently, soil microbes and biochar decreased Pb^{2+} translocation from roots-to-shoots as well as grains through rhizosphere interactions (Andrey et al., 2019). The synergistic impacts of beneficial microbes and biochar lead to favourable biochemical alterations in the rhizosphere (Saeed et al., 2021). Consequently, biochar addition indirectly decreases the accumulation of Pb^{2+} in grains and shoots by decreasing rhizosphere Pb^{2+} mobility and availability.

The elevated concentrations of organic molecules in biochar significantly improve the soil's capacity to adsorb and sequester toxic metals, including cadmium, mercury, copper, and lead, by facilitating the development of chelates and complexes with stable surface functional groups. Furthermore, biochar modifies the pH of the soil and releases organic compounds, which may decrease soil lead concentration, bioavailability, and mobility, restricting crop uptake. The augmentation of cation exchange capacity and pH were likely the primary factors constraining the bioavailability of Pb^{2+} in the soil; furthermore, complexation with functional groups and precipitation/co-precipitation on the biochar surface and within soil aggregates were the principal mechanisms immobilizing this metal ion. Likewise, biochar improved Pb^{2+} tolerance in plants by facilitating heavy metals adsorption and deposition, resulting in biochar-Pb^{2+} complexes that mitigate Pb^{2+} toxicity (Acosta-Luque et al., 2023). By altering rhizosphere associations, biochar may indirectly lessen grain and shoot Pb^{2+} accumulation (Su et al., 2023). Translocation of Pb^{2+} to the apical parts is constrained via root barrier/ active efflux transporter pathways. Biochar treatment may enhance this mechanism, decreasing the accumulation of Pb^{2+} in grains and shoots (Noreen et al., 2024). Biochar and N-based fertilizers contribute to mechanisms including vacuolar sequestration, chelation, and compartmentalization, enhancing apoplastic barriers and hence inhibiting translocation of Pb^{2+} from the roots to aerial parts (Algethami et al., 2023). Suwunwong et al. (2021) similarly found that in *Zea mays*, biochar reduced Pb^{2+} levels in shoots compared to the roots, due to its capacity to bind Pb^{2+} as well as form complexes, thereby mitigating its toxicity. The combination of biochar and allantoin can decrease the accumulation of Pb^{2+} by elevating soil pH, accelerating precipitation, facilitating electrostatic interactions, boosting the root adsorption process, and increasing ion exchange efficiency (Gul et al., 2024). These modifications affect the metal fractionation in roots and decrease the availability of Pb^{2+} (Huang et al., 2023). Application of fertilizers and biochar are essential in mitigating the toxicity of Pb^{2+}, they promote cell wall deposition of Pb^{2+} or the creation of Pb^{2+}-chelation complexes in crops, hence reducing the uptake of Pb^{2+} from roots and Pb^{2+} translocation to shoots (Geetha et al., 2023).

3.6. *Biochar improves uptake of essential nutrients*

Biochar treatments may influence the absorption of vital nutrients. The nutrient uptake of barley was greatly increased in applied treatments using biochar, allantoin, and their combination vs. the control (Fig. 5). The treatments with allantoin alone and biochar alone resulted in increased nitrogen uptake. Under Pb-induced stress, treatments of biochar, allantoin, and their combination attained nitrogen uptake of 94.4, 89.3, and 110 % vs. the control, respectively, but nitrogen uptake was decreased under Pb-induced stress alone (Fig. 5a, d). Under Pb-caused stress, the biochar, allantoin, and their combination resulted in phosphorus concentrations that were 18.4, 20.1, and 21.7 % higher than the control, while phosphorus concentrations under Pb-induced stress alone were 6.2 % vs. the control (Fig. 5b, e). The activity of uptake absorption was elevated in the biochar, allantoin, and their combined application vs. other treatments (Fig. 5c). The findings indicate that biochar addition improves crop uptake of total nitrogen, total phosphorus, and total potassium by enhancing soil mineralization through the introduction of cohesive forces and negatively charged ions generation (Islam et al., 2016).

Seaweed-derived biochar was found to improve the uptake of vital nutrients due to its increased carbon contents, which increases the population of rhizosphere bacteria and facilitates the soil nitrification process. Invasive weeds-biochar enhances the SOM (soil organic matter) and total nitrogen level, hence promoting the growth of plants under environmental stress due to its exceptional porous nature and increased levels of ash (Palansooriya et al., 2019; Saleem et al., 2022). This exceptionally porous structure greatly enhances water retention and soil aeration, improving the structure of the soil and enabling plant roots to develop deeper and absorb nutrients effectively (Batool et al., 2022; Watanabe et al., 2014). The enhanced soil structure facilitates retention and gradual release of vital minerals including nitrogen, phosphorus, and potassium, hence increasing their availability to crops. Conversely, allantoin dissolves swiftly in the soil, liberating nitrogen as well as improving the soil structure through synergistic interactions with biochar (Kaur et al., 2023). The synergistic incorporation of allantoin and biochar fosters a desirable soil condition for the uptake of nutrients.

3.7. *Impact of biochar application on after-harvest soil and its Pb^{2+} bioavailability*

The level of nutrients in soil after harvesting was greatly increased in amended plants compared to the control group. Total nitrogen, phosphorus, and potassium in post-harvest soil were greatly increased in the allantoin-alone and biochar-alone application compared to the control (see Table 4), which is comparable to the findings by Chen et al. (2024). Under treatment of Pb-induced stress alone, total nitrogen, phosphorus, and potassium in post-harvest soil were considerably reduced by 29, 49, and 60 %, respectively, compared to the control. Compared to the control group, the EC and pH of after-harvest soil were increased

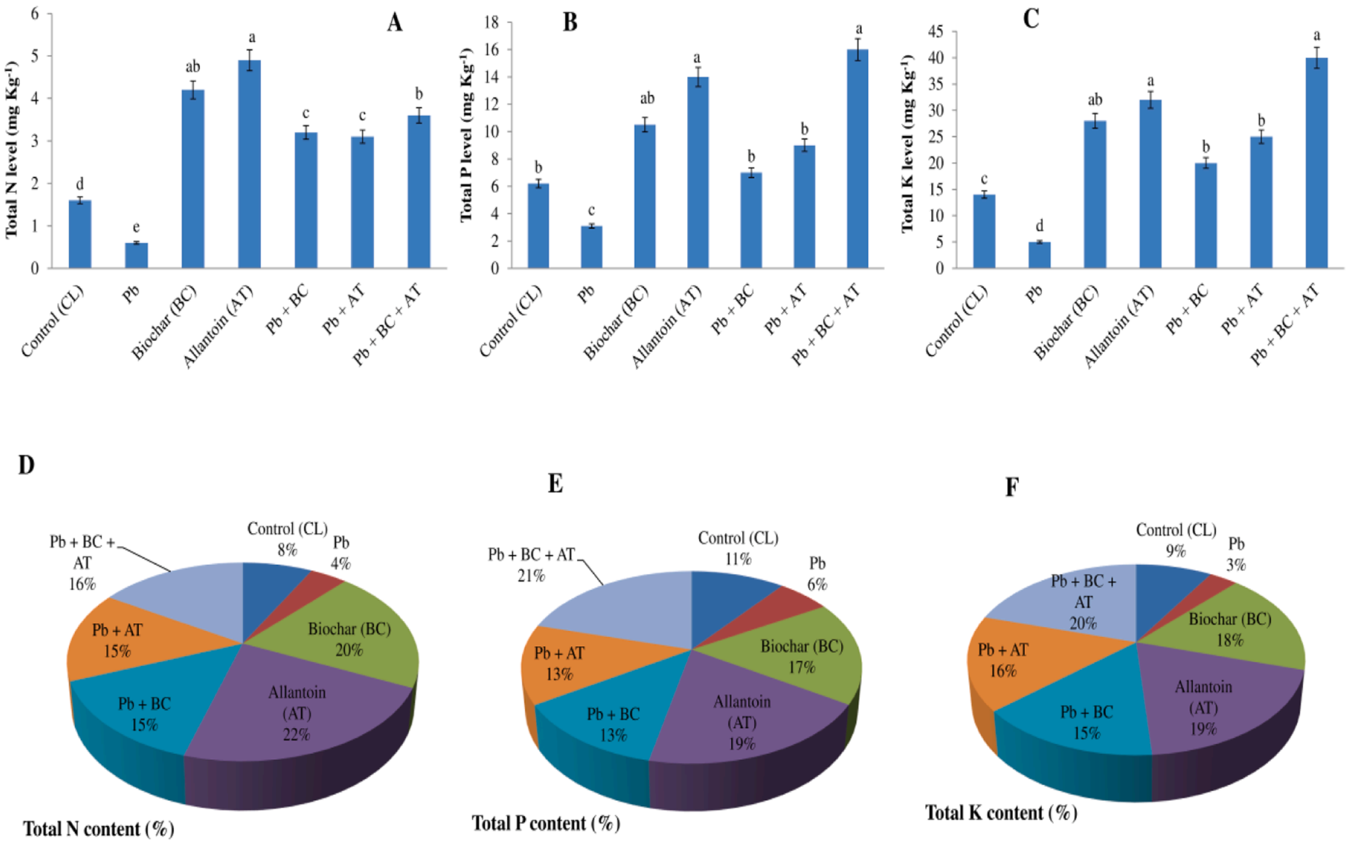

Fig. 5. Application of biochar, allantoin and their combination to examine the response of barely to Pb stress (a-c); Total concentrations of nitrogen, phosphorus, and potassium (d-f); Relative total concentrations of nitrogen, phosphorus, and potassium, respectively (expressed as a percentage). Various letters denote groups that exhibit significant differences at $p < 0.05$. Vertical bars denote the standard deviation (SD) based on 3 replicates. Tukey HSD and ANOVA tests were conducted ($p < 0.05$).

significantly in the allantoin-alone and biochar-alone application, but decreased under Pb stress. Under Pb-induced stress, biochar, allantoin, and their combined application significantly improved soil EC and pH vs. Pb stress alone and the control (see Table 4). Allantoin and biochar greatly decreased the Pb^{2+} bioavailability in after-harvest soil. The Pb^{2+} content in after-harvest soil was minimal in the biochar application without Pb stress. Under Pb-induced stress, the after-harvest soil Pb^{2+} level was found be be decreased after biochar, allantoin, and their combined application (see Table 4). Several studies have indicated a decrease in the bioavailability of Pb^{2+} after biochar treatment (Hamid et al., 2024). Yin et al. (2016) demonstrated that the application of 5 % biochar considerably decreases the availability of Pb^{2+} in soil. This decrease could be because of biochar's capacity to modify the chemical form of lead, rendering it less accessible and hence less hazardous to crops.

The simultaneous application of biochar and allantoin decreases the bioavailability of Pb^{2+} in post-harvest soil via many processes. Administered biocharadsorbs and immobilizes Pb^{2+}, decreases its solubility by modifying the pH of the soil, and generates stable biochar-Pb complexes. Furthermore, biochar improves the structure and fertility of soil while enhancing microbial growth. Collectively, these mechanisms reduce the bioavailability of Pb^{2+}, improve the quality of soil, and alleviate Pb hazards in cropping systems (Gong et al., 2022). The biochar-allantoin combination improved nutrient-use performance, increasing vital nutrient concentrations in the soil and decreasing toxicity of Pb^{2+} in agricultural systems. The observed enhancement was probably attributable to the improvement of soil qualities, including water retention, aggregation ability, and porosity resulting from the application of allantoin and biochar to metal-contaminated soil (Kaur et al., 2021). The biochar accelerates the mineralization of soil, potentially increasing the nitrogen, phosphorus, and potassium values (Chen et al., 2024). Due to its exceptionally permeable composition, which includes ammonium, nitrate, and phosphate, biochar enhances the fertility of the soil for crops (Hamilton, 2024). Compartmentalization is a mechanism that mitigates the toxicity of Pb by sequestering Pb in the vacuoles, especially inside the roots; Pb attaches to crop tissue, thereby decreasing its mobility (Dannhauser et al., 2024). The presence of heavy metals in soil results in modifications to critical physiological and biochemical functions, encompassing alterations in gene expression, protein variations, and changes in metabolite composition, all of which are essential for

Table 4
Uptake of vital nutrients and soil EC and pH as well as Pb^{2+} concentration.

Parameters	Control (CL)	Pb	Biochar (BC)	Allantoin (AT)	Pb + BC	Pb + AT	Pb + BC + AT
EC ($dS*m^{-1}$)	3.20^d	2.79^e	4.19^a	3.69^b	3.39^c	3.40^c	3.50^c
pH	7.30^d	7.40^c	7.65^a	7.50^b	7.40^c	7.42^b	7.60^a
Pb level (mg/kg^{-1})	0.40^c	1.20^a	0.20^c	0.22^c	0.70^b	0.79^b	0.70^c
Total P (mg/kg^{-1})	15^c	6.79^c	17.9^a	19.9^a	16.4^b	18.6^a	19.8^a
Total K (mg/kg^{-1})	30^c	15^d	40^{ab}	45^a	34^b	36.1^b	39^{ab}
Total N (mg/kg^{-1})	2.6^d	1.8^e	5.30^b	6.30^a	3.30^d	3.30^d	4.7^c

generating signals that activate defence and tolerance mechanisms in plants subjected to heavy metal toxicity and detoxification processes that may vary among different plants and their exposure to various metals or metalloids (Berni et al., 2019). Our data suggest that the biochar's elevated pH may facilitate the reduction of the Pb^{2+} content in barley. Furthermore, biochar enhances the mineralization of soil and facilitates Pb^{2+} uptake through plants, hence alleviating the toxicity of Pb^{2+} (Haiying et al., 2022). Biochar enhances the nutrient retention capacity of soil, contingent upon its porosity and surface charge. Biochar enhances nitrogen retention in soil by mitigating leaching and gaseous loss, while simultaneously augmenting phosphorus availability by diminishing the leaching process in soil (Xiang et al., 2022). In alignment with our results, Chen et al. (2022) observed that a substantial rise in the pH level of soil enhances the electronegativity and quantity of soil colloid binding areas, which are both essential for the immobilization of Pb^{2+} through adsorption and precipitation. The pH of the soil is closely associated with metal bioavailability (Haiying et al., 2022; Pengshun et al., 2024). As the pH of the soil rises, an increase in negatively charged ions on organic matter occurs, which enhances the adsorption of positively charged Pb^{2+} ions, hence decreasing their bioavailability in soil (Chen et al., 2023). Elevating the pH of the soil improves Pb^{2+} precipitation by fostering metallic-organic complex interactions on the surface of the soil, thereby facilitating the synthesis of Pb^{2+} complexes with hydroxides and carbonates (Natasha et al., 2022). The nitrogen release from urea in soil greatly decreases the pH level of soil, hence increasing its acidity level (Motasim et al., 2024).

3.8. *Correlation between Pb^{2+} concentration and barley growth*

Mantel tests were carried out to analyse pairwise correlations among root, shoot, and grain Pb^{2+} levels and various crop growth-related parameters (including length of roots, shoots, and spikes, dry and fresh weight, and grain quantity); physiological attributes; gas exchange-related parameters; and concentrations of vital nutrients. The Pb^{2+} presence in grains had a positive correlation with the growth of plants and the amount of essential nutrients, whereas it demonstrated a negative correlation with electrolyte leakage and malondialdehyde levels (Fig. 6). The Pb^{2+} concentration in roots and shoots exhibited a robust positive correlation with the growth of the plant and vital nutrient amounts, a weak positive correlation with catalase, peroxidase, superoxide dismutase as well as with the soil Pb^{2+} level, and a significant negative correlation with electrolyte leakage and malondialdehyde levels (Fig. 6). Nonetheless, soil Pb^{2+} content exhibited a less robust positive connection with root length, fresh weight ratio, dry weight ratio, the activity of antioxidant enzymes, and levels of flavonoids (Fig. 6). Talebzadeh and Valeo (2022) indicated a negative correlation between soil and leaf Pb^{2+} concentration and leaf carotenoid and chlorophyll levels, while a positive correlation was observed between leaf peroxidase, malondialdehyde, antioxidant activity, and Pb^{2+} content, aligning with our present results. Consequently, our results indicate that levels of Pb^{2+} in soil influence crop growth and output.

This study underscores the critical need to utilize *Achyranthes japonica* derived biochar in combination with allantoin to enhance growth attributes, development and resilience to Pb-induced stress. *Achyranthes japonica* biochar enhances soil physicochemical qualities, increases the availability of nutrients and antioxidant activity levels, and alleviates the detrimental impacts of Pb^{2+} stress. Similarly, allantoin serves as an accessible source of N, which can promote growth attributes and development of barely plants. Allantoin fosters robust plant development, which indirectly enhances the absorption of vital nutrients and ameliorates soil quality, potentially decreasing Pb^{2+} availability through heightened crop uptake and competing for nutrients from the soil. The simultaneous addition of biochar and allantoin is an excellent strategy for boosting barley growth and alleviating Pb-induced stress, consequently fostering sustainable enhancements in agricultural quality and productivity. Assessing the precise impacts of biochar and allantoin without Pb^{2+} stress is demanding. This knowledge gap is essential for comprehensively understanding the unique functions of biochar and allantoin, hence offering a more nuanced perspective on their synergistic actions under Pb-induced stress.

4. Conclusion

This study indicates the positive impact of integrating allantoin and *Achyranthes japonica*-biochar on the growth and yield of barley, potentially mitigating the potential risk of Pb^{2+} transfer within the food

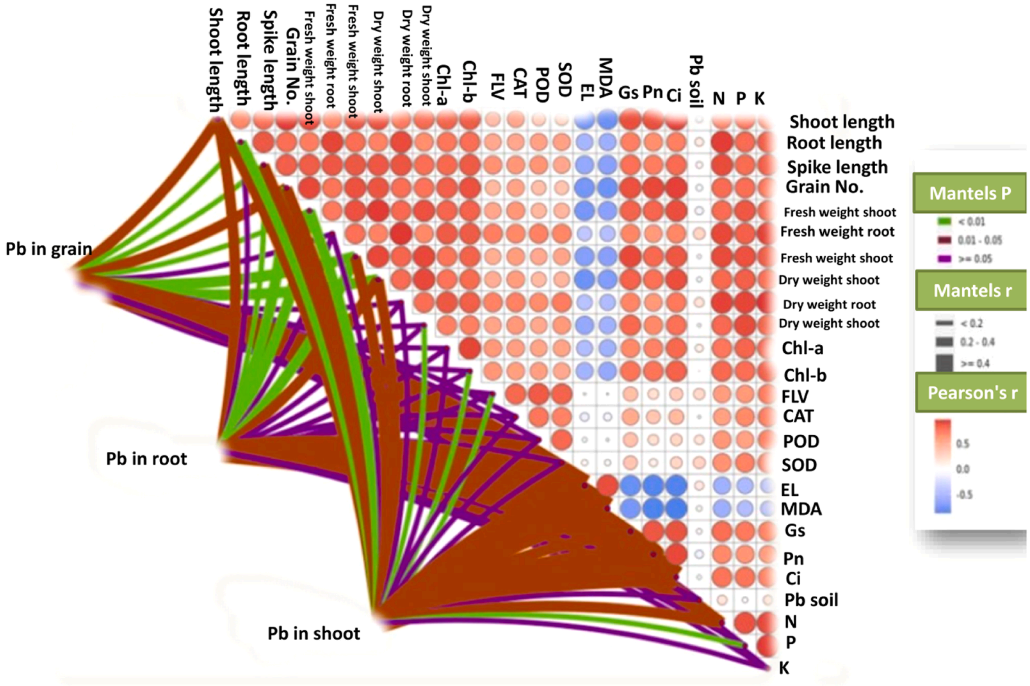

Fig. 6. Mantel evaluations for correlations between the levels of lead in barley roots, shoots, and grains, alongside various growth-related parameters.

system. The allantoin and *Achyranthes japonica*-biochar combo improved barley's biochemical and physiological attributes/responses to Pb^{2+} and mitigated its detrimental impacts. The application of biochar alone, and combined with allantoin, greatly mitigated the toxicity of Pb^{2+} by decreasing its translocation from roots to shoots and from roots to grains compared to Pb-induced stress alone. The treatment of allantoin-biochar greatly reduced Pb^{2+} accumulation in various barley tissues and alleviated Pb^{2+} toxicity via lowering the levels of hydrogen peroxide and MDA. A combination of allantoin-biochar offers an environmentally sustainable method for mitigating the toxicity of Pb^{2+} and remediating contaminated soil. Further research is needed to investigate the molecular processes and possible impacts of *Achyranthes japonica*-biochar as an amendment to the soil for other horticultural crops.

Author's contribution

All authors contributed equally to the conception, designing, writing, reviewing, and approval of the final version of the manuscript.

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CRediT authorship contribution statement

Deng Gang: Writing – review & editing, Software, Resources, Project administration, Investigation, Data curation, Conceptualization. **Ahmed Zeeshan:** Writing – review & editing, Software, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Iqbal Javed:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Elshikh Mohamed S:** Writing – review & editing. **Lackner Maximilian:** Data curation, Formal analysis, Funding acquisition, Resources, Software, Writing – review & editing. **Murtaza Ghulam:** Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Hassan Najmaldin Ezaldin:** Writing – review & editing, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Usman Muhammad:** Writing – review & editing, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rizwana Humaira:** Writing – review & editing, Software, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Ali Basharat:** Writing – review & editing, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Iqbal Rashid:** Writing – review & editing, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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