Environmental Technology & Innovation 38 (2025) 104121

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Synergistic effects of biochar and abscisic acid improved root morphology, antioxidant defense system and decreased availability and bioaccumulation of cadmium in *Triticum aestivum* (L.) under cadmium stress

Feng Lin [a], Zaffar Malik [b], Nasir Masood [c], Muhammad Rizwan [d,*], Yousef Alhaj Hamoud [e], Hiba Shaghaleh [f], Sana Noreen [b], Jean Wan Hong Yong [g,*]

[a] School of Environmental Engineering, Nanjing Institute of Technology, Nanjing 211167, China
[b] Department of Soil Science, The Islamia University of Bahawalpur, Bahawalpur, Punjab 63100, Pakistan
[c] Department of Biosciences, COMSATS University Islamabad Islamabad Campus, Tarlai kalan chak shehzad Park Road, Islamabad 45550, Pakistan
[d] Department of Environmental Sciences, Government College University Faisalabad, Faisalabad 38000, Pakistan
[e] College of Hydrology and Water Resources, Hohai University, Nanjing 210098, China
[f] College of Environment, Hohai University, Nanjing 210098, China
[g] Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Alnarp 23456, Sweden

ARTICLE INFO

Keywords:
Cd-contaminated soil
Biochar
Abscisic acid
Photosynthesis
Oxidative injury
Antioxidant enzymatic activities

ABSTRACT

Biochar (BC) and abscisic acid (ABA) may deliver positive physiological effects on heavy metal-stressed plants but their interactive role for regulating cadmium (Cd) availability in agricultural soils is unclear. This study revealed that the Cd-induced oxidative stress significantly reduced the growth of wheat, physiology and antioxidant responses. Interestingly, the co-application of BC (2.5 %) and ABA (20 μmol L^{-1}) restored the growth of wheat plants by minimizing Cd accumulation and translocation than their single use. The co-application of these amendments significantly increased the tissues biomass by 36 %, total root volume (29 %), root surface area (44 %), foliar Chl-a and Chl-b by 59 % and 55 % at 10 mg kg^{-1} Cd than control. Elevated Cd levels increased the proline, MDA and H_2O_2 contents, while BC and ABA applications ameliorated the Cd-induced oxidative injury by boosting the enzymatic activities of catalase by 46 %, ascorbate-peroxidase by 46 % and peroxidase by 37 % at 10 mg kg^{-1} Cd. The Cd treatment also increased Cd levels in soil, root and shoot tissues of wheat plants. The co-application BC and ABA reduced DTPA-extractable soil Cd by about 3-fold at 5 mg kg^{-1} and by about 1.8-fold at 10 mg kg^{-1}, as compared to respective controls. The combined BC + ABA treatment reduced Cd biological accumulation by 35 % and 33 %; and Cd translocation by 21 % and 9 % at 5 and 10 mg kg^{-1} Cd levels than control. It was concluded that the combined BC+ABA application restored the growth, physiology, antioxidant enzymatic activities and minimized Cd bioaccumulation in wheat tissues.

* Corresponding authors.
E-mail addresses: mrazi1532@yahoo.com (M. Rizwan), jean.yong@slu.se (J.W.H. Yong).

https://doi.org/10.1016/j.eti.2025.104121
Received 18 January 2025; Received in revised form 18 February 2025; Accepted 2 March 2025
Available online 7 March 2025

1. Introduction

Anthropogenic activities like agriculture and industrial wastes and utilization of untreated wastewater from industry and households have increased heavy metals and metalloids (HMs) contamination in our terrestrial environment (Clemens and Ma, 2016; Ghuge et al., 2023; Goncharuk and Zagoskina, 2023; Yang et al., 2023). Excess levels of HMs have significantly impacted soil, water and air quality (Khaliq et al., 2024; Khan et al., 2022; Li et al., 2023a; Li et al., 2023b; Rizwan et al., 2016; Wang et al., 2020, 2023). Agricultural inputs in the form of pesticides, synthetic fertilizers and wastewater for crop production has compelled humans to consume adulterated food due to HMs uptake by the plants (Ghuge et al., 2023; Liu et al., 2012; Shaghaleh et al., 2024). Moreover, these pollutants had affected the crop tolerance threshold and resulted in an alteration to plant genetic structure, with adverse consequences for humans and animals (Li et al., 2023a, 2024; Liu et al., 2020; Riseh et al., 2023). In addition, HMs especially cadmium (Cd), will alter tissue morphology and disrupt many essential physiological functions such as photosynthesis, respiration, and water and nutrients uptake from the soil (Goncharuk and Zagoskina, 2023; Lan et al., 2024; Li et al., 2023b; Song et al., 2019).

Cadmium has been recognized as one of the most hazardous substances owing to its high mobility and extreme toxicity even at low concentrations (Fan et al., 2023). High levels of Cd were reported to cause cancer and abnormalities in bones, liver and reproductive organs (Haider et al., 2021). The average dose of 3 μg L^{-1} in drinking water, and 25 μg kg^{-1} body weight is the recommended monthly limit for Cd consumption (Li et al., 2023a). The high levels of Cd in vegetables, cereal crops and grains, and trees, have adversely affected the normal functionality of living species (Aslam et al., 2023; Li et al., 2024; Liu et al., 2012; Shaghaleh et al., 2024). Despite the variable physiochemical properties of soil, Cd remains bioavailable to plants and can move freely in soil solutions (Rizwan et al., 2016).

Wheat (*Triticum aestivum* L.) is a staple food for many countries and is a source of essential nutrition for humans and livestock (Shiferaw et al., 2013). The annual production of wheat was reported around 600 million tons globally and its production was increased to 779.76 million tons cultivated on 222.11 million hectares (Zhang et al., 2023). It has been estimated that wheat demand may increase up to 60 % in the coming decades (Grote et al., 2021; Ahmad et al., 2022; Li et al., 2022; Shiferaw et al., 2013). This enormous demand for wheat and other cereals has compelled farmers in developing countries to utilize marginal or contaminated land; harnessing even polluted wastewater to fulfil crop water requirements, which, in turn, may create health issues after consuming adulterated wheat grains (Rahman et al., 2023; Shaghaleh et al., 2024). The high concentrations of Cd in aerial tissues of plants, especially grains, can cause serious health problems due to dietary Cd intake (Abbas et al., 2017). Apart from Cd uptake and its bioaccumulation, Cd was found to perturb many physiological functions of plants like photosynthesis, protein production, antioxidants activities and root morphology (Rizwan et al., 2016; Song et al., 2019). The soil characteristics i.e. pH, redox potential and soil organic material, affect the Cd absorption by the plant root and enhance uptake even at lower soil Cd concentrations (Zulfiqar et al., 2023). It was highlighted in many studies that Cd phytotoxicity would alter morphology, root development, and photosynthetic apparatus of plants (Haider et al., 2021; Zulfiqar et al., 2023).

For mitigation of Cd influence on environment and plant functions, ecofriendly and cost-effective strategies must be introduced. The organic and inorganic amendments and their consortium have been used to alleviate Cd toxicity to plant and its mobility in soil (Abbott et al., 2018; Afzal et al., 2024; Aslam et al., 2023; Fan et al., 2023; Ghuge et al., 2023). Extensive application of biochar (BC) in agricultural lands has been found to successfully decrease HMs toxicity to plants and their availability in soil (Shaghaleh et al., 2024; Yang et al., 2023). The physiochemical properties associated with BC such as high surface area and porous structure had enhanced adsorption capacity of HMs in soil (Sani et al., 2023; Zhong et al., 2023). The high organic matter contents along with numerous elements had increased soil fertility leading to high growth and yield in metals contaminated soils. The BC preparation techniques, its organic source and combination with other minerals had tremendously increased its ability for HMs adsorption and bioavailability in soil (Gong et al., 2022). Moreover, the abundance of functional groups (-COOH and OH-) on BC surfaces, its porosity, high pH and liming effects are few properties which significantly enhanced HMs (Cd, Cu, As, Ni, Pb as well as Zn) adsorption and immobilization in soil and decreased their uptake by plant (Danso et al., 2023; Li et al., 2024).

The improvement in growth and yield of plants has been reported in many studies after the addition of BC (including nanobiochar) in polluted soils (Kamran et al., 2019; Nafees et al., 2023; Sani et al., 2023). The application of BC to polluted soils has improved soil physiochemical properties and decreased soil extractable/bioavailable concentration of HMs (Afzal et al., 2024; Senthilkumar and Prasad, 2020). Many soil physical properties including soil porous structure, WHC, aggregate formation and saturated hydraulic conductivity along with chemical (CEC and pH) (Singh et al., 2023) and biological (soil enzymes, microbial biomass C and N, and microbial community structure) properties were reported to improve many folds with addition of BC in contaminated soil and might promote plant growth by decreasing HMs phytotoxicity (Khan et al., 2022; Su et al., 2024; Tan et al., 2017; Zong et al., 2023; Senthilkumar and Prasad, 2020).

A search of literature revealed that many biological research focuses on individual application of selected amendment to overcome a specific stress factor curtailing plant performance and yield. Conversely, several research revealed that the combination of amendments, as compared to their individual application, has demonstrated the capacity to produce more substantial enhancements in growth performance, yield, and eco-physiological resilience to unfavourable condition (Abbott et al., 2018; Hussain et al., 2022; Sani and Yong, 2022). Thus, for this study, the co-application of BC with various inorganic compounds and biostimulants (including plant hormones, amino acids) might provide effective solution to remediate HMs contaminated soils (Dai et al., 2023; Dami and Zhang, 2023; Danso et al., 2023; de Bang et al., 2021). The application of external abscisic acid (ABA), a well known phytohormone, can restore plant growth under biotic and abiotic stress (Ali et al., 2020; Kocaman, 2023; Suzuki et al., 2014). Specifically, the endogenous levels of ABA regulate stomatal conductance, whole-plant transpiration, and osmotic systemto to help plants to cope with the prevailing growth conditions (Ali et al., 2020; Chen et al., 2020; Yong et al., 2010). From literature, ABA increased plant growth by

decreasing oxidative stress caused by Cd toxicity (Tao et al., 2021; Zhao et al., 2023). Apart from physiological and biochemical modification, ABA has participated in genetic levels to down regulate HMs associated genes expression (*IRT1* and *HMA*) and enhanced *GSH1* expression for decreasing Cd toxicity on plants through reduced transpiration and metal ion chelation (Sun et al., 2023). The foliar application of ABA facilitated optimized plant antioxidant activities by producing SOD, APX and CAT enzymes to scavenge reactive oxygen species (ROS) produced by elevated levels of MDA, H_2O_2 and proline under Cd contamination (Tao et al., 2021); and efficiently decreased metals uptake, translocation from root to shoot and bioaccumulation factors by closing and opening of stomata and osmotic potential (Kamran et al., 2021). Thus, ABA has significantly improved plant growth in HMs contaminated media by improving physiological and biochemical functions (Hu et al., 2020). The growth of many plant species like *Oryza sativa*, *Brassica napus*, *Lactuca sativa*, and *Solanum tuberosum* (Tang et al., 2023; Yu et al., 2023) was improved with ABA application as ABA modulated the transpiration rate, osmolyte production and activated enzymatic activities to deter Cd phytotoxicity and to increase plant biomass (Liao et al., 2023).

The phytotoxicity of HMs for sustainable agriculture require the exploration of various innovative approaches to mitigate their toxicity and prevent their entry into the food chain. In the present study, we hypothesized that the combination of rice-husk BC and ABA could emerge as a promising strategy to reduce Cd translocation and bioaccumulation in wheat plants by reducing the Cd bioavailability in the soil. This study investigated the effects of individual and combined application of biochar and abscisic acid (ABA) on the following: (i) wheat growth performance, physiological traits, and antioxidant enzyme activities; (ii) Cd translocation from soil to plants. Hopefully, the results from this study could provide a comprehensive approach for the effective management of Cd contamination in agricultural soils.

2. Material and methods

2.1. Experimental design

The soil for this experiment was obtained from the agricultural farm area at 0–15 and 15–30 cm depth. The soil sampled at different depths was mixed and a composite sample was prepared and brought to the laboratory. The soil was dried in shade, sieved in 2 mm mesh size to remove gravels, roots and debris. The soil basic physical and chemical properties were measured by routine protocol as shown in Table 1. The soil used in the study has clay loam texture, pH 8.1, EC 3.7 dSm^{-1}, OM 0.4 %, and available N, P and K concentrations of 11.3, 17.6 and 86.3 mg kg^{-1}, respectively. Our study treatments were comprised of 5 and 10 mg kg^{-1} cadmium (Cd) levels along with 2.5 % biochar (BC) and 20 μmol L^{-1} abscisic acid (ABA) application. The soil was spiked with 5 and10 mg kg^{-1} concentration of Cd solution using $CdCl_2$ solution. After spiking, the soil samples were placed in shade and regularly mixed for 1 month. After aging, Cd contaminated soil (5 and10 mg kg^{-1} concentration) was mixed with 2.5 % level of rice husk biochar. The biochar incorporated Cd polluted soils were shifted to green house and filled in 10 kg clay pots. The treatments include: 2.5 % BC; 20 μmol L^{-1} ABA; and 2.5 % BC + 20 μmol L^{-1} ABA used in combination of various Cd levels i.e., Cd 0 mg kg^{-1}; Cd 5 mg kg^{-1}; and Cd 10 mg kg^{-1}. The experiment was set up in factorial arrangement with completely randomized design having three replicates per treatment. The pots were watered with distilled water at about 60 % of soil water holding capacity for one month and after that wheat seeds were grown in pots.

2.2. Biochar and soil analysis

The rice husk biochar used in the current study was produced at 550 ℃ for 2 hours. The properties of biochar are summarized in Table 1. The routine standard laboratory methods were used to determine soil and biochar physical and chemical characteristics. The BC was characterized for SEM, FTIR and XRD (Fig. 1b,c,d). The percentages of sand, silt and clay were measured with bouyoucos hydrometer method devised by Bouyoucos, (1962). Soil texture class was examined through USDA soil textural triangle. Soil saturation percentage was determined with the procedure of Grewal et al. (1990). The soil and biochar pH were determined by making a suspension of 1:1 (soil: water) and 1:5 (biochar: water), shaken and stirred for 30 minutes and pH was measured through pH meter (AB33 pH meter, OHAUS, Switzerland), respectively. The soil Cd contents were measured by digesting the sample with HNO_3 and $HClO_4$ acids

Table 1
The physical and chemical characterization of the soil and biochar used in the current study.

Characteristics	Units	Soil	Biochar
Texture	–	Clay Loam	-
Sand	%	30	-
Silt	%	43	-
Clay	%	27	-
Saturation Percentage	%	34	-
EC_e	(dS $m^{-1)}$	3.7	5.4
pHs	–	8.1	9.7
Available K	mg kg^{-1}	86.3	261.3
Available P	mg kg^{-1}	17.6	1.4
Available N	mg kg^{-1}	14.3	19.3
Organic Matter	%	0.4	-

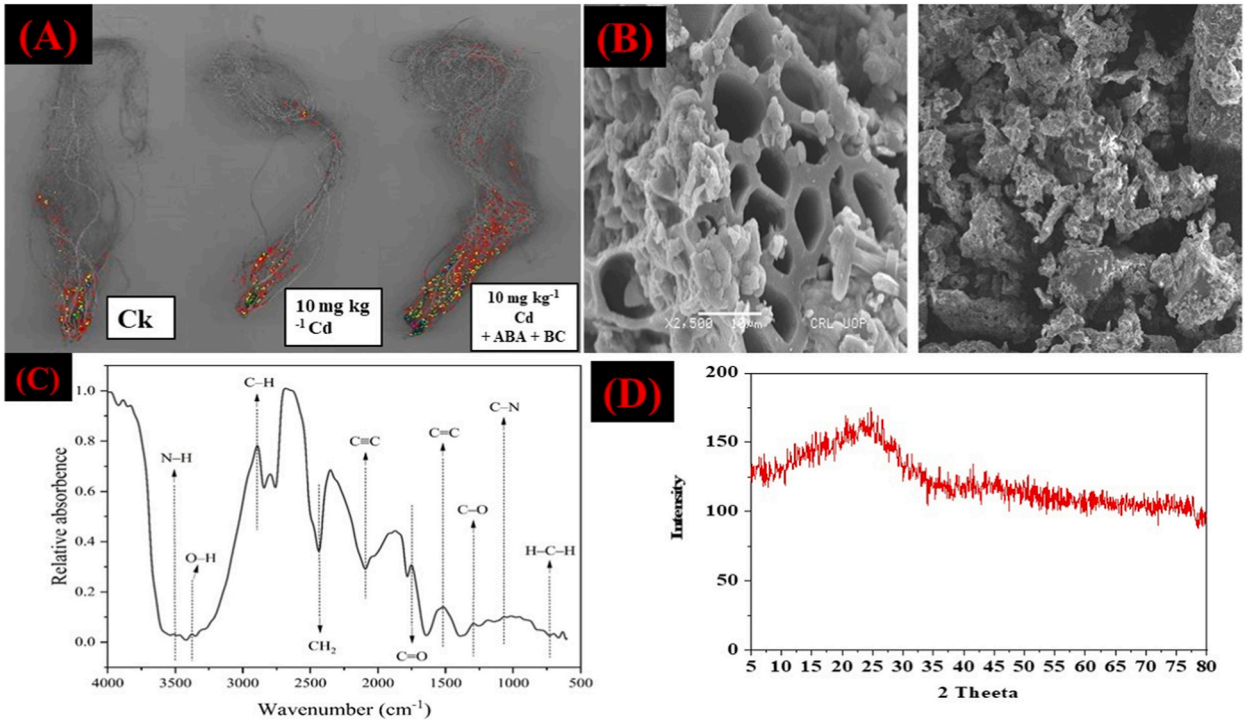

Fig. 1. The wheat root morphological traits (**A**) represented by root scanning, (**B**) SEM, (**C**) FTIR spectra and (**D**) XRD pattern of biochar used in the study. The FTIR spectra indicated different functional groups like (0-H), (H-C-H), (C-H), (C═C), (C═N), (N-H) and (C═O) for exchangeable sites for Cd adsorption.

with 2:1 ratio as described previously (Jones and Case, 1990). Briefly, a sample of soil (0.5 g) was digested with acids on a hot plate at 350 °C to colorless fumes. The digested sample was transferred to 50 ml flask with several washings and filtered through filter paper (Whatman no. 40); and stored for Cd analysis. The soil DTPA extractable Cd contents after harvesting were measured by method of Chen et al. (2021). Briefly, 2.0 g soil was gently shaked with 20 ml solution of DTPA (0.005 mol l^{-1} + 0.1 mol L^{-1} triethanolamine (TEA) and 0.01 mol L^{-1} $CaCl_2$) and centrifuged at 259 rpm min^{-1} and extract was transferred to 25 ml flask. The soil Cd and DTPA contents were measured on atomic absorption spectrophotometer (Analytikjena, novAA 300, Germany).

2.3. Plant growth condition and application of ABA

The wheat plants were grown in green house condition during the growth of wheat season. The healthy and uniform wheat seeds of local varieties were surface sterilized with 10 % H_2O_2 for 10 minutes and then washed with distilled water. The sterilized seeds were sown directly into clay pots with different treatments. After the emergence of seedling, 5 plants per pot were maintained and watered with distilled water to maintain 45 % WHC The green house temperature was maintained at 25/19 ℃ (day/night) with 16/8-h of light/dark period and 65–70 % relative humidity throughout the growth period. The pots were also supplied with recommended dose of NPK (60, 30 and 30 kg ha^{-1}) into two growth stages to maintain the plant health and vigor. The ABA (20 μmol L^{-1}) was dissolved in 5 % ethanol solution with 0.05 % tween 20 as wetting agent and was applied to wheat plant at three critical growth stages to boost crop growth (Jamalian et al., 2013). The 1st foliar application was done at two leaf stage, 2nd on tillering stage and 3rd spray was applied on heading stage, respectively.

2.4. Harvesting and measurement of plant morphological and physiological traits

The various morphological and physiological parameters of the wheat plants were performed before and after the crop harvesting. The wheat plants were harvested at maturity stage, brought to laboratory, washed, dried and separated into root and shoot tissues. The plant fresh biomass (root and shoot) was measured by electrical balance (Chen et al., 2021). Plant dry biomass (roots and shoots) weas measured by placing the samples in oven at 65 ℃ till constant weight. Following which, the dried samples were ground passed through 0.5 size mesh and plant Cd contents were measured in root and shoot (Zhao et al., 2021). The morphological traits of roots (root volume, average root diameter, no. of root tips etc.) were determined by scanning fresh root samples using a specialized scanner (WinRHIZO Pro, Netherlands) (Malik et al., 2023). Plant SPAD value was determined by selecting the expanded leaves and reading were taken at 5 representative points and average was calculated by SPAD-502 (Minolta, Minolta Co, Ltd, UK) (Kandel, 2020). Plant relative water contents were determined in fully expanded leaves by taking their fresh weight (FW). After that, leaf samples were

placed in distilled water for 16–18 hrs and leaf turgid (TW) weight was recorded (Malik et al., 2023). The samples were then oven dried at 65 ℃ and leaves dry weight (DW) was again measured and plant relative water content was measured by following formula:

Leaf relative water contents (%) = (FW-DW) / (TW-DW) X 100

The membrane stability index (MSI) was measured using the steps outlined by Ahmad et al. (2022). The fresh leaf samples were washed in distilled water several times and placed in hot bath at 40 ℃ in 10 ml water for 30 minutes to calculate EC1. Again, these samples were heated at 100 ℃ in hot bath to measure EC2 and MSI was computed by following formula:

Membrane stability index (MSI) = [1 – (EC1 / EC2)] x 100

Mature and healthy wheat leaves were identified and harvested freshly; following which the foliar chlorophyll *a*, *b* and carotenoids contents were determined spectrophotometically (Liu et al., 2020; Yong et al., 2014). Specifically, plant leaf extracts (0.05 g sample) were ground using the pre-iced mortar and pestle. The leaf extracts were mixed with 10 ml dimethyl sulphoxide and heated at 65 ℃ for 4 hours. Following which, the foliar chlorophyll a, b was determined at 645 nm and 665 nm and carotenoids contents were recorded at 470 nm in spectrophotometer (Agilent Technologies, Cary 60, UV VIS).

2.5. Determination of plant oxidative stress indicators

Plant biochemical assays were determined in mature and freshly harvested leaves. The fresh leaves were cut, packed in zip bags with ice and brought to the laboratory. Plant biochemical assays were performed on spectrophotometer. The plant leaf tissues (0.5 g) were immersed in solution containing 2 ml ninhydrin, 5 ml sulfosalicylic acid and 2 ml glacial acetic acid prior to heating at 80 ℃ in hot water bath for 30 min. The solution was cooled and centrifugation was performed at 10000 rpm for 10 min. The supernatant was separated and reading was obtained at 520 nm after drawing the standard curve to calculate proline contents (Ye et al., 2015). Plant MDA contents were measured by taking fresh leaves and grounding them (0.5 g) in 10 ml TBA solution (0.25 %) which was in 10 % trichloroacetic acid (TCA). The extract was subjected to heating at 95 ℃ prior to ice-cooling to stop the reaction. The extract was centrifuged (10000 rpm) and the absorbance was checked at 532 nm and 600 nm. MDA contents were obtained through extinction co-efficient (155 mM^{-1} cm^{-1}) (Ali et al., 2018).

The polyphenol peroxidase (PPO) concentration in leaves samples were determined by Esterbauer et al. (1977). Simply, 0.5 ml leaf extract was mixed with 2 ml phosphate buffer solution (PMBS) and 1 ml catechol (0.1 mM) and pH was adjusted at 6.0. In a similar manner, a blank sample was also prepared with all the reagents except leaf extract for the validation of the results. All the samples were run on spectrophotometer at 495 nm after gently shaken and PPO contents were recorded. The Velikova et al. (2000) method was used to find H_2O_2 contents in plant leaves samples. For this purpose, 1 ml of 0.1 % TCA solution was mixed with 0.2 g leave extract and centrifugation was done at 8000 rpm for 15 min. The supernatant was obtained in 5 ml Teflon tube and 0.5 ml K-P buffer solution (10 mM), 1 ml potassium iodide (1 M) was added and gently shaken. The absorbance was measured at 390 nm with spectrophotometer to calculate H_2O_2 concentration.

2.6. Estimation of antioxidant enzymatic activities

The plant leaf extract was obtained in the presence of distilled water, phosphate buffer (PBS) (pH 7.8) and sodium bicarbonate after crushing the fresh leaves in pre iced mortar and pestle. Then, the solution was later centrifuged at 8000–13000 rpm for 20 minutes (Nasirzadeh et al., 2021). After that, supernatant was obtained in 5 ml 5 ml centrifugation tubes and stored at 4 °C in the refrigerator for the estimation of antioxidant enzyme activities. The leaf catalase (CAT) concentration was measured by adopting Tyagi et al. (2021) method. The leaf extract, PBS and H_2O_2 (10 mM) were mixed (2 ml, 2.8 ml and I ml, respectively) and shake manually. After that, CAT activity was measured in prepared sample along with blank on spectrophotometer at 240 nm wavelength. The leaf peroxidase (POD) activity was estimated by following Saberi et al. (2021) method. The plant leaves extract was dissolved in 300 mM H_2O_2, 1.5 % guaicol, and 50 mM PBS, respectively. The reaction solution consisted of 2.8 ml PBS, 0.1 ml leaf extract, 0.1 ml guaicol and 0.1 ml H_2O_2, respectively. A blank sample including reagents but without leaf extract was also made. All the prepared samples were assessed on spectrophotometer at 470 nm for POD estimation.

The 0.5 ml leaf extract with 0.1 ml H_2O_2 (10 mM), 0.5 ml ascorbic-acid (0.5 mM), 0.25 ml EDTA and 2.7 ml PBS (50 mM), respectively was used for measurement of peroxidase ascorbate (APX). A blank sample was also prepared for results accuracy. The samples were run on spectrophotometer at 290 nm as described by Asghar et al. (2023). Similarly, peroxidase (POX) activity was found by adopting Gorin and Heidema, (1976) analysis method. A solution composed of leaf extract, Pyrogallol, PBS and H_2O_2 with 0.5 ml, 1 ml, 2 ml and 1 ml quantity, respectively was made. The prepared mixture was further mixed with 1 ml H_2SO_4 (2.5 M) at 25°C and waited for 5 minutes. The POX activity was measured at 420 nm on spectrophotometer.

2.7. Plant Cd contents, translocation and bioaccumulation

The plant (root & shoot) Cd contents were measured by converting the plant material into ash by placing them into furnace at 450–500 °C for 12 hours in boron free silica crucible. The plants ash samples were removed from furnace and 10 ml of 1 M HNO_3 was added. The crucibles were placed for 1 hour at room temperature and the mixture was heated at 350 °C for 2–3 hours until solution become clear. After that, the liquid was transferred into 50 ml flask and filtration was done with Whatman No. 42 filter paper. The

solutions concentrations (Cd root and shoot contents) were recorded on atomic absorption spectrophotometer (Analytikjena, novAA 300, Germany) as mentioned by Akinyele and Shokunbi, (2015). Based on soil and plant Cd concentration, bioaccumulation factor (BAC), bioconcentration factor (BCF) as well as translocation factor (TF) for Cd was determined by following formulas as described by Kamran et al. (2019):

$$BAC = \text{Cd concentration in shoots/ Cd concentration in soil} \quad (1)$$

$$BCF = \text{Cd concentration in roots/ Cd concentration in soil} \quad (2)$$

$$TF = \text{Cd concentration in shoots/ Cd concentration in roots} \quad (3)$$

2.8. Statistical analysis

Data analysis carried out to calculate ANOVA results using SPSS 17 software. The average data and standard deviation (SD) were computed by the three replications. Tukey HSD test ($P < 0.05$) was used to measure the differences between means of each treatment. The graphs were created using Origin Pro software. The principal component analysis (PCA) analysis was performed on R package with the help of factoextra, ggplot2, and dplyr libraries.

3. Results

3.1. Effects of ABA and BC on plant morphological traits

The effects of Cd toxicity lowered the different growth parameters of wheat (Table 2) and altered the root morphology (Fig. 1a). However, biochar, ABA, and their combination significantly decreased the negative impacts of excess Cd. The combined application of biochar and ABA under 10 mg kg^{-1} Cd treatment resulted in the maximum significant improvement in shoot length (28 %), root length (34 %), shoot fresh (48 %) and root fresh biomass (47 %), and root and shoots dry weights (47 and 73 %) as compared to the un-amended control. Similarly, significant improvements in average diameter of roots (AVGRD) (44 %), total volume of roots (TRV) (29 %) and surface area of roots (RSA) (44 %) were observed when treated with BC+ABA under 10 mg kg^{-1} than unamended control.

Table 2
Effects of abscisic acid (ABA) and biochar (BC) on the growth parameters and root morphological traits of wheat plants at 0, 5 and 10 mg kg^{-1} of Cd levels. Data are means (± standard deviation) of 3 values. Treatment means not sharing common letters within the same column are statistically different ($P < 0.05$) from each other, based on LSD test.

Treatments		Shoot Length (cm)	Root Length (cm)	Shoot Fresh Biomass (g)	Shoot Dry Biomass (g)	Root Fresh biomass (g)	Root Dry Biomass (g)	Average Root Diameter (AVGRD)	Total root volume (TRV) (cm^3)	Root Surface Area (SA) (cm^2)
0 Cd	Unamended	35 gh ± 1.01	18.3c ± 0.8	23.5 d ± 0.7	6.8 de ± 0.3	3.4 cd ± 0.06	0.4 cd ± 0.07	2.6 d ± 0.1	25 bcd ± 0.5	55.7 de ± 1.6
	ABA	40.7 cde ± 1.1	23b ± 1.4	35 bc ± 1.8	9.2 b ± 0.25	3.9c ± 0.2	0.5 bc ± 0.1	3.0c ± 0.07	29.2 b ± 0.8	66.5c ± 2.3
	BC	43.3 bc ± 1.9	24.7 b ± 1.5	36 bc ± 1.6	10 b ± 0.23	4.7 b ± 0.2	0.6 ab ± 0.1	3.5 b ± 0.04	37.3 a ± 1.1	76.7b ± 0.8
	ABA+ BC	53 a ± 1.82	32.8 a ± 1.3	46 a ± 2.2	11.5 a ± 0.5	5.8 a ± 0.5	0.7 a ± 0.1	3.8 a ± 0.03	39 a ± 1.1	85.7 a ± 0.6
5 Cd	Unamended	32.7 h ± 1.1	16.7 cd ± 0.3	2 de ± 0.9	5 fg ± 0.2	3 d ± 0.07	0.3 de ± 0.02	1.6 g ± 0.03	23 cde ± 1.1	48.7 fg ± 1.01
	ABA	40.7 de ± 0.8	18.3c ± 0.8	23.5 d ± 0.7	7.3 cd ± 0.9	3.4 cd ± 0.05	0.4 cd ± 0.02	1.9 f ± 0.06	26 bcd ± 1	60.1 d ± 1.1
	BC	42.3 cd ± 0.8	22.8 b ± 1.4	32c ± 1	8c ± 0.1	3.9c ± 0.2	0.5 bc ± 0.05	2.3 e ± 0.06	28.2 b ± 1.3	67.4c ± 1
	ABA+ BC	45.8 b ± 0.5	24.7 b ± 1.5	36 b ± 1.6	9.3 b ± 0.3	4.7 b ± 0.2	0.6 ab ± 0.02	2.6 d ± 0.03	35a ± 2.3	74.8 b ± 1.4
10 Cd	Unamended	29.5 i ± 0.3	15 d ± 0.5	16.5 e ± 0.9	4.1 g ± 0.2	2.03 e ± 0.3	0.2e ± 0.02	1.2 h ± 0.02	18.4e ± 1	29 i ± 0.8
	ABA	36.7 fg ± 1.	16.7 cd ± 0.3	20 de ± 1.1	5.2 f ± 0.3	2.9 d ± 0.1	0.23 de ± 0.02	1.6 g ± 0.05	21 de ± 0.8	36.5 h ± 1.4
	BC	38.7 ef ± 1.3	18.3 b ± 0.8	23 d ± 0.7	5.9 ef ± 0.2	3.4 cd ± 0.05	0.3 cd ± 0.05	1.6 f ± 0.04	23cde ± 1.7	45.6 g ± 1.5
	ABA+ BC	42 cd ± 0.4	22.8 b ± 1.4	32 c ± 1	7.2 cd ± 0.3	3.9 c ± 0.2	0.37 bc ± 0.1	2.2 e ± 0.03	26 bc ± 2.1	52.6ef ± 1.3

3.2. Effects of ABA and BC on plant physiological parameters

The Cd stress negatively impacted the wheat physiological functions, whereas BC and ABA application had modulated these functions (Fig. 2). The maximum improvement in the Chl. a & b (59 % & 55 %), carotenoids (52 %), RWC (26 %), MSI (40 %) along with SPAD value (44 %) was observed with BC + ABA combination at 10 mg kg^{-1} Cd stress than unamended soil. Similar results were also found at 5 mg kg^{-1} Cd level for BC+ABA application in unamended soil.

3.3. Effects of ABA and BC on plant oxidative stress indicators

The Cd toxicity had significantly increased proline, MDA, PPO and H_2O_2 content at 5 & 10 mg kg^{-1} Cd treatments in unamended soil compared to control (Fig. 3). The use of BC and ABA reduced the production of these stress indicators. The significant low values for proline (18 %), MDA (26 %), PPO (26 %) and H_2O_2 (20 %) were observed for co-application of BC and ABA, respectively, at 5 & 10 mg kg^{-1} Cd stress.

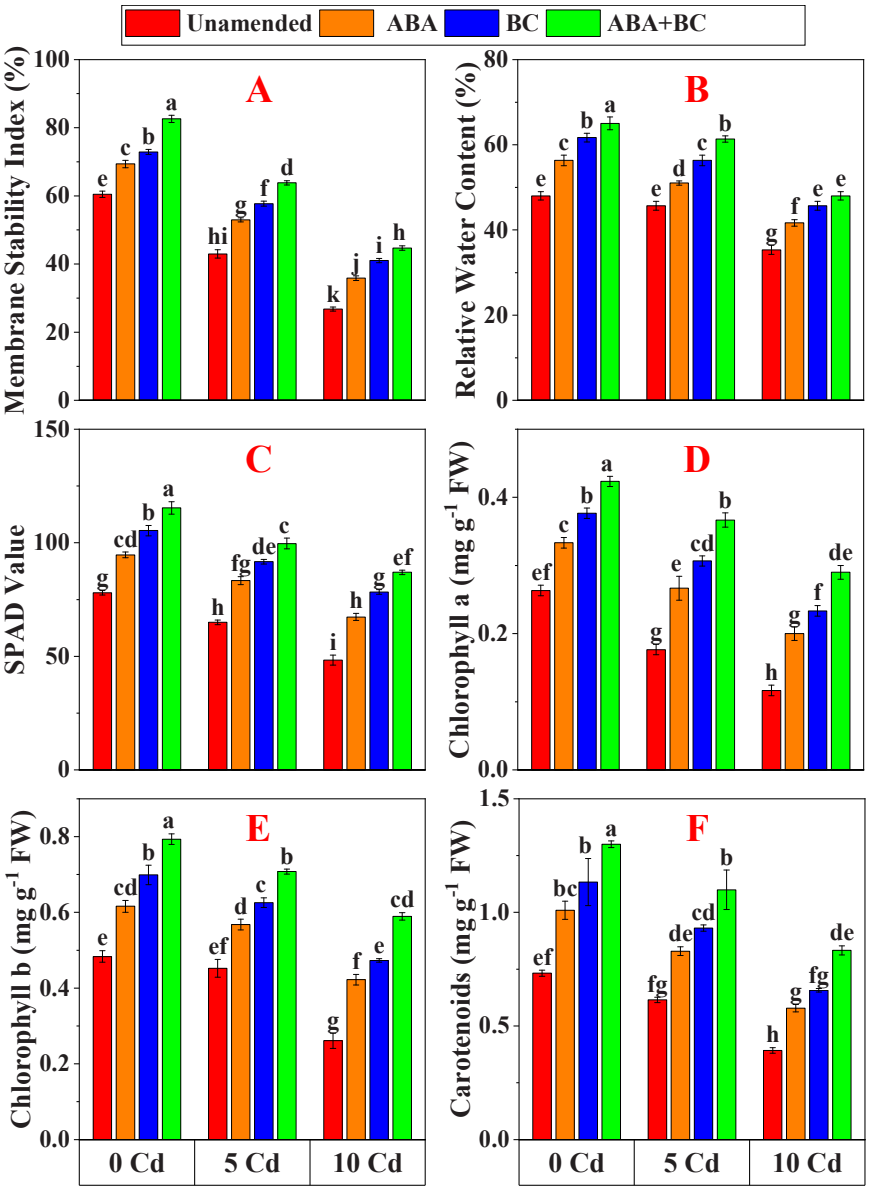

Fig. 2. Effects of biochar (BC) and exogenous application of abscisic acid (ABA) on wheat (**A**) membrane stability index (MSI), (**B**) relative water content (RWC), (**C**) SPAD value, (**D**) chlorophyll a, (**E**) chlorophyll *b*, and (**F**) carotenoids contents at 0, 5 and 10 mg kg^{-1} of Cd levels. The values are means ± standard deviation of 3 replicates. Different letters on vertical bars indicate significant difference at probability level $P < 0.05$.

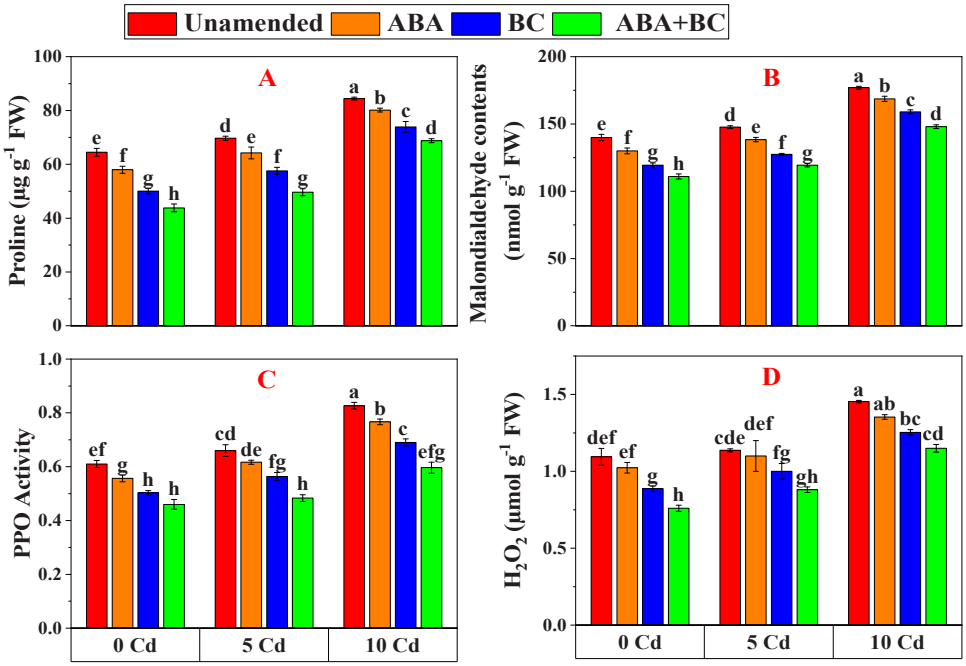

Fig. 3. Effects of biochar (BC) and exogenous application of abscisic acid (ABA) on wheat oxidative stress (**A**) proline contents, (**B**) malondialdehyde contents, (**C**) polyphenol peroxidase (PPO) contents, and (**D**) H_2O_2 contents at 0, 5 and 10 mg kg^{-1} of Cd levels. The values are means ± standard deviation of 3 replicates. Different letters on vertical bars indicate significant difference at probability level $P < 0.05$.

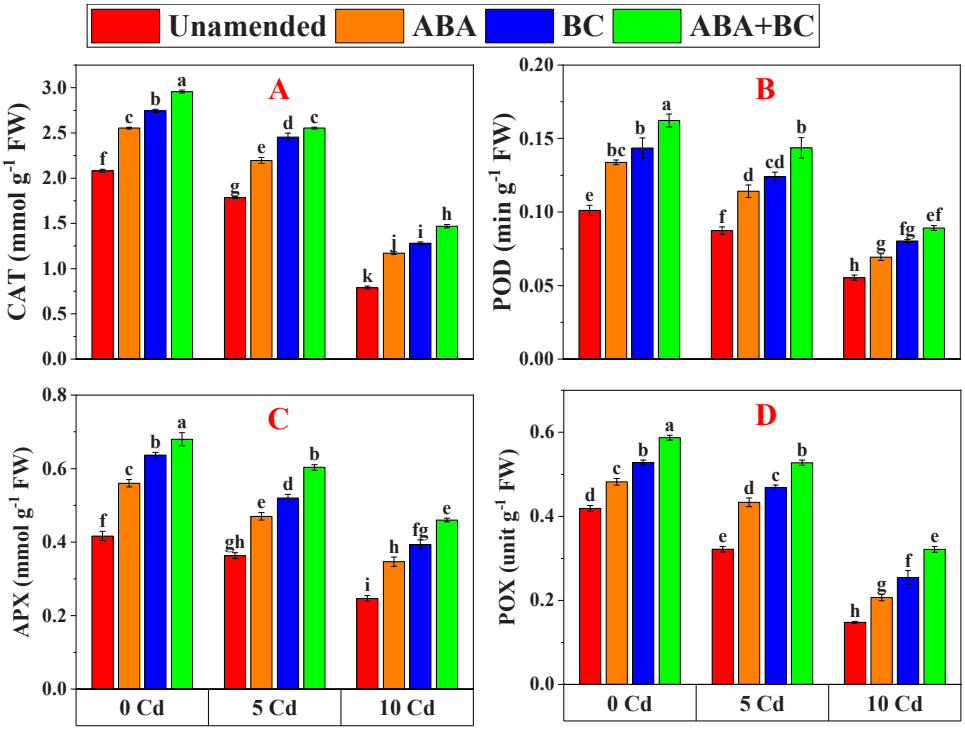

Fig. 4. Effects of biochar (BC) and exogenous application of abscisic acid (ABA) on wheat leaf antioxidant enzymatic activities (**A**) catalase (CAT) activity, (**B**) peroxidase (POD) activity, (**C**) peroxidase ascorbate (APX) activity, and (**D**) peroxidase (POX) activity at 0, 5 and 10 mg kg^{-1} of Cd levels. The values are means ± standard deviation of 3 replicates. Different letters on vertical bars indicate significant difference at probability level $P < 0.05$.

3.4. Effects of BC and ABA on antioxidant enzymatic activities

The results demonstrated low antioxidant enzymatic activities at 5 & 10 mg kg^{-1} Cd level in unamended soil compared to control (Fig. 4). The results also showed that the application of BC and ABA had increased catalyze (CAT), ascorbate (APX), guaicol peroxidase (POD) and peroxidase (POX) activities in wheat plants grown in 5 and 10 mg kg^{-1} Cd stress, respectively, compared to unamended soils. The significant results were observed with BC+ABA application for wheat shoot CAT, APX, POD and POX activities by 43 %, 46 % and 39 %, 46 % and 39 %, 37 % and 38 %, 54 % at 5 and 10 mg kg^{-1} Cd levels compared to unamended soils.

3.5. Effects of ABA and BC on Cd uptake, translocation and bioaccumulation

The results demonstrated that soil DTPA extractable Cd contents significantly increased at 5 & 10 mg kg^{-1} Cd treatments in unamended soils compared to control (Fig. 5). While BC and ABA significantly reduced ($P < 0.05$) Cd available Cd contents in soil. The maximum reduction in soil DTPA extractable Cd was observed at ABA + BC application (69 % & 43 %) at 5 & 10 mg kg^{-1} Cd levels, respectively, then unamended soil. Fig. 5 also represented that in unamended soil, the root & shoots Cd contents increased significantly

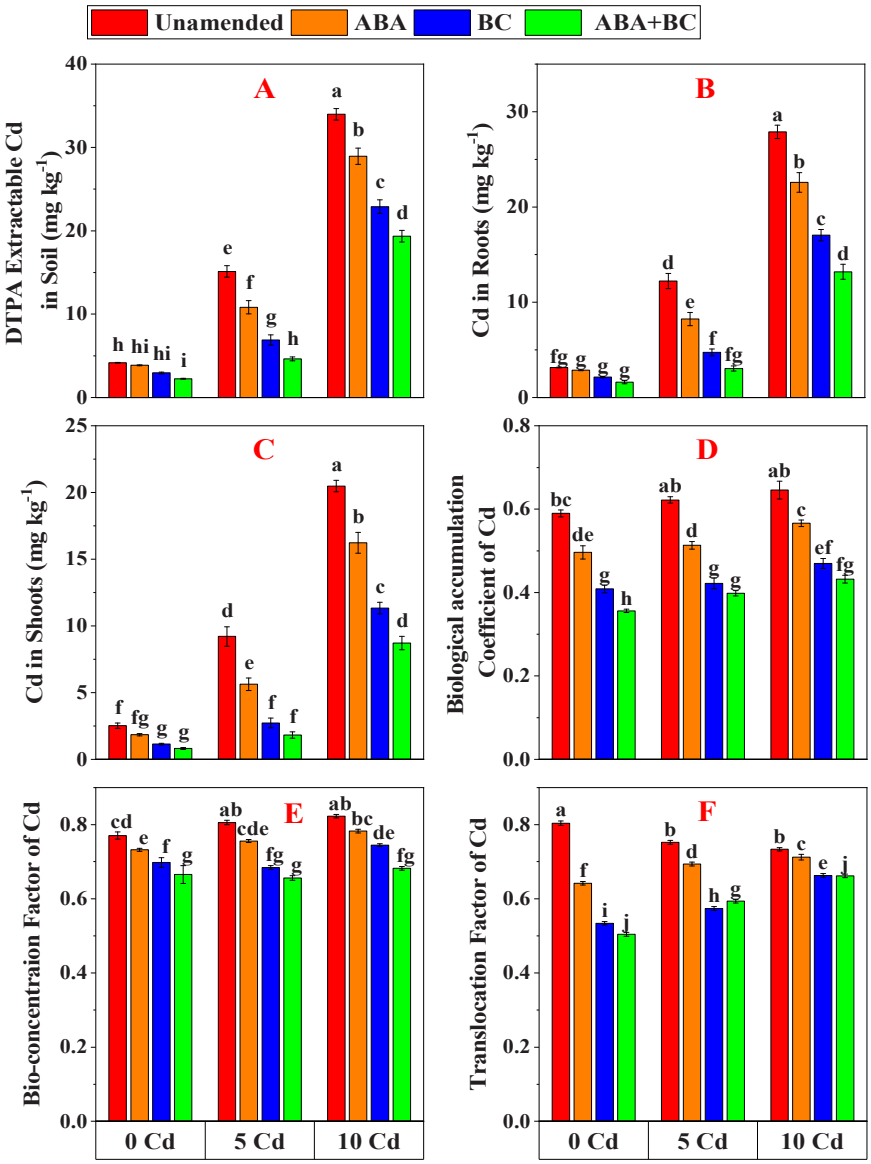

Fig. 5. Effects of abscisic acid (ABA) and biochar (BC) on the (**A**) Cd concentration in soil, (**B**) Cd concentration in root (**C**) Cd concentration in shoot (**D**) bioaccumulation factor (BAC), (**E**) bioconcentration factor (BCF) and (**F**) translocation factor (TF) at 0, 5 and 10 mg kg^{-1} of Cd levels. The values are means ± standard deviation of 3 replicates. Different letters on vertical bars indicate significant difference at probability level $P < 0.05$.

at 5 & 10 mg kg^{-1} Cd levels. The BC and ABA application to unamended soils decreased Cd contents in wheat plants. The highest improvement was observed in BC + ABA combination, where, root and shoot contents decreased by 75 %, 52 % and 80 %, 57 % at 5 and 10 mg kg^{-1} Cd levels, respectively compared to unamended soils. Moreover, co-application of BC and ABA successfully reduced the possibility of Cd bioaccumulation and its translocation in wheat plants. The results highlighted that BC + ABA combination decreased BAC, BCF and TF values by 35 %, 33 % and 18 %, 17 % and 21 %, 9 % respectively, at 5 and 10 mg kg^{-1} Cd stress compared with unamended soils.

3.6. *Principal component analysis: factors controlling wheat growth under Cd toxicity*

Principal component analysis (PCA) was conducted to assess whether wheat growth, reactive oxygen species (ROS) generation, activities of antioxidant enzymes, and Cd translocation from soil to root and shoot showed distinct responses to varying levels of Cd toxicity, and whether these responses were mitigated by the application of biochar and abscisic acid (ABA) amendments (Fig. 6). PC1 and PC2, accounted for 86.7 % and 7.3 % of total variance, respectively. The PCA plot revealed that all treatments (control, ABA, biochar, and ABA + BC) were distinctly separated at each Cd toxicity level. For all levels of Cd toxicity, the treatments with ABA, BC, and ABA + BC formed clear clusters, which were distinct from their respective unamended controls, indicating that all amendments enhanced wheat tolerance to Cd toxicity. The clustering ellipses for the 0 and 5 g kg^{-1} Cd toxicity levels were positioned along the positive PC1 axis, suggesting that ABA and BC, whether applied individually or in combination, had a greater impact in alleviating lower Cd toxicity levels. However, the clustering for the 10 g kg^{-1} Cd level aligned with the negative regions of PC1 and PC2, reflecting its detrimental effects on wheat growth. In the PCA plot, various variables are represented by the spread of vectors. Dry weights of shoots (SDW) and root dry weights (RDW) were positively correlated with total chlorophyll concentrations, membrane stability-index (MSI), RWC, catalase (CAT), and peroxidase (POD), but negatively correlated with malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline, polyphenol oxidase (PPO), and Cd translocation factor (Cd-TF). Additionally, soil-Cd was positively associated with shoot-Cd and root-Cd concentrations.

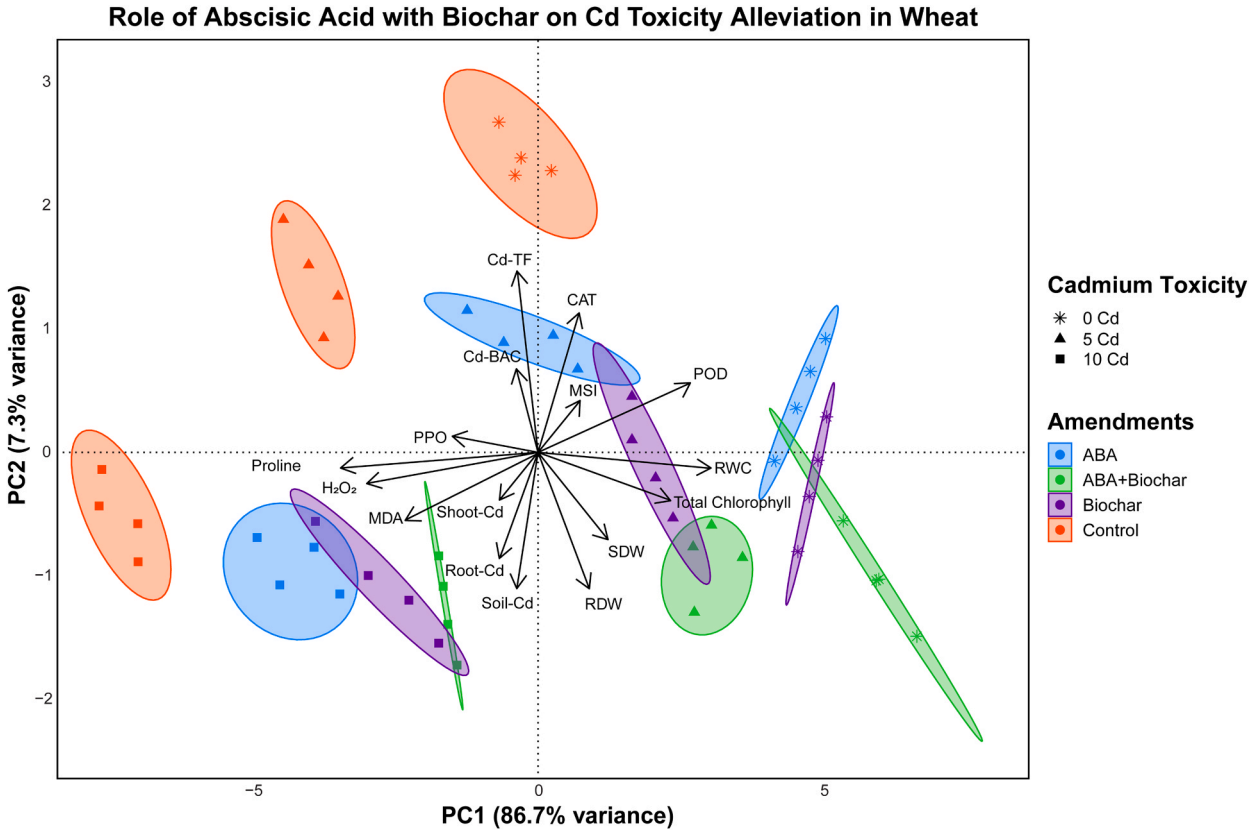

Fig. 6. Biplot of principal component analysis (PCA) displaying scores in the first two components i.e., PC1 and PC2 for the selected parameters related to growth, physiology, ROS generation, and Cd translocation, and its concentration in wheat (Triticum aestivum L.) grown under various Cd toxicity levels (0, 5, and 10 mg kg^{-1} soil). Abbreviations: SDW: shoot dry weight; RDW: root dry weight; RWC: leaf relative water contents; MSI: membrane stability index; MDA: malondialdehyde contents; H2O2: hydrogen peroxide contents; Soil-Cd: Cd concentration in soil; Shoot-Cd: Cd concentration in shoot tissues; Root-Cd: Cd concentration in root tissues; Cd-BAC: Cd biological accumulation coefficient; Cd-TF: Cd translocation factor; PPO: polyphenol oxidase activity; CAT: catalase activity; POD: peroxidase activity.

4. Discussion

This study examined the effects of elevated Cd on wheat growth and physiology under various applications of amendments and control treatment. Specifically, it was demonstrated that 5 and 10 mg kg^{-1} Cd levels decreased length and wheat biomass production. The Cd toxicity to plant includes chlorosis, stunted growth, rotting of roots and low yield even at low level (0.05 μM) (Zaid et al., 2020). Wheat plant growth parameters such as roots and shoots length, biomass, photosynthetic activities and ion homeostasis was severely affected by Cd under 10 μM levels (Jiang et al., 2023). It was found that Cd toxicity had hindered normal plant functioning by disrupting nutrient uptake, initiating oxidative stress and taking part in genotoxicity (Li et al., 2023b; Wang et al., 2020). The wheat crop root morphological traits were drastically impacted by Cd toxicity as highlighted in Table 2. Plants roots are the first organ to encounter the presence of Cd toxicity and Cd buildup in roots decreased carbon availability and increased ROS production (Aslam et al., 2023). Plant roots hair adsorbed Cd^{2+} ions and root volume were found to BE correlated with Cd phytotoxicity and undergone root tip modification to survive Cd toxicity (Kohanová et al., 2018; Li et al., 2023a). Wheat is a staple crop of many countries and Cd toxicity is going to be the main reason to diminish yield and negatively impact human health by Cd bioaccumulation in grains (Zhou et al., 2020). Therefore, it is imperative to improve growth of crops cultivated on Cd contaminated lands and to restrict Cd accumulation in edible parts by adopting suitable measures. Several HMs removal techniques like desorption, immobilization, phyto-extraction along with combination of organic and inorganic treatments have been employed for the minimization of HMs toxicity in plants (Afzal et al., 2024; Jiang et al., 2023; Li et al., 2023b). Addition of biochar to immobilize HMs and to supply essential nutrients for the growth of plants had been acknowledged in many studies (Su et al., 2024). Apart from the use of BC to soil, foliar application of plant growth regulators (abscisic acid) to promote the growth of plants and to sustain abiotic stress especially HMs toxicity was an effective method to increase yield and lower toxic levels of metals to plant edible organs (Haider et al., 2024). Application of biochar to HMs contaminated soils had greatly enhanced plant growth, biochemical traits and nutrient's ability of soils (Das and Ghosh, 2023; Rassaei, 2023). Plant shoot and root morphological characteristics were improved by biochar application (Malik et al., 2023; Wan et al., 2023).

Wheat physiological traits were severely impacted under Cd toxicity, however, BC and ABA and their combination had significantly corrected chlorophyll a and b, carotenoids, SPAD values, RWC and MSI impairments (Fig. 2). The addition of biochar had decreased ROS production and increased foliar chlorophyll, carotenoid and antioxidant capacity of plant under Zn and Cd contamination in soil (Anbuganesan et al., 2024). The utilization of biochar to ameliorate abiotic stresses was facilitated by regulating key physiological functions like transpiration (by increasing soil W.H.C), photosynthesis, stomatal conductance and chlorophyll content by enhancing nutrient pool in soil and reducing the production of ROS by effectively restraining the Cd mobility in soil (Haider et al., 2022; Jiang et al., 2022; Lan et al., 2024). Osmoregulators helped the plant to adapt to abiotic stress by adjusting their osmotic balance and BC was found to increase osmoregulators in maize crops to cope better against soil environmental variations (Cong et al., 2023). Moreover, high nutrients availability with BC in soil had helped wheat plants to survive Cd stress by enhancing its physiological characteristics. Biochar has improved biomass, yield and chlorophyll contents in plants grown under abiotic stresses by increasing nutrient and water uptake and improving soil physio-chemical properties (Kamal et al., 2024). The application of ABA significantly mitigated Cd phyto-toxicity by increasing biomass, photosynthetic pigments and chlorophyll *a* and *b* levels and suppression of Cd toxicity genes (Liao et al., 2023). The ABA had improved mung bean growth by regulating the lipid, photosynthesis and stress response genes under Cd toxicity. It was revealed by transcriptome analysis that ABA promoted the hormone signaling and transport responsible genes and modulated the photosynthetic activity to curb Cd phytotoxicity (Leng et al., 2024). The Cd toxicity was reported to hinder plant transpiration by disturbing stomata opening and closing and chlorophyll contents by promoting enzyme degradation. The phytohormones like ABA promoted plant growth by improving nutrient uptake, cell wall division and biochemical parameters (Dai et al., 2023). Foliar and root zone application of ABA were found to detoxify HMs stress by improving plant biomass and increasing antioxidant activity (Kamran et al., 2021). The ABA role in mitigating Cd toxicity was also observed on lettuce production, where, ABA had increased biomass, physiological and biochemical aspects and reduced Cd uptake in lettuce edible parts (Tang et al., 2023). The exogenous ABA had significantly up-regulated genes responsible for cell wall formation and enhanced the cellulose and hemicellulose production in wheat roots. The thickened cell wall of roots served as a barrier to restrict HMs (Hg) to upper parts and hence indirectly promoted the photosynthesis and physiological functions (Wang et al., 2023). The application of plant growth regulators especially ABA has increased rice growth by mitigating oxidative stress in Cr contaminated soil (Alwutayd et al., 2024). The foliar spray of ABA was reported to increase biomass and N assimilation in *Arabidopsis* through by modulating tricarboxylic acid cycle (Khoshniat et al., 2023). Moreover, ABA had overexpressed the crucial transporter genes like *AIT1*, which decreased the metals uptake (Cd, Zn, Pb and Ni) and promoted the plant physiological characteristics and photosynthesis (Zhu et al., 2024). Cadmium stress increased the production of proline, MDA, and H_2O_2 contents in wheat tissues, indicating that the plant underwent oxidative stress (Fig. 3). Moreover, activities of antioxidant (SOD, CAT, POD, and POX) enzymes in plant tissues significantly improved with BC and ABA utilization, scavenging ROS and improving plant growth (Fig. 4). However, the addition of BC and ABA decreased the production of these oxidative stress indicators. The Cd stress in our experiment had increased the proline contents. The production of primary and secondary metabolites especially proline had important role in osmotic adjustment. The biochar has increased the plant osmo-protectants by providing high water holding capacity (WHC) and nutrient absorption to withstand Cd stress. The increased plant root and shoot biomass with biochar has also validated its role to improve osmo-protectants exposed to Cd pollution (Hussain et al., 2022).

The BC had increased the antioxidant enzymatic activities to cope enhanced ROS production to safeguard plant from membrane damage, nucleic acid rupture and protein synthesis (Koramutla et al., 2021). The toxic level of Cd exposure in plant promoted MDA, O^{-2}, and H_2O_2 production and in response plant initiated defensive mechanisms to cope toxic effect of these ROS by increasing levels of SOD, CAT, POD in shoots (Unsal et al., 2020). The improved antioxidant defense system controlled many important plant functions and

played key role in alleviating HMs stress (Chen et al., 2022). The utilization of amendment especially biochar had maintained plant health through minimizing lipid peroxidation of cell membrane and improving antioxidant capacity of plant. The biochar application has enhanced the antioxidant enzymes production under excess Cd and improved plant vigor by regulating peroxidase activity through CAT and decomposition of superoxide was controlled through SOD by converting them into H_2O_2 (Riaz et al., 2021). The POD production had aided the plant respiration under HMs induced stress and effectively diminished ROS activities (Khan et al., 2022). Similarly, ABA application also took part in producing antioxidant enzymatic activities to reduce Cd phytotoxicity in wheat plants. The ABA application increased the SOD and POD activities in Cd-stressed B. napus cultivar and declined the ROS production to control oxidative stress. The closure of stomata in guard cells under ABA influxes is one of the functions to abate oxidative stress (Liao et al., 2023). The antioxidant enzymatic activities like SOD, APX, CAT and POD were increased with ABA application to control ROS and protect cell membranes in mung bean and *Brassica campestris* L. (Leng et al., 2021; Li et al., 2023a). The supply of ABA was found to increase CAT, SOD and POD activities in strawberry leaves suggested that ABA had protected the leaf membranes from lipid peroxidation (Kocaman, 2023). The upregulation of antioxidant enzyme genes like *MnSOD, CAT, APX and POX* were reported to enhance plant ability to resist abiotic stresses (Hu et al., 2021). The ABA utilization increased fenugreek resistant against HM stress by producing NO which then can combine with ABA and H_2O_2 to participate in activation of mitogen protein kinases and upregulated the antioxidant encoding genes (Parwez et al., 2023). The ABA application to HMs stressed plants often found to have a positive correlation with antioxidant enzymes and protected the important proteins from damage caused by HMs (Sun et al., 2023). This co-application of BC and ABA is a suitable approach to improve growth and quality of crops by significantly decreasing oxidative stress.

The elevated levels of Cd had increased soil DTPA extractable and plant (root and shoot) Cd contents. The biological accumulation factors and translocation factors like BAC, BCF and TF factors were all high under Cd pollution. While, ABA and BC were found to mitigate Cd phytotoxicity by significantly reducing the Cd translocation and bioaccumulation (Fig. 5). The effectiveness of ABA for mitigation of Cd phytotoxicity was acknowledged by many studies where ABA had controlled Cd accumulation and translocation of Cd in many plant species via reducing transpiration rate (Dai et al., 2023). The application of ABA had been reported to affect Cd uptake in plant by regulation of *HMA* gene expression (Chen et al., 2021). The ABA might obstruct HMA2 expression and thus control Cd transport from lower to upper parts of the plant (Liu et al., 2022). The ABA upregulated the expression of the *bZIP* transcription to produce protein for the inhibition of *IRT1* expression and reduced the Cd accumulation (Liu et al., 2024). The exogenous ABA application was found to control Cd uptake, translocation, and accumulation by decreasing hemicellulose production in root cell wall, along with reducing the transcription levels of genes (*IRT1, HMA2, HMA4, ZIP1*) involved in Cd uptake and accumulation (*AIT1 and PDR8*) while, restricting the Cd movement by controlling the transpiration rate (Meng et al., 2022). In another study ABA significantly enhanced tolerance of Cd in *Arabidopsis* plants by upregulated the expression of *ZAT6* to express GSH1, GSH2, PCS1, and PCS2 for metal ion chelation (Liu et al., 2023).

The ABA application to S. *alfredii*, P. *americana* and durum wheat had reduced Cd mobility and uptake by strengthening apoplastic barriers in roots stele. Similar findings were reported by Cheng at al. (2022), where ABA had decreased Cd accumulation and movement via apoplastic pathway by decreasing stomatal conductance and transpiration. The ABA has decreased Cd uptake and its accumulation in roots by the minimization of transpiration rate through modification in stomata size, aperture and density in leaves (Deng et al., 2021). Therefore, low transpiration rate in leaves under ABA influence might be the dominant factors in reducing Cd translocation and bioaccumulation in upper parts of the plants (Tao et al., 2021).

The BC application rates, and quality produced from different feed stocks were reported decrease Cd bioavailability owing to its high pH and availability of macronutrients for promoting plants resistance against the stresses (Algethami et al., 2023). Several factors, like electrostatic attraction, precipitation, ion exchange, complexation and conversion of inorganic forms into organic forms for HMs were reported for HMs adsorption/immobilization with the addition of BC in HMs contaminated soil. Moreover, biochar application as an amendment to contaminated and problematic soils have improved soil fertility and enhanced crop growth (Wang et al., 2023). This amendment can also improve plant root structure, soil hydraulic conductivity and water retention and thus restrict HMs entry through roots by increasing nutrient absorption (Haider et al., 2022). Apart from this function, biochar was also reported to reduce HMs phytotoxicity by passivation methods like coprecipitation and complex formation. The high surface area, abundant functional groups and high CEC associated with biochar has promoted transformation of exchangeable fractions of HMs to fixed or non-bioavailable forms. Wang et al., 2023, demonstrated that surface adsorption, chemical binding with ions on biochar surface, surface complexation and precipitation with phosphorus was mainly few mechanisms responsible for HMs immobilization in soil (Senthilkumar and Prasad, 2020). However, these mechanisms can vary with each metal as Cd and Zn and those mainly immobilized by the surface adsorption while Cu was found to form chemical bonding on biochar surface whereas Pb showed affinity towards complexation with functional groups. Moreover, biochar had increased organic matter bound fraction than acid extracted fraction of HMs in soil and thus reduced availability of these HMs. The high pH and increased total soluble salts might also reduce HMs solubility in soil, therefore, biochar incorporation to HMs contaminated soil effectively reduced their mobility and bioavailability owing elevated EC, CEC, and pH (Awad et al., 2021). Our study showed that ABA and biochar have significantly reduced plant available Cd concentration in soil and effectively controlled the bioaccumulation and translocation factors. Similar findings were also reported in other study where biochar had reduced Cd uptake compared to untreated soil. Biochar had improved growth and yield of wheat and decreased the concentrations of potentially hazard elements in grains grown using industrial wastewater (Irfan et al., 2022). The reduction in Cd translocation and bioaccumulation in cereals and vegetables had significantly reduced health risks in humans (Naeem et al., 2020). Certain mechanisms were reported associated with unitization of biochar to immobilize HMs toxicity in soil. Biochar also releases abundance of cations likely Ca and Mg which might also enhance adsorption of HMs by the process of CEC. The negatively charged surfaces of biochar provide electrostatic forces for positively charged metals (Cd) to adsorb on its surfaces produced by the high pH biochar in soil (Ahmad et al., 2018). The formation of complexes between biochar associated functional groups (OH, COOH) as well as inorganic ions like Si, S

and Cl might combine with HMs to reduce their mobility and solubility in soils (Tan et al., 2017). The HMs precipitation is another process of reduction of bioavailability of HMs in soil likely when inorganic P released from biochar interact with HMs (Cd) (Yu et al., 2024). The enhanced organic carbon contents owing to biochar application in HMs contaminated soils were reported to effectively reduce metals mobility and plant uptake by converting the labile forms into non labile forms. The combination of ABA and BC was proven effective in restoring root growth, nutrient uptake and crop vigor by regulating physiological traits, reducing oxidative stress, mitigating Cd bioaccumulation and translocation in wheat, thereby ensuring cleaner and safer crop production.

5. Conclusion

This study demonstrated that individual and/or co-application of BC and abscisic acid (ABA) improved wheat growth by enhancing biomass, root morphological traits, increase in photosynthetic pigments, and relative water contents, when cultivated under Cd-stress. It was interesting that BC and ABA delivered synergistic and positive effects in alleviating Cd phytotoxicity, at 10 mg kg^{-1} Cd level, compared to the control (unamended soil). Specifically, the co-application of BC and ABA significantly decreased the proline, H_2O_2 and MDA contents and increased CAT, POD, APX and POX activities (37–46 %) to mitigate Cd-mediated oxidative stress. Concomitantly, these amendments decreased the DTPA-extractable soil Cd contents in soil and effectively lowering the Cd contents in roots and shoots. The Cd translocation factors, biological accumulation coefficient and bioconcentration factors were reduced with these amendments. Thus, it can be concluded that the combination of BC and ABA restored wheat growth under Cd stress. Moving forward, field studies with various HMs should be performed to examine the effectiveness of the combination of BC and ABA for the long-term, practical and cleaner cultivation of wheat in these contaminated soils.

CRediT authorship contribution statement

Hamoud Yousef Alhaj: Writing – review & editing, Validation, Software, Resources, Methodology. **Noreen Sana:** Writing – review & editing, Resources, Data curation. **Shaghaleh Hiba:** Writing – review & editing, Visualization, Investigation. **Yong Jean Wan Hong:** Writing – review & editing, Visualization, Validation, Software, Funding acquisition. **Malik Zaffar:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Lin Feng:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Rizwan Muhammad:** Writing – review & editing, Visualization, Software, Project administration, Funding acquisition. **Masood Nasir:** Writing – review & editing, Visualization, Validation.

Declaration of Competing Interest

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

Acknowledgements

This work was supported by the Islamia University of Bahawalpur, Punjab, Pakistan and the Government College University Faisalabad, Pakistan.

Data availability

Data will be made available on request.

References

Abbas, T., Rizwan, M., Ali, S., Zia-Ur-Rehman, M., Qayyum, M.F., Abbas, F., Hannan, F., Rinklebe, J., Ok, Y.S., 2017. Effect of biochar on cadmium bioavailability and uptake in wheat (*Triticum aestivum* L.) grown in a soil with aged contamination. Ecotoxicol. Environ. Saf. 140, 37–47. https://doi.org/10.1016/j.ecoenv.2017.02.028.

Abbott, L.K., Macdonald, L.M., Wong, M.T.F., Webb, M.J., Jenkins, S.N., Farrell, M., 2018. Potential roles of biological amendments for profitable grain production – A review. Agric. Ecosyst. Environ. 256, 34–50. https://doi.org/10.1016/j.agee.2017.12.021.

Afzal, S., Alghanem, S.M., Alsudays, I.M., Malik, Z., Abbasi, G.H., Ali, A., Noreen, S., Ali, M., Irfan, M., Rizwan, M., 2024. Effect of biochar, zeolite and bentonite on physiological and biochemical parameters and lead and zinc uptake by maize (*Zea mays* L.) plants grown in contaminated soil. J. Hazard. Mater. 2, 133927. https://doi.org/10.1016/j.jhazmat.2024.133927.

Ahmad, A., Aslam, Z., Javed, T., Hussain, S., Raza, A., Shabbir, R., Tauseef, M., 2022. Screening of wheat (Triticum aestivum L.) genotypes for drought tolerance through agronomic and physiological response. Agronomy 12 (2), 287.

Ahmad, Z., Gao, B., Mosa, A., Yu, H., Yin, X., Bashir, A., Ghoveisi, H., Wang, S., 2018. Removal of Cu(II), Cd(II) and Pb(II) ions from aqueous solutions by biochars derived from potassium-rich biomass. J. Clean. Prod. 437–449. https://doi.org/10.1016/j.jclepro.2018.01.133.

Akinyele, I.O., Shokunbi, O.S., 2015. Comparative analysis of dry ashing and wet digestion methods for the determination of trace and heavy metals in food samples. Food Chem. 173, 682–684. https://doi.org/10.1016/j.foodchem.2014.10.097.

Algethami, J., Irshad, S., Javed, M.K., Alhamami, W.M.A., Ibrahim, M., 2023. Iron-modified biochar improves plant physiology, soil nutritional status and mitigates Pb and Cd-hazard in wheat (*Triticum aestivum* L.). Front. Plant Sci. 14, 1221434.

Ali, S., Gill, R.A., Ulhassan, Z., Najeeb, U., Kanwar, M.K., Abid, M., Mwamba, T.M., Huang, Q., Zhou, W., 2018. Insights on the responses of Brassica napus cultivars against the cobalt-stress as revealed by carbon assimilation, anatomical changes and secondary metabolites. EEB 156, 183–196. https://doi.org/10.1016/j.envexpbot.2018.07.004.

Ali, Q., Shahid, S., Nazar, N., Hussain, A.I., Ali, S., Chata, S.A.S., Perveen, R., Naseem, J., Haider, M.Z., Hussain, B., Hussain, S.M., 2020. Use of phytohormones in conferring tolerance to environmental stress. In: Hasanuzzaman, M. (Ed.), Plant ecophysiology and adaptation under climate change: mechanisms and perspectives II. Springer Singapore, Singapore, pp. 245–355. https://doi.org/10.1007/978-981-15-2172-0_11.

Alwutayd, K.M., Alghanem, S.M., Alwutayd, R., Alghamdi, S.A., Alabdallah, N.M., Al-Qthanin, R.N., Sarfraz, W., Khalid, N., Naeem, N., Ali, B., Saleem, M.H., 2024. Mitigating chromium toxicity in rice (*Oryza sativa* L.) via ABA and 6-BAP: Unveiling synergistic benefits on morphophysiological traits and ASA-GSH cycle. Sci. Total Environ. 908, 168208. https://doi.org/10.1016/j.scitotenv.2023.168208.

Anbuganesan, V., Vishnupradeep, R., Mehnaz, N., Kumar, A., Freitas, H., Rajkumar, M., 2024. Synergistic effect of biochar and plant growth promoting bacteria improve the growth and phytostabilization potential of Sorghum bicolor in Cd and Zn contaminated soils. Rhizosphere 29, 100844. https://doi.org/10.1016/j.rhisph.2023.100844.

Asghar, M.A., Balogh, E., Ahres, M., Szalai, G., Gondor, O.K., Darkó, É ., Kocsy, G., 2023. Ascorbate and hydrogen peroxide modify metabolite profile of wheat differently. J. Plant Growth Regul. 42 (10), 6155–6170. https://doi.org/10.1007/s00344-022-10793-0.

Aslam, M.M., Okal, E.J., Waseem, M., 2023. Cadmium toxicity impacts plant growth and plant remediation strategies. J. Plant Growth Regul. 99 (3), 397–412. https://doi.org/10.1007/s10725-022-00917-7.

Awad, M., Liu, Z., Skalicky, M., Dessoky, E.S., Brestic, M., Mbarki, S., El Sabagh, A., 2021. Fractionation of heavy metals in multi-contaminated soil treated with biochar using the sequential extraction procedure. Biomol 11 (3), 448. https://doi.org/10.3390/biom11030448.

Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analyses of soils. J. Agron, 54 (5), 464–465. https://doi.org/10.2134/agronj1962.00021962005400050028x.

Chen, H., Zhang, Q., Lv, W., Yu, X., Zhang, Z., 2022. Ethylene positively regulates Cd tolerance via reactive oxygen species scavenging and apoplastic transport barrier formation in rice. Environ. Pollut. 119063. https://doi.org/10.1016/j.envpol.2022.119063.

Chen, R., Cheng, N., Ding, G., Ren, F., Lv, J., Shi, R., 2021. Predictive model for cadmium uptake by maize and rice grains on the basis of bioconcentration factor and the diffusive gradients in thin-films technique. Environ. Pollut. 289, 117841. https://doi.org/10.1016/j.envpol.2021.117841.

Chen, K., Li, G.J., Bressan, R.A., Song, C.P., Zhu, J.K., Zhao, Y., 2020. Abscisic acid dynamics, signaling, and functions in plants. J. Integr. Plant Biol. 62, 25–54. https://doi.org/10.1111/jipb.12899.

Chen, S., Li, X., Yang, C., Yan, W., Liu, C., Tang, X., Gao, C., 2021. Genome-wide Identification and characterization of FCS-like zinc finger (FLZ) family genes in maize (*Zea mays*) and functional fnalysis of ZmFLZ25 in plant abscisic acid response. Int. J. Mol. Sci. 22, 3529.

Clemens, S., Ma, J.F., 2016. Toxic heavy metal and metalloid accumulation in crop plants and foods. Annu. Rev. Plant Biol. 67, 489–512. https://doi.org/10.1146/annurev-arplant-043015-112301.

Cong, M., Hu, Y., Sun, X., Yan, H., Yu, G., Tang, G., Chen, S., Xu, W., Jia, H., 2023. Long-term effects of biochar application on the growth and physiological characteristics of maize. Front. Plant Sci. 14, 1172425. https://doi.org/10.3389/fpls.2023.1172425.

Dai, Z.H., Guan, D.X., Bundschuh, J., Ma, L.Q., 2023. Roles of phytohormones in mitigating abiotic stress in plants induced by metal (loid) s As, Cd, Cr, Hg, and Pb. Crit. Rev. Environ. Sci. Technol. 1310–1330. https://doi.org/10.1080/10643389.2022.2134694.

Dami, I., Zhang, Y., 2023. Variations of freezing tolerance and sugar concentrations of grape buds in response to foliar application of abscisic acid. Front. Plant Sci. 14, 1084590. https://doi.org/10.3389/fpls.2023.1084590.

Danso, O.P., Acheampong, A., Zhang, Z., Song, J., Wang, Z., Dai, J., Zhu, R., 2023. The management of Cd in rice with biochar and selenium: Effects, efficiency, and practices. Carbon Res 2 (1), 41. https://doi.org/10.1007/s44246-023-00073-1.

de Bang, Husted, S., Laursen, K.H., Persson, D.P., Schjoerring, J.K., 2021. The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. New Phytol. 229, 2446–2469. https://doi.org/10.1111/nph.17074.

Deng, B., Zhang, W., Yang, H., 2021. Abscisic Acid Decreases Cell Death in Malus hupehensis Rehd. Under Cd Stress by Reducing Root Cd^{2+} Influx and Leaf Transpiration. J. Plant Growth Regul. 639–646. https://doi.org/10.1007/s00344-021-10327-0.

Esterbauer, H., Schwarzl, E., Hayn, M., 1977. A rapid assay for catechol oxidase and laccase using 2-nitro-5-thiobenzoic acid. Anal. Biochem. 486–494. https://doi.org/10.1016/0003-2697(77)90262-7.

Fan, P., Wu, L., Wang, Q., Wang, Y., Luo, H., Song, J., Chen, S., 2023. Physiological and molecular mechanisms of medicinal plants in response to cadmium stress: Current status and future perspective. J. Hazard. Mater. 450, 131008. https://doi.org/10.1016/j.jhazmat.2023.131008.

Ghuge, S.A., Nikalje, G.C., Kadam, U.S., Suprasanna, P., Hong, J.C., 2023. Comprehensive mechanisms of heavy metal toxicity in plants, detoxification, and remediation. J. Hazard. Mater. 450, 131039. https://doi.org/10.1016/j.jhazmat.2023.131039.

Goncharuk, E.A., Zagoskina, N.V., 2023. Heavy metals, their phytotoxicity, and the role of phenolic antioxidants in plant stress responses with focus on cadmium. Mol 28 (9), 3921. https://doi.org/10.3390/molecules28093921.

Gong, H., Zhao, L., Rui, X., Hu, J., Zhu, N., 2022. A review of pristine and modified biochar immobilizing typical heavy metals in soil: applications and challenges. J. Hazard. Mater. 432, 128668. https://doi.org/10.1016/j.jhazmat.2022.128668.

Gorin, N., Heidema, F.T., 1976. Peroxidase activity in Golden Delicious apples as a possible parameter of ripening and senescence. J. Agric. Food Chem. 200-1.

Grewal, K.S., Buchan, G.D., Tonkin, P.J., 1990. Estimation of field capacity and wilting point of some New Zealand soils from their saturation percentages. N. Z. J. Crop. Hortic. Sci. 18 (4), 241–246. https://doi.org/10.1080/01140671.1990.10428101.

Grote, U., Fasse, A., Nguyen, T.T., Erenstein, O., 2021. Food security and the dynamics of wheat and maize value chains in Africa and Asia. Front. Sustain. Food Syst. 4 (4), 1–17. https://doi.org/10.3389/fsufs.2020.617009.

Haider, F.U., Liqun, C., Coulter, J.A., Cheema, S.A., Wu, J., Zhang, R., Wenjun, M., Farooq, M., 2021. Cadmium toxicity in plants: Impacts and remediation strategies. Ecotoxicol. Environ. Saf. 211, 111887. https://doi.org/10.1016/j.ecoenv.2020.111887.

Haider, F.U., Virk, A.L., Rehmani, M.I.A., Skalicky, M., Ata-ul-Karim, S.T., Ahmad, N., Soufan, W., Brestic, M., Sabagh, A.E.L., Liqun, C., 2022. Integrated Application of Thiourea and Biochar Improves Maize Growth, Antioxidant Activity and Reduces Cadmium Bioavailability in Cadmium-Contaminated Soil. Front. Plant. Sci.. 12, 809322. https://doi.org/10.3389/fpls.2021.809322.

Haider, F.U., Khan, I., Farooq, M., Cai, L., Li, Y., 2024. Co-application of biochar and plant growth regulators improves maize growth and decreases Cd accumulation in cadmium-contaminated soil. J. Clean. Prod. 440, 140515. https://doi.org/10.1016/j.jclepro.2023.140515.

Hu, B., Deng, F., Chen, G., Chen, X., Gao, W., Long, L., Xia, J., Chen, Z.H., 2020. Evolution of Abscisic Acid Signaling for Stress Responses to Toxic Metals and Metalloids. Front. Plant Sci. 11, 909. https://doi.org/10.3389/fpls.2020.00909.

Hu, W., Zhang, J., Yan, K., Zhou, Z., Zhao, W., Zhang, X., Pu, Y., Yu, R., 2021. Beneficial effects of abscisic acid and melatonin in overcoming drought stress in cotton (*Gossypium hirsutum* L. Physiol. Plant. 173 (4), 2041–2054.

Hussain, S., Irfan, M., Sattar, A., Hussain, S., Ullah, S., Abbas, T., Yang, J., 2022. Alleviation of cadmium stress in wheat through the combined application of boron and biochar via regulating morpho-physiological and antioxidant defense mechanisms. Agron 12 (2), 434. https://doi.org/10.3390/agronomy12020434.

Irfan, M., Dawar, K., Fahad, S., Mehmood, I., Alamri, S., Siddiqui, M.H., Nasim, W., 2022. Exploring the potential effect of *Achnatherum splendens* L.–derived biochar treated with phosphoric acid on bioavailability of cadmium and wheat growth in contaminated soil. ESPR 37676–37684. https://doi.org/10.1007/s11356-021-17950-0.

Jamalian, S., Gholami, M., Esna-Ashari, M., 2013. Abscisic acid-mediated leaf phenolic compounds, plant growth and yield is strawberry under different salt stress regimes. Theor. Exp. Plant Physiol. 25, 291–299. ⟨https://www.scielo.br/j/txpp/a/QCZPKP6P3xRnpnPwv7k8bFd/?lang=en⟩.

Jiang, W., Xu, L., Liu, Y., Su, W., Yan, J., Xu, D., 2022. Effect of biochar on the growth, photosynthesis, antioxidant system and cadmium content of Mentha piperita 'Chocolate'and Mentha spicata in cadmium-contaminated soil. Agron 12 (11), 2737. https://doi.org/10.3390/agronomy12112737.

Jiang, Y., Wei, C., Jiao, Q., Li, G., Alyemeni, M.N., Ahmad, P., Shah, T., Fahad, S., Zhang, J., Zhao, Y., Liu, F., 2023. Interactive effect of silicon and zinc on cadmium toxicity alleviation in wheat plants. J. Hazard. Mater. 458, 131933. https://doi.org/10.1016/j.jhazmat.2023.131933.

Kamal, M.Z., Sarker, U., Roy, S.K., Alam, M.S., Azam, M.G., Miah, M.Y., Hossain, N., Ercisli, S., Alamri, S., 2024. Manure-biochar compost mitigates the soil salinity stress in tomato plants by modulating the osmoregulatory mechanism, photosynthetic pigments, and ionic homeostasis. Sci. Rep. 14 (1), 21929. https://doi.org/10.1038/s41598-024-73093-5.

Kamran, M., Malik, Z., Parveen, A., Zong, Y., Abbasi, G.H., Rafiq, M.T., Shaaban, M., Mustafa, A., Bashir, S., Rafay, M., Mehmood, S., 2019. Biochar alleviates Cd phytotoxicity by minimizing bioavailability and oxidative stress in pak choi (*Brassica chinensis* L.) cultivated in Cd-polluted soil. J. Environ. Manag. 250, 109500. https://doi.org/10.1016/j.jenvman.2019.109500.

Kamran, M., Danish, M., Saleem, M.H., Malik, Z., Parveen, A., Abbasi, G.H., Jamil, M., Ali, S., Afzal, S., Riaz, M., Rizwan, M., 2021. Application of abscisic acid and 6-benzylaminopurine modulated morpho-physiological and antioxidative defense responses of tomato (*Solanum lycopersicum* L.) by minimizing cobalt uptake. Chemosphere 263, 128169. https://doi.org/10.1016/j.chemosphere.2020.128169.

Kandel, B.P., 2020. Spad value varies with age and leaf of maize plant and its relationship with grain yield. BMC Res. Notes 13, 1–4. https://doi.org/10.1186/s13104-020-05324-7.

Khaliq, M.A., Alsudays, I.M., Alhaithloul, H.A.S., Rizwan, M., Yong, J.W.H., Rahman, S.U., Sagir, M., Bashir, S., Ali, H., Zuo, H., 2024. Biochar impacts on carbon dioxide, Methane emission, and cadmium accumulation in rice from Cd-contaminated soils: a meta-analysis.Ecotoxicology and. Environ. Saf. 274, 116204. https://doi.org/10.1016/j.ecoenv.2024.116204.

Khan, Z., Fan, X., Khan, M.N., Khan, M.A., Zhang, K., Fu, Y., Shen, H., 2022. The toxicity of heavy metals and plant signaling facilitated by biochar application: Implications for stress mitigation and crop production. Chemosphere 308, 136466. https://doi.org/10.1016/j.chemosphere.2022.136466.

Khoshniat, P., Rafudeen, M.S., Seifi, A., 2023. ABA spray on *Arabidopsis* seedlings increases mature plants vigor under optimal and water-deficit conditions partly by enhancing nitrogen assimilation. Physiol. Plant. 175 (4), e13979. https://doi.org/10.1111/ppl.13979.

Kocaman, A., 2023. Effects of foliar application of abscisic acid on antioxidant content, phytohormones in strawberry shoots, and translocation of various heavy metals. Sci. Hortic. 314, 111943. https://doi.org/10.1016/j.scienta.2023.111943.

Kohanová, J., Martinka, M., Vaculík, M., White, P.J., Hauser, M.T., Lux, A., 2018. Root hair abundance impacts cadmium accumulation in *Arabidopsis thaliana* shoots. Ann. Bot. 122 (5), 903–914. https://doi.org/10.1093/aob/mcx220.

Koramutla, M.K., Negi, M., Ayele, B.T., 2021. Roles of glutathione in mediating abscisic acid signaling and its regulation of seed dormancy and drought tolerance. Genes 12, 1620. https://doi.org/10.3390/genes12101620.

Lan, Y., Burca, G., Yong, J.W.H., Johansson, E., Kuktaite, R., 2024. New insights into the bio-chemical changes in wheat induced by Cd and drought: what can we learn on Cd stress using neutron imaging? Plants 13 (554), 13040554. https://doi.org/10.3390/plants13040554.

Leng, Y., Li, Y., Ma, Y.H., He, L.F., Li, S.W., 2021. Abscisic acid modulates differential physiological and biochemical responses of roots, stems, and leaves in mung bean seedlings to cadmium stress. ESPR 28 (5), 6030–6043. https://doi.org/10.1007/s11356-020-10843-8.

Leng, Y., Wu, P.M., Li, S.W., Zhang, X.J., 2024. Foliar application of abscisic acid alleviates cadmium stress by modulating differential physiological and transcriptome response in leaves, stems, and roots of mung bean seedlings. J. Plant Growth Regul. 4904–4922. https://doi.org/10.1007/s00344-024-11443-3.

Li, Y., Rahman, S.U., Qiu, Z., Shahzad, S.M., Nawaz, M.F., Huang, J., Naveed, S., Li, L., Wang, X., Cheng, H., 2023b. Toxic effects of cadmium on the physiological and biochemical attributes of plants, and phytoremediation strategies: A review. Environ. Pollut., 121433 https://doi.org/10.1016/j.envpol.2023.121433.

Li, S., Wang, S., Shi, J., Tian, X., Wu, J., 2022. Economic, energy and environmental performance assessment on wheat production under water-saving cultivation strategies. Energy 261, 125330. https://doi.org/10.1016/j.energy.2022.125330.

Li, M., Heng, Q., Wang, Z., Jiang, Y., Wang, X., He, X., Yong, J.W.H., Dawoud, T.M., Rahman, S.U., Fan, J., Zhang, Y., 2024. Phytoremediation efficiency of poplar hybrid varieties with diverse genetic backgrounds in soil contaminated by multiple toxic metals (Cd, Hg, Pb, and As). Sci. Total Environ. 283, 116843. https://doi.org/10.1016/j.ecoenv.2024.116843.

Li, G., Yan, L., Chen, X., Lam, S.S., Rinklebe, J., Yu, Q., Sonne, C., 2023a. Phytoremediation of cadmium from soil, air and water. Chemosphere, 138058. https://doi.org/10.1016/j.chemosphere.

Liao, Y., Tang, Y., Wang, S., Su, H., Chen, J., Zhang, D., Wu, J., Zhou, D., Yan, M., Liu, L., 2023. Abscisic acid modulates differential physiological and biochemical responses to cadmium stress in *Brassica napus*. Environ. Pollut. Bioavailab. 35 (1), 2168216. https://doi.org/10.1080/26395940.2023.2168216.

Liu, C., Chang, J., Yang, J., Li, H., Wu, J., Wu, J., Xia, Z., 2024. Overexpression of NtDOGL4 improves cadmium tolerance through abscisic acid signaling pathway in tobacco. J. Hazard. Mater. 465, 133462. https://doi.org/10.1016/j.jhazmat.2024.133462.

Liu, L., Zhang, Q., Hu, L., Tang, J., Xu, L., Yang, X., Yong, J.W.H., Chen, X., 2012. Legumes can increase cadmium contamination in neighboring crops. PLOS ONE 7, e42944. https://doi.org/10.1371/journal.pone.0042944.

Liu, H., Zhu, Q., Pei, X., Xing, G., Ou, X., Li, H., 2020. Comparative analysis of the photosynthetic physiology and transcriptome of a high-yielding wheat variety and its parents. Crop J. 8 (6), 1037–1048. https://doi.org/10.1016/j.cj.2020.01.004.

Liu, J., Yang, L., Wen, Y., Li, Y., Shabala, S., Zhang, J., Song, W.Y., 2023. The roles of abscisic acid and ethylene in cadmium accumulation and tolerance in plants. Plant Soil 1–12. https://doi.org/10.1007/s11104-023-05886-y.

Liu, S., Li, L., Deng, Y., Bai, Y., Sun, C., Huang, S., Zhou, J., Shi, L., Yang, X., Li, L., et al., 2022. BrpNAC895 and BrpABI449 coregulate the transcription of the efflux-type Cd transporter BrpHMA2 in *Brassica parachinensis*. Hortic. Res., uhac044 https://doi.org/10.1093/hr/uhac044.

Malik, Z., Malik, N., Noor, I., Kamran, M., Parveen, A., Ali, M., Sabir, F., Elansary, H.O., El-Abedin, T.K., Mahmoud, E.A., Fahad, S., 2023. Combined effect of rice-straw biochar and humic acid on growth, antioxidative capacity, and ion uptake in maize (*Zea mays* L.) grown under saline soil conditions. J. Plant Growth Regul. 42 (5), 3211–3228. https://doi.org/10.1007/s00344-022-10786-z.

Meng, Y., Huang, J., Jing, H., et al., 2022. Exogenous abscisic acid alleviates Cd toxicity in Arabidopsis thaliana by inhibiting Cd uptake, translocation and accumulation, and promoting Cd chelation and efflux. Plant Sci. 325, 111464. https://doi.org/10.1016/j.plantsci.2022.111464.

Naeem, M.A., Shabbir, A., Amjad, M., Abbas, G., Imran, M., Murtaza, B., Tahir, M., Ahmad, A., 2020. Acid treated biochar enhances cadmium tolerance by restricting its uptake and improving physio-chemical attributes in quinoa (*Chenopodium quinoa* Willd.). Ecotoxicol. Environ. Saf. 191, 110218. https://doi.org/10.1016/j.ecoenv.2020.110218.

Nafees, M., Rizwan, M., Ahmad, S., Ali, S., Obaid, W.A., Alsubeie, M.S., Abeed, A.H., 2023. Combined effect of Zinc lysine and biochar on growth and physiology of wheat (*Triticum aestivum* L.) to alleviate salinity stress. Front. Plant Sci. 13, 1017282. https://doi.org/10.3389/fpls.2022.1017282.

Nasirzadeh, L., Sorkhilaleloo, B., Majidi Hervan, E., Fatehi, F., 2021. Changes in antioxidant enzyme activities and gene expression profiles under drought stress in tolerant, intermediate, and susceptible wheat genotypes. Cereal Res. Commun. 49, 83–89. https://doi.org/10.1007/s42976-020-00085-2.

Parwez, R., Aftab, T., Khan, M.M., Naeem, M., 2023. Exogenous abscisic acid fine-tunes heavy metal accumulation and plant's antioxidant defence mechanism to optimize crop performance and secondary metabolite production in Trigonella foenum-graecum L. under nickel stress. Plant Sci. 332, 111703. https://doi.org/10.1016/j.plantsci.2023.111703.

Rahman, K.U., Hussain, A., Ejaz, N., Shang, S., Balkhair, K.S., Khan, K.U.J., Rehman, N.U., 2023. Analysis of production and economic losses of cash crops under variable drought: A case study from Punjab province of Pakistan. IJDRR 85, 103507. https://doi.org/10.1016/j.ijdrr.2022.103507.

Rassaei, F., 2023. Biochar effects on rice paddy cadmium contaminated calcareous clay soil: a study on adsorption kinetics and cadmium uptake. PAWE 389–400. https://doi.org/10.1007/s10333-023-00937-7.

Riaz, M., Kamran, M., Rizwan, M., Ali, S., Parveen, A., Malik, Z., Wang, X., 2021. Cadmium uptake and translocation: synergetic roles of selenium and silicon in Cd detoxification for the production of low Cd crops: a critical review. Chemosphere 273, 129690. https://doi.org/10.1016/j.chemosphere.2021.129690.

Riseh, R.S., Vazvani, M.G., Hajabdollahi, N., Thakur, V.K., 2023. Bioremediation of heavy metals by Rhizobacteria. Appl. Biochem. Biotechnol. 195 (8), 4689–4711. https://doi.org/10.1007/s12010-022-04177-z.

Rizwan, M., Ali, S., Abbas, T., Zia-ur-Rehman, M., Hannan, F., Keller, C., Ok, Y.S., 2016. Cadmium minimization in wheat: a critical review. Ecotoxicol. Environ. Saf. 130 43–53. https://doi.org/10.1016/j.ecoenv.2016.04.001.

Saberi, R.R., Dashti, H., Gholizadeh Vazvani, M., Dini, A., 2021. Changes in the activity of enzymes phenylalanine ammonia-lyase, polyphenol oxidase, and peroxidase in some wheat genotypes against take-all disease. JAST 23 (4), 929–942. ⟨http://jast.modares.ac.ir/article-23-43587-en.html⟩.

Jones, Jr.J.B., Case, V.W., 1990. In soil testing and plant analysis, SSSA Special Publ. Series No. 2 49 – 58. Madison, Wis.: Soil Sci. Soc. Amer. Part Two. 3:389-427. https://doi.org/10.2136/sssabookser3.3ed.c15.

Senthilkumar, R., Prasad, D.M.R., 2020. Sorption of heavy metals onto biochar. In: applications of biochar for environmental safety. IntechOpen, Rijeka, Croatia.doi. https://doi.org/10.5772/intechopen.92346.

Sani, M.N.H., Amin, M., Siddique, A.B., Nasif, S.O., Ghaley, B.B., Ge, L., Wang, F., Yong, J.W.H., 2023. Waste-derived nanobiochar: a new avenue towards sustainable agriculture, environment, and circular bioeconomy. Sci. Total Environ. 905, 166881. https://doi.org/10.1016/j.scitotenv.2023.166881.
Sani, M.N.H., Yong, J.W.H., 2022. Harnessing synergistic biostimulatory processes: aplausible approach for enhanced crop growth and resilience in organic farming. Biology 11, 41. https://doi.org/10.3390/biology11010041.
Shaghaleh, H., Azhar, M., Zia-ur-Rehman, M., Hamoud, Y.A., Hamad, A.A.A., Usman, M., Rizwan, M., Yong, J.W.H., Alharby, H.F., Al-Ghamdi, A.J., Alharbi, B.M., 2024. Effects of agro based organic amendments on growth and cadmium uptake in wheat and rice crops irrigated with raw city effluents: Three years field study. Environ. Pollut. 344, 123365. https://doi.org/10.1016/j.envpol.2024.123365.
Shiferaw, B., Smale, M., Braun, H-J., Duveiller, E., Reynolds, M., Muricho, G., 2013. Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Secur. 5, 291–317. https://doi.org/10.1007/s12571-013-0263-y.
Singh, S., Luthra, N., Mandal, S., Kushwaha, D.P., Pathak, S.O., Datta, D., Sharma, R., Pramanick, B., 2023. Distinct behavior of biochar modulating biogeochemistry of salt-affected and acidic soil: a review. J. Soil Sci. Plant Nutr. 2981–2997. https://doi.org/10.1007/s42729-023-01370-9.
Song, J., Finnegan, P.M., Liu, W., Xiang, L., Yong, J.W.H., Xu, J., Zhang, Q., Wen, Y., Qin, K., Guo, J., Li, T., Zhao, C., Zhang, Y., 2019. Mechanisms underlying enhanced Cd translocation and tolerance in roots of *Populus euramericana* in response to nitrogen fertilization. Plant Sci. 287, 110206. https://doi.org/10.1016/j.plantsci.2019.110206.
Su, J.Z., Zhang, M.Y., Xu, W.H., Xu, W.M., Liu, C., Rui, S., Tuo, Y.F., He, X.H., Xiang, P., 2024. Preparation and applications of iron/biochar composites in remediation of heavy metal contaminated soils: Current status and further perspectives. Environ. Technol. Innov., 103671 https://doi.org/10.1016/j.eti.2024.103671.
Sun, X., Wang, S., Tian, J., Xiang, X., Zheng, H., Liu, H., Fang, Z., Tian, Z., Liu, L., Zhu, Y., Du, S., 2023. Synergistic interplay between ABA-generating bacteria and biochar in the reduction of heavy metal accumulation in radish, pakchoi, and tomato. Environ. Pollut. 333, 122084. https://doi.org/10.1016/j.envpol.2023.122084.
Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E., Mittler, R., 2014. Abiotic and biotic stress combinations. New Phytol 203, 32–43. https://doi.org/10.1111/nph.17074.
Tan, Z., Wang, Y., Zhang, L., Huang, Q., 2017. Study of the mechanism of remediation of Cd-contaminated soil by novel biochars. Environ. Sci. Pollut. Control. 24844–24855. https://doi.org/10.1007/s11356-017-0109-9.
Tang, W., Liang, L., Xie, Y., Li, X., Lin, L., Huang, Z., Sun, B., Sun, G., Tu, L., Li, H., Tang, Y., 2023. Foliar application of salicylic acid inhibits the cadmium uptake and accumulation in lettuce (*Lactuca sativa* L.). Front. Plant Sci. 14, 1200106. https://doi.org/10.3389/fpls.2023.1200106.
Tao, Q., Jupa, R., Dong, Q., et al., 2021. Abscisic acid-mediated modifications in water transport continuum are involved in cadmium hyperaccumulation in *Sedum alfredii*. Chemosphere 268, 129339. https://doi.org/10.1016/j.chemosphere.2020.129339.
Tyagi, S., Singh, K., Upadhyay, S.K., 2021. Molecular characterization revealed the role of catalases under abiotic and arsenic stress in bread wheat (*Triticum aestivum* L.). J. Hazard. Mater. 403, 123585. https://doi.org/10.1016/j.jhazmat.2020.123585.
Unsal, V., Dalkıran, T., Çiçek, M., Kölükçü, E., 2020. The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: a review. Adv. Pharm. Bull. 10 (2), 184. https://doi.org/10.34172/apb.2020.023.
Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants protective role of endogenous polyamines. Plant Sci. 151, 59–66. https://doi.org/10.1016/S0168-9452(99)00197-1.
Wan, H., Liu, X., Shi, Q., Chen, Y., Jiang, M., Zhang, J., Cui, B., Hou, J., Wei, Z., Hossain, M.A., Liu, F., 2023. Biochar amendment alters root morphology of maize plant: Its implications in enhancing nutrient uptake and shoot growth under reduced irrigation regimes. Front. Plant Sci. 14, 1122742. https://doi.org/10.3389/fpls.2023.1122742.
Wang, F., Zhang, X., Zhang, S., Zhang, S., Adams, C.A., Sun, Y., 2020. Effects of co-contamination of microplastics and Cd on plant growth and Cd accumulation. Toxics 8 (2), 36. https://doi.org/10.3390/toxics8020036.
Wang, J., Gao, J., Zheng, L., Fu, Y., Ji, L., Wang, C., Kang, G., 2023. Abscisic acid alleviates mercury toxicity in wheat (*Triticum aestivum* L.) by promoting cell wall formation. J. Hazard. Mater. 449, 130947. https://doi.org/10.1016/j.jhazmat.2023.130947.
Yang, T., Xu, Y., Sun, G., Huang, Q., Sun, Y., Liang, X., Wang, L., 2023. Application of ferromanganese functionalized biochar simultaneously reduces Cd and Pb uptake of wheat in contaminated alkaline soils. Ecotoxicol. Environ. Saf. 257, 114930. https://doi.org/10.1016/j.ecoenv.2023.114930.
Ye, T.T., Shi, H.T., Wang, Y.P., Chan, Z.L., 2015. Contrasting changes caused by drought and submergence stresses in bermuda grass (*Cynodon dactylon*). Front. Plant Sci. https://doi.org/10.3389/fpls.2015.00951.
Yu, X., Yang, L., Fan, C., Hu, J., Zheng, Y., Wang, Z., Liu, Y., Xiao, X., Yang, L., Lei, T., Jiang, M., 2023. Abscisic acid (ABA) alleviates cadmium toxicity by enhancing the adsorption of cadmium to root cell walls and inducing antioxidant defense system of Cosmos bipinnatus. Ecotoxicol. Environ. Saf. 261, 115101. https://doi.org/10.1016/j.ecoenv.2023.115101.
Yong, J.W.H., Letham, D.S., Wong, S.C., Farquhar, G.D., 2010. Effects of root restriction on growth and associated cytokinin levels in cotton. Funct. Plant Biol. 37, 974–984. https://doi.org/10.1071/FP10009.
Yong, J.W.H., Letham, D.S., Wong, S.C., Farquhar, G.D., 2014. *Rhizobium*-induced elevation in xylem cytokinin delivery in pigeonpea induces changes in shoot development and leaf physiology. Funct. Plant Biol. 41, 1323–1335. https://doi.org/10.1071/FP14066.
Yu, X., Wang, X., Sun, M., Liu, H., Liu, D., Dai, J., 2024. Cadmium immobilization in soil using phosphate modified biochar derived from wheat straw. Sci. Total Environ. 926, 171614. https://doi.org/10.1016/j.scitotenv.2024.171614.
Zaid, A., Mohammad, F., Fariduddin, Q., 2020. Plant growth regulators improve growth, photosynthesis, mineral nutrient and antioxidant system under cadmium stress in menthol mint (*Mentha arvensis* L.). Physiol. Mol. Biol. Plants 26 (1), 25–39. https://doi.org/10.1007/s12298-019-00715-y.
Zhang, D.Y., Luo, H.S., Cheng, T., Li, W.F., Zhou, X.G., Gu, C.Y., Diao, Z., 2023. Enhancing wheat *Fusarium* head blight detection using rotation Yolo wheat detection network and simple spatial attention network. Comput. Electron Agr. 211, 107968. https://doi.org/10.1016/j.compag.2023.107968.
Zhao, D., Gao, S., Zhang, X., Zhang, Z., Zheng, H., Rong, K., Khan, S.A., 2021. Impact of saline stress on the uptake of various macro and micronutrients and their associations with plant biomass and root traits in wheat. PSE 67 (2). https://doi.org/10.17221/467/2020-PSE.
Zhao, Y., Wang, J., Huang, W., Zhang, D., Wu, J., Li, B., Yan, M., 2023. Abscisic-acid-regulated responses to alleviate cadmium toxicity in plants. Plants 12 (5), 1023. https://doi.org/10.3390/plants12051023.
Zhong, M., Li, W., Jiang, M., Wang, J., Shi, X., Song, J., Zhang, W., Wang, H., Cui, J., 2023. Improving the ability of straw biochar to remediate Cd contaminated soil: KOH enhanced the modification of K_3PO_4 and urea on biochar. Ecotoxicol. Environ. Saf. 262, 115317. https://doi.org/10.1016/j.ecoenv.2023.115317.
Zhou, J., Zhang, C., Du, B., Cui, H., Fan, X., Zhou, D., Zhou, J., 2020. Effects of zinc application on cadmium (Cd) accumulation and plant growth through modulation of the antioxidant system and translocation of Cd in low-and high-Cd wheat cultivars. Environ. Pollut. 265, 115045. https://doi.org/10.1016/j.envpol.2020.115045.
Zhu, Y., You, Y., Zheng, S., Li, J., Wang, Y., Wu, R., Fang, Z., Liu, H., Du, S., 2024. ABA-importing transporter (AIT1) synergies enhances exogenous ABA minimize heavy metals accumulations in *Arabidopsis*. J. Hazard. Mater., 134718 https://doi.org/10.1016/j.jhazmat.2024.134718.
Zong, Y., Liu, Y., Li, Y., Ma, R., Malik, Z., Xiao, Q., Li, Z., Zhang, J., Shan, S., 2023. Changes in salinity, aggregates and physico-mechanical quality induced by biochar application to coastal soil. Arch. Agron. Soil Sci. 69 (13), 2721–2738. https://doi.org/10.1080/03650340.2023.2172167.
Zulfiqar, U., Haider, F.U., Maqsood, M.F., Mohy-Ud-Din, W., Shabaan, M., Ahmad, M., Shahzad, B., 2023. Recent advances in microbial-assisted remediation of cadmium-contaminated soil. Plants 12 (17), 3147. https://doi.org/10.3390/plants12173147.