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Biological insights into alleviating heavy metal toxicity through the simultaneous supply of biochar biofilters derived from the *Mikania micrantha* and molybdenum nanoparticles

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Abstract

Cadmium (Cd) contamination in the environment is widespread, making it crucial to reduce Cd accumulation in cereal crops like wheat. However, strategies that not only mitigate Cd pollution but also address other environmental challenges, such as invasive species management, remain unclear. This study introduces an innovative approach combining molybdenum nanoparticles (Mo NPs, 1 μ M) and biochar biofilters derived from the invasive plant *Mikania micrantha* (IPMM), targeting the biochemical and molecular responses of wheat under Cd stress (100 μ M). Our findings showed that this novel combination significantly improved wheat physiological characteristics, growth, root architecture, elemental profile, osmoregulation, carotenoid, chlorophyll, gas exchange, and photosynthetic efficiency. Remarkably, simultaneous supply of IPMM biochar biofilters and Mo NPs substantially modulated the Cd translocation, reducing its accumulation in root (30.54%) and shoot (53.59%). Additionally, this strategy not only preserved mesophyll cell structures and the membrane integrity, but also strengthened and activated the oxidative defense systems through the regulation of genetic expressions. This synergistic approach advances the Cd alleviating techniques and offers a sustainable solution for utilizing invasive plants as a potential resource. By addressing both heavy metal pollution and ecological challenges, it provides a promising solution for safer crop production in Cd-contaminated environments.

Highlights

- IPMM biochar biofilter along with Mo NPs supply improved the membrane integrity and osmoregulation under Cd stress.
- IPMM biochar biofilter and Mo NPs played a protective role in oxidative defense system through regulating the genetic expressions under Cd toxicity.
- IPMM biochar biofilter together with Mo NPs improved the elemental profile and minimized the nutrient reductions in roots and shoots.
- IPMM biochar biofilter and Mo NPs preserved the ultrastructure of leaf and root architecture.
- IPMM biochar biofilter together with Mo NPs could be use as safeguard under HMs toxic conditions.

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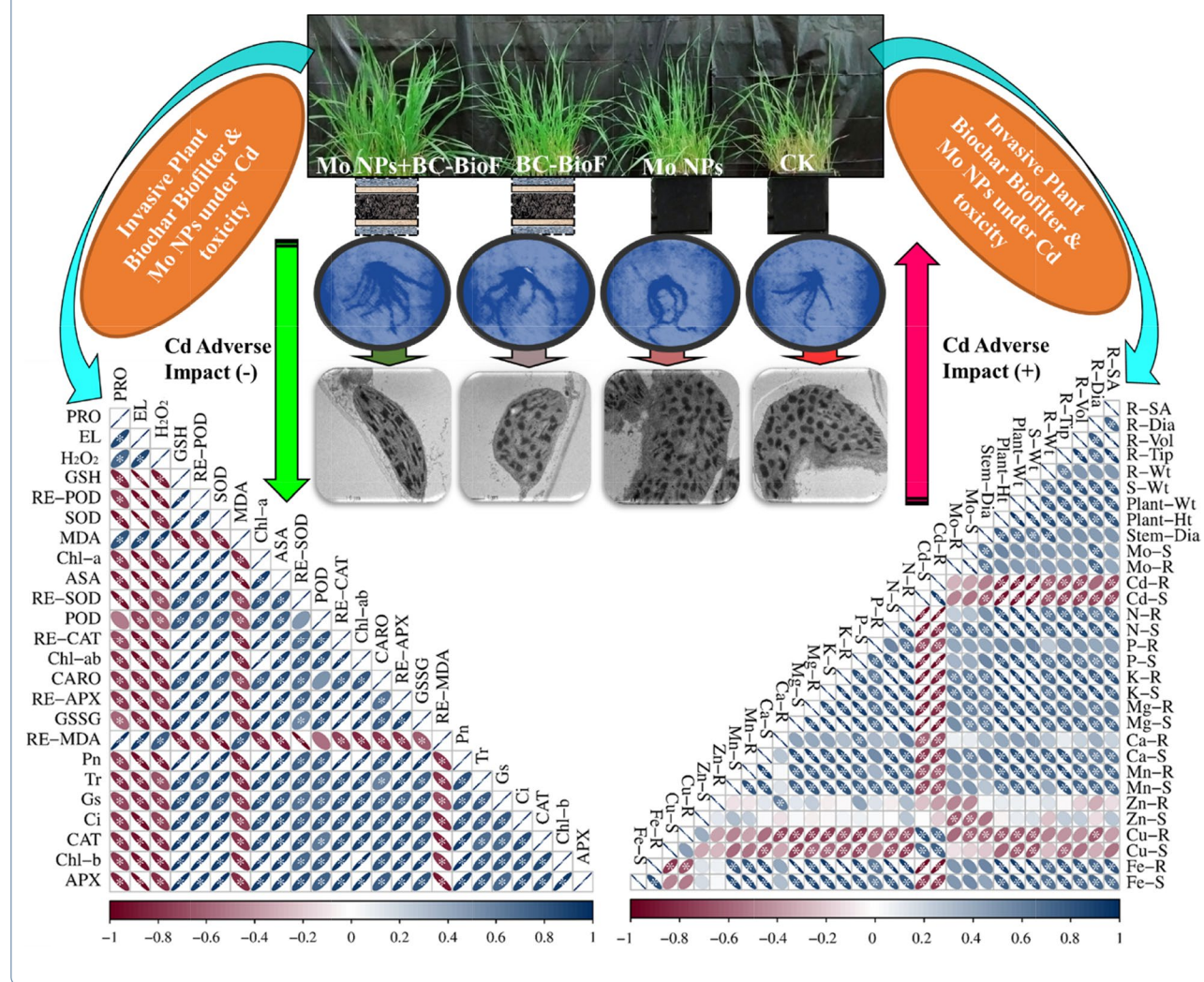
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Keywords Biochar biofilters, Invasive plant, HMs toxicity, Environmental remediation, Mo NPs

Graphical Abstract



1 Introduction

Recently, heavy metals (HMs) accumulation has emerged as a significant global concern (Yao et al. 2021; Zhang et al. 2023). The accumulation of Cd in soil, sediments, and water has raised significant environmental and health concerns due to its cumulative toxic effects and ability to mobilize through plant species and the food chain (Yotsova et al. 2020). For example, in the US and EU countries, over 100,000 and 250,000 contaminated sites, respectively, have been identified

and require urgent remediation (Connell and Messerschmidt 2005; Mench et al. 2010). Additionally, 20% of agricultural land in China is contaminated with HMs, with over 130,000 hectares specifically polluted by Cd (Khan et al. 2017). The issue of remediation is relatively important because the plants readily absorb Cd from contaminated soil and accumulate it in their tissues. Consequently, Cd can enter the human body directly or indirectly through the food chain (Gu et al. 2020; Zhang et al. 2023). Given the extensive soil and

water pollution in China and around the world, there is an urgent need to develop green and sustainable remediation techniques and technologies, particularly for addressing HMs-contaminated agricultural soils through native resource utilization. Besides HMs toxic effect on the environment, invasive plants also significantly impact the global ecological environment due to their robust adaptability and rapid proliferation, leading to detrimental effects on local ecosystems (Li et al. 2023). Invasive plant *Mikania micrantha* Kunth has caused a great threat to native ecosystem in South China and current methods used to control invasive plants typically involve physical, chemical, and biological approaches, such as mechanical removal and pesticide spraying (Abdellah et al. 2024, 2023). However, these methods incur substantial costs and contribute to environmental pollution (Hua 2015; Poovey et al. 2005). For example, the chemical compounds used often contain trace metals like copper (Cu), Cd, arsenic (As), and lead (Pb), which can leach into watersheds, posing threats to aquatic ecosystems and human health.

Therefore, there is an urgent need to explore cost-effective and practical strategies for managing the HMs contamination and invasive plants. In this context, biochar (BC) emerges as a promising, environment friendly material that can help to address both the issue of invasive plants (Abdellah et al. 2024, 2023) and Cd pollution (Wang et al. 2024b). The use of invasive plants as reserves for BC production not only reduces the waste but also helps control the spread of these plants to some degree. Moreover, BC has been widely known for its characteristics to absorb HMs, offering a dual advantage of managing biological invasions and HMs (Yang et al. 2022). Therefore, BC production from invasive plants offers an innovative approach for simultaneously addressing the environmental concerns posed by invasive species and HMs pollution. Furthermore, the combined use of BC with stress-relieving nutrients like Mo could further enhance its effectiveness in alleviating the HMs toxicity. Previous studies have demonstrated the beneficial effects of Mo (Rana et al. 2020a, 2020c, 2020d) on plants and in alleviating Cd stress damage in both plants and animals (Ismael et al. 2018; Liao et al. 2018). Additionally, Mo is widely recognized for its role as a stress-resistant nutrient, which can enhance plant tolerance to oxidative stress induced by salinity, drought, low temperature, ammonium stress, and reactive oxygen species (ROS) homeostasis, including increased activity and transcriptional abundance of oxidative enzymes (Imran et al. 2020). Current experiments have showed the positive effects of Mo in alleviating Cd stress damage in several species such as *Cannabis sativa* L. (Ali et al. 2023) and *Brassica napus* L. (Ismael et al. 2018). Recent studies have

explored the changes of soil microorganisms to explore the mechanism of BC for reducing the HMs toxicity in plants (Gorovtsov et al. 2020). Biochar can increase the microbial diversity, leading to increased microbial activity which can immobilize the HMs. These changes in soil could contribute to reduced bioavailability and improved plant growth. Meanwhile, the changes in soil physical and chemical properties, as well as enzymes activities are also worthy of consideration (Qu et al. 2024b). However, there remains a research gap regarding the combined use of IPMM BC biofilters and Mo NPs to minimize the toxic effects of HMs. This combined approach could potentially offer a novel and effective solution for managing HMs contamination in soils.

Although numerous laboratory studies have demonstrated the potential of BC to remove HMs ions, there has been limited exploration of how addressing one problem, such as the invasive plant *M. micrantha*, can simultaneously help to mitigate other environmental pollutants. The novelty of this study is in using BC derived from the IPMM as biofilters equipped with Mo NPs. This approach aims to explore the mechanisms responsible for Cd-detoxification to reduce the Cd-induced damage in cell organelles. Therefore, the key objectives of current study were to: (1) characterize IPMM BC biofilters with and without Mo NPs in a Cd-contaminated environment, followed by an in-depth analysis of the morphological, physiological, and biochemical responses of wheat to exposure; (2) investigate root architecture, cell ultrastructural changes, and elemental deteriorations accompanying with Cd toxicity in hydroponically cultivated wheat; (3) evaluate alterations in plant defense mechanisms and their impact on wheat Cd distribution; (4) assess the IPMM BC biofilters and Mo NPs immobilization effect on photosynthetic activity and its impact on molecular and biological processes through gene expression analysis. The findings of this research may offer new perspectives on developing functional materials and associated technologies for the green and sustainable remediation of HMs-polluted environments.

2 Materials and methods

2.1 Invasive plant-derived biochar biofilter preparation

The raw material of the invasive plant *M. micrantha* was sourced from South China Agricultural University, Guangzhou, China. Prior to carbonization, the plants underwent multiple washes with tap water and were subsequently dried. The dried raw materials were then crushed and sieved through a 2.0 mm sieve. Carbonization was conducted at 500 °C for 2 h under oxygen-deficient conditions. The physicochemical properties of the IPMM BC were assessed using methods described in previous studies (Zhang and Luo 2014). The

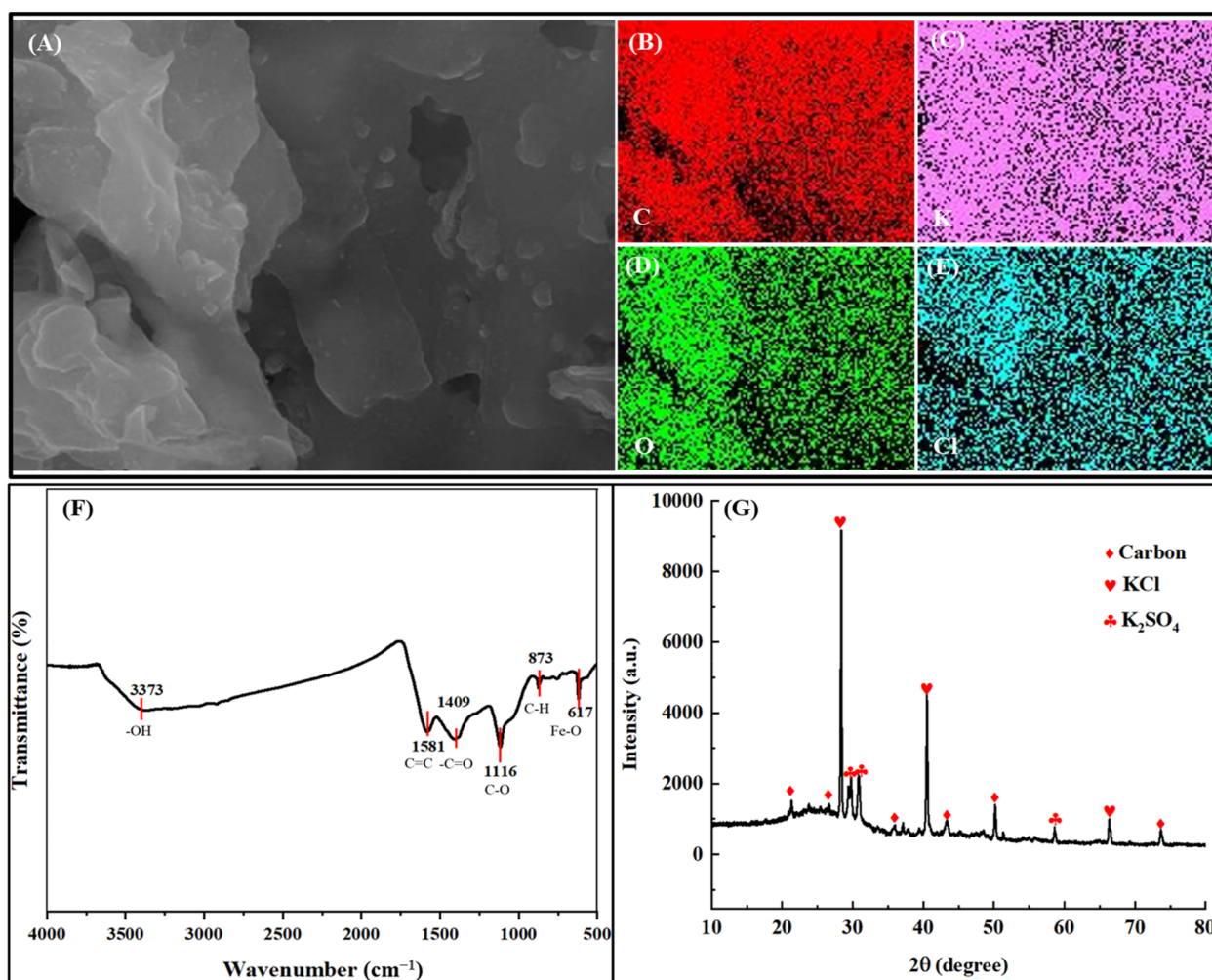


Fig. 1 A SEM image of IPMM BC, B C, C K, D O, E Cl elemental mapping, F FT-IR spectra and G XRD patterns

morphology of the BC was examined using scanning electron microscopy (SEM). Figure 1 illustrates a scanning electron micrograph of the BC derived from IPMM, depicting its properties of rough, irregular, and porous surface. IPMM BC biofilters were assembled by filling a perforated plastic tube (5 cm in length and 3 cm in diameter) with 1.0 g of IPMM BC sample. To prevent BC loss, a layer of sand (0.5 cm thick, <2 mm size) was placed above and below the BC layer. Additionally, gravel layers (0.5 cm thick) were added both above and below to facilitate uniform flow distribution. Cheesecloth sheets were used to separate and secure the IPMM BC, sand, and gravel layers. A schematic diagram illustrating the IPMM BC biofilter design is depicted in Fig. S1.

2.2 Characterization

The characterization of BC was carried out by the previously described methods (Qu et al. 2024a, 2024b). The microstructure and functional groups of the BC were investigated via field emission scanning electron microscope (SEM) (Hitachi, SU8010, Tokyo, Japan) and Fourier transform infrared spectroscopy (FTIR) on a Nicolet 7600 spectrometer (Nicolet Instrument Corporation, Madison, WI, USA). Crystal structures of BC were detected by X-ray diffraction (XRD) on a Bruker D8 Advance diffractometer (Bruker, Germany). The surface elemental compositions and distribution were determined using an energy dispersive X-ray spectroscopy (EDS, HORIBA EMAX mics2, Hitachi, Tokyo, Japan) detector attached to an SEM. Furthermore, abundant C, O, K and Cl elements were uniformly distributed on the BC surface (Fig. 1A, B, C, D, E). As presented in Fig. 1F, the peaks at 3373 cm^{-1} , 1581 cm^{-1} , 1409 cm^{-1} , 1116 cm^{-1} , 873 cm^{-1}

and 617 cm^{-1} were identified as $-\text{OH}$, $\text{C}=\text{C}$, $-\text{C}=\text{O}$, $\text{C}-\text{O}$, $\text{C}-\text{H}$, and $\text{Fe}-\text{O}$, respectively. The crystal structure of the materials showing the C peaks has been presented in Fig. 1G.

2.3 Experimental treatments, plant material and growth conditions

The wheat seeds were germinated on cheese cloth in a growth chamber set at $28 \pm 2^\circ\text{C}$ for 5 days. Uniform seedlings of wheat were then transplanted into plastic pots filled with a one-quarter concentration of Hoagland's nutrient solution. The growth chamber conditions were maintained at $28 \pm 2^\circ\text{C}$, with a photon density of $820\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ and a relative humidity of 65–70%. Each plastic pot, sized $13\text{ cm} \times 9\text{ cm} \times 12\text{ cm}$, had 96 holes in the lid and contained 1 L of the respective nutrient solution. Each treatment was replicated three times to ensure consistency and reliability of the experimental results.

The wheat seedlings were fixed into the lid holes and initially grown in 1/4 strength Hoagland nutrient solution for the initial two days, followed by a 1/2 strength solution for the next two days. The combinations of treatments included: CK ($100\text{ }\mu\text{M CdCl}_2$), Mo NPs ($1\text{ }\mu\text{M K}_2\text{MoO}_4 + 100\text{ }\mu\text{M CdCl}_2$), BC-BioF (IPMM BC biofilter + $100\text{ }\mu\text{M CdCl}_2$), and BC-BioF + Mo NPs (IPMM BC biofilter + Mo NPs $1\text{ }\mu\text{M K}_2\text{MoO}_4 + 100\text{ }\mu\text{M CdCl}_2$). The treatment with sole CdCl_2 was designated as CK. Seedlings were supplemented with full-strength Hoagland nutrient solution for 30 days. The modified Hoagland nutrient solution contained: $9.1\text{ }\mu\text{M MnSO}_4$, $1\text{ mM }(\text{NH}_4)_2\text{SO}_4$, $20\text{ }\mu\text{M Fe-EDTA}$, $1\text{ mM KH}_2\text{PO}_4$, $1\text{ mM MgSO}_4 \cdot 7\text{H}_2\text{O}$, $1\text{ mM Ca }(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $2\text{ mM Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$, $1\text{ }\mu\text{M ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $0.1\text{ }\mu\text{M CuSO}_4 \cdot 5\text{H}_2\text{O}$, and $10\text{ }\mu\text{M H}_3\text{BO}_3$. The pH of solution was adjusted daily to 6.5 ± 0.05 by adding 0.1 mM HCl or NaOH . This solution provides a balanced nutrition essential for plant growth, making it easier to study the effect of HMs or NPs exposure. Moreover, the Mo NPs supplementation was provided in the relevant treatments as compared to other treatments as per designed experiment. The solution was changed every 3 days throughout the experiment. After 30 days, the plants were harvested, and their physiological characteristics were recorded. Half of the plants from each pot were oven-dried, while the other half was stored at -80°C for chemical and molecular analysis.

2.4 Root architecture, morphology and vegetative characteristic measurements

At the harvest stage (30 days after planting), representative wheat samples (25 plants) were carefully chosen from each treatment to record the plant height. The samples were then separated into shoots and roots and gradually washed with distilled water. Root architecture and wheat

morphological characteristics, including cumulative root length, surface area, volume, mean diameter, and root tips, were studied using the WinRhizo system v. 4.0b (Regent, Canada), following the method described by Yue et al. (2019). Subsequently, the samples were dried in an oven at 80°C , and the oven-dried weights of shoots and roots were recorded.

2.5 Determination of osmoregulation attributes, gas exchange and photosynthetic pigments

For gas exchange measurements, fully stretched mature leaves from each plant were utilized. Five plants per treatment were randomly selected for each replicate, and their measurements were averaged. The transpiration rate (Tr), net photosynthesis rate (Pn), intercellular CO_2 concentration (Ci), and stomatal conductance (Gs) of leaves were assessed using a photosynthesis system (LI-6400XT, Li-Cor, Inc., Lincoln, NE, USA) following methods outlined by Austin (1990) and Saleem et al. (2020).

Fresh wheat leaves were taken to measure the chlorophyll a, chlorophyll b, and carotenoids following a previously described method (Imran et al. 2019a). Briefly, the fresh wheat leaves were extracted with 95% ethanol, and the absorbance was measured at wavelengths of 470 nm, 649 nm, and 665 nm using a spectrophotometer (UV-VIS 2550, Shimadzu, Japan).

The protein content of the leaves was determined using the G-250 method. Fresh leaf samples (0.2 g) were ground in 50 mM sodium phosphate buffer (pH 7.0) with 2% polyvinylpyrrolidone-40 (PVP-40) and 1 mM EDTA- Na_2 . The homogenate was centrifuged at $10,000\text{ g}$ (15 min; 4°C), and the absorbance of the resulting supernatant was measured at 595 nm in triplicate using a spectrophotometer (UV-VIS 2550, Shimadzu, Japan). The ninhydrin method was applied to measure proline level. The reaction mixture was extracted through 5 mL of toluene, and the absorbance of the red chromophore in the toluene phase was measured at 520 nm. Proline was quantified by equating the absorbance with a standard curve.

2.6 Measurement of reactive oxygen species and lipid peroxidation

Fresh leaf samples (0.2 g) were finely ground using liquid N, then normalized with 1 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at $12,000\text{ g}$ (20 min; 4°C) to quantify hydrogen peroxide (H_2O_2). The reaction mixture included 1 mL of potassium iodide (1 M), 0.5 mL of potassium phosphate buffer (pH 6.8, 100 mM), and 0.5 mL of the supernatant. H_2O_2 levels were recorded using a spectrophotometer (UV-VIS 2550, Shimadzu, Japan) at a wavelength of 390 nm (Valentovic et al. 2006). Fresh leaves were harvested and promptly homogenized in cold TCA (0.1% w/v). The homogenate was

subsequently centrifuged at 12,000 g (20 min; 4 °C) to assess the malondialdehyde (MDA) content. The reaction mixture consisted of 2.5 mL of 0.5% thiobarbituric acid (TBA) solution dissolved in 20% TCA, along with 0.5 mL of the supernatant. After the reaction mixture was boiled for 30 min, it was allowed to cool and then centrifuged at 12,000×g for 5 min. The MDA content was determined by calculating the difference in absorbance values at 532 nm and 600 nm, through an extinction coefficient (155 mM cm⁻¹). To measure the electrolyte leakage (EL), leaf tissues were incubated in 10 mL of deionized water for 6 h at 25 °C. The percentage of EL was measured using an electrical conductivity (EC) meter (SX-650, Sanxin, China). Subsequently, the same procedure was repeated with incubation at 90 °C for 2 h to obtain EC2. The EL% was measured using the formula: EL (%) = (EC1/EC2)*100 (Imran et al. 2020; Valentovic et al. 2006).

2.7 Transmission electron microscopy

Fresh leaf samples (1–3 mm in length) were immersed in a 4% glutaraldehyde solution containing 0.2 mol L⁻¹ sodium phosphate buffer (PBS, pH 7.2) for 8 h. They were then treated with 1% OsO₄ (Osmium tetroxide) for 1 h and rinsed in 0.2 mol L⁻¹ PBS (pH 7.2) for 2 h. The samples underwent dehydration using a classified series of ethanol (50%, 60%, 70%, 80%, 90%, 95%, and 100%) followed by acetone. Subsequently, they were infiltrated and embedded in Spurr's resin. Ultrathin sections were prepared using an ultramicrotome to cut samples from the embedded resin block. Briefly, the samples were placed in the sample holder of the ultramicrotome. The ultrathin sections were then prepared by moving the sample toward the knife while maintaining a controlled and consistent speed. Afterward, the ultrathin sections were collected and placed onto copper grids, ensuring that the sections were properly oriented for observation under a transmission electron microscope. (Roland and Vian 1991).

2.8 Measuring the enzymatic and non-enzymatic oxidative defense attributes

Fresh leaves were pulverized using liquid nitrogen and homogenized with cold sodium phosphate buffer (pH 7.8). The homogenate was then centrifuged at 4000 rpm for 10 min at 4 °C, and the supernatant was collected, and separated from the crude fibres. The reaction mixture for measuring superoxide dismutase (SOD; EC 1.15.1.1) activity included the enzyme extract, sodium phosphate buffer (pH 7.8), nitro blue tetrazolium (NBT), riboflavin, methionine, EDTA-Na₂, and distilled water. The absorbance corresponding to SOD activity was recorded at 560 nm through a spectrophotometer (UV–VIS 2550, Shimadzu, Japan) (Imran et al. 2023). To measure the

activity of peroxidase (POD; EC 1.11.1.7), fresh samples were ground with a cold extraction buffer containing sodium phosphate (50 mM, pH 5.5) and then centrifuged (3000 rpm; 10 min) to collect the supernatant. The reaction mixture for POD activity measurement included enzyme extract, sodium phosphate buffer (50 mM, pH 5.5), H₂O₂ (0.3%), and guaiacol (0.2%). The absorbance was recorded at 470 nm using a spectrophotometer. For catalase (CAT; EC 1.11.1.6) activity measurement, fresh plant samples were ground in a sodium phosphate extraction buffer (50 mM, pH 7.8). The resulting mixture was then centrifuged at 4000 rpm for 15 min at 4 °C to collect the supernatant. The reaction mixture for CAT activity assessment included distilled water, sodium phosphate buffer (50 mM, pH 7.8), H₂O₂ (0.1 M), and the enzyme extract. The absorbance was measured at 240 nm using a spectrophotometer. To determine the activity of ascorbate peroxidase (APX; EC 1.11.1.11), leaves were homogenized in sodium phosphate buffer (50 mM, pH 5.5) containing EDTA-Na₂ (0.2 mM). The homogenate was then centrifuged at 10,000 rpm for 15 min at 4 °C, and the resulting supernatant was used to measure APX activity. For determining the levels of reduced glutathione (GSH) and oxidized glutathione (GSSG), commercial kits 'A006-1' and 'A061-2' were used, respectively (www.njjcbio.com).

2.9 Total RNA extraction and qRT-PCR analysis

Fresh wheat leaves were harvested for total RNA extraction using TRIzol reagent (Invitrogen, Carlsbad, California, USA). Following extraction, standard protocols were rigorously followed to assess the quality and quantity of the total RNA, performed cDNA synthesis, and Real Time PCR experiments, as previously described (Imran et al. 2019b). Details such as nucleotide sequences are provided in Table S1. We utilized three biological replicates for each sample and normalized the Ct (cycle threshold) values relative to the ACTIN Ct values. This normalization allowed calculation of the expression levels for each gene using the 2^{-ΔΔCt} method for quantification (Pfaffl 2001).

2.10 Mineral elements assays

Plant samples were first dried, ground, and weighed. They were then steeped in H₂SO₄ and digested in a fume hood (180 °C; 3 h). H₂O₂ was subsequently added. The nitrogen (N) concentrations were determined using the previously described methods (Barbano and Clark 1990; Rana et al. 2020b, 2018). Potassium (K) concentration in plant samples was analysed using a flame photometer (Model 410, USA). For phosphorus (P) content analysis, the digested plant tissues were assessed colorimetrically using the Mo-blue method, as described by Lu et al.

(1999). This method involves the formation of a blue complex between orthophosphate and molybdate under acidic conditions, which is then measured spectrophotometrically to quantify P content in the samples. Dried plant samples were processed using a microwave oven with a mixture of nitric acid (HNO_3) and perchloric acid (HClO_4) in a ratio of 5:1 (v/v). This digestion method was used to determine the concentrations of calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn), Mo, and copper (Cu) in the plant samples. The acids dissolve the plant tissue and release the metals for subsequent analysis using techniques such as Inductively Coupled Plasma (ICP) spectroscopy or other suitable analytical methods. The concentrations of Cd and other elements (Ca, Mg, Zn, Fe, Mn, and Cu) in the digested samples were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) with a Vista-PRO instrument (Varian, Inc., Palo Alto, CA, USA) and ICP-mass spectrometry (ICP-MS) (ELAN DRC-e, Perkin-Elmer Sciex, Wilmington, DE, USA).

2.11 Statistical analysis

The data were statistically analysed following a Completely Randomized Design (CRD) using Statistix 8.1 software (Analytical Software, Tallahassee, FL, USA). Mean variances were assessed using the least significant difference (LSD) test at $P \leq 0.05$. Graphs were generated using GraphPad Prism software.

3 Results

3.1 Integrative effect of IPMM biochar biofilter and Mo NPs on plant physiological characteristics in Cd contaminated environment

Compared to treatment with Cd alone (100 μM), the incorporation of an IPMM BC biofilter, particularly in conjunction with Mo NPs, significantly alleviated Cd detrimental effects on wheat plant growth (Table 1). The addition of Mo NPs and the IPMM BC biofilter notably enhanced root and shoot length as well as dry weight ($P \leq 0.05$). These changes were markedly different from

those observed in the control (LSD test). As indicated in Table 1, the presence of the IPMM BC biofilter and Mo NPs led to substantial increases in stem diameter (5.77%), biomass dry weight (67.64% and 66.0% for roots and shoots, respectively), and height (33.99%) compared to conditions without the IPMM BC biofilter and Mo NPs. However, applying only Mo NPs or the IPMM BC biofilter minimally mitigated the adverse effects of Cd on wheat plant growth (Table 1). Meanwhile Mo NPs and IPMM BC biofilters individually increased biomass by 23.84% and 46.15%, and plant height by 8.78% and 17.36%, respectively. The individual effectiveness of Mo NPs and IPMM BC biofilters in reducing Cd toxicity was less pronounced than when used together. In contrast, stem diameter increased by 1.67% in the IPMM BC biofilter treatment compared to the control. This suggests a potential synergistic effect of Mo NPs and the IPMM BC biofilter on enhancing plant physiological traits.

3.2 IPMM biochar biofilter and Mo NPs induced effect on root architecture in Cd contaminated environment

Root morphological attributes such as cumulative root length, number of root tips, surface area, average diameter, and root volume underscore the advantageous role of the IPMM BC biofilter (Fig. 2). The combined application of IPMM BC biofilter and Mo NPs increased the total root length by 59.70% compared to CK, whereas the Mo NPs showed an increase of 9.26% but the results were non-significant ($P \leq 0.05$). The highest root surface area of 27.62 cm^2 was recorded in the IPMM BC biofilter+Mo NPs treatment followed by IPMM BC biofilter (22.50 cm^2), Mo NPs (18.35 cm^2), and CK (15.32 cm^2). The CK treatment significantly ($P \leq 0.05$) reduced the root average diameter as compared to other treatments. The average root diameter increased by 9.97%, 7.45%, and 13.0% in the Mo NPs, IPMM BC biofilter, and IPMM BC biofilter+Mo NPs treatment, respectively, but the results were not statistically significant ($P \leq 0.05$). Additionally, the combined treatment of IPMM BC biofilter and Mo NPs showed a substantial increase in root

Table 1 Integrative effect of IPMM biochar biofilters and Mo NPs on plant physiological characteristics in Cd contaminated environment

Treatments	Plant growth characteristics		Plant biomass yield characteristics (g plant^{-1})		
	Plant height (cm)	Stem diameter (mm)	Root	Shoot	Total
CK	21.25 $\pm$ 0.71c	1.79 $\pm$ 0.02b	0.011 $\pm$ 0.002b	0.07 $\pm$ 0.006c	0.078 $\pm$ 0.007d
Mo NPs	23.12 $\pm$ 0.99bc	1.85 $\pm$ 0.04ab	0.014 $\pm$ 0.002ab	0.08 $\pm$ 0.004b	0.097 $\pm$ 0.005c
BC-BioF	24.94 $\pm$ 0.55b	1.82 $\pm$ 0.03ab	0.015 $\pm$ 0.002ab	0.10 $\pm$ 0.001a	0.114 $\pm$ 0.003b
BC-BioF + Mo NPs	28.47 $\pm$ 0.68a	1.89 $\pm$ 0.02a	0.019 $\pm$ 0.001a	0.11 $\pm$ 0.002a	0.129 $\pm$ 0.002a

The data are the average of three replicates $\pm$ SE from different independent treatments. Different lowercase (a, b) represent significant differences by the LSD test ($P \leq 0.05$) among different treatment

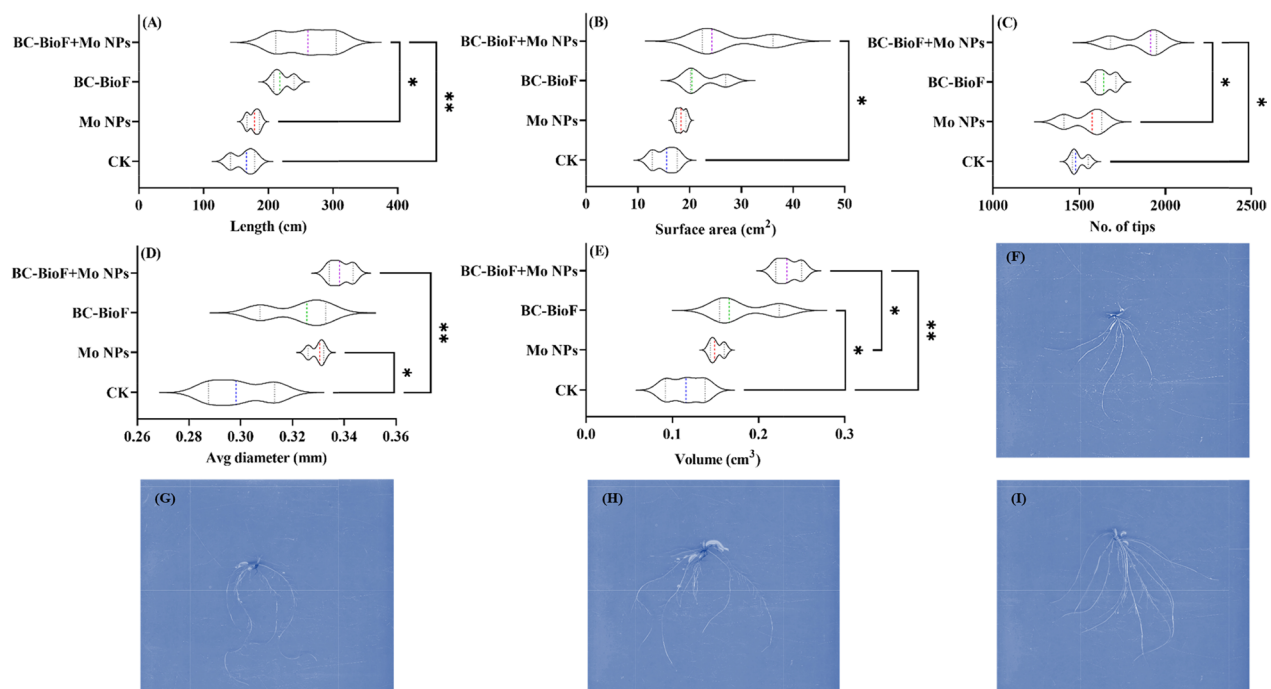


Fig. 2 Integrative effect of IPMM biochar biofilters and Mo NPs on root physiological indicators and root architecture. **A** root length, **B** surface area, **C** number of tips, **D** average diameter, **E** root volume, and root architecture of **F** CK, **G** Mo NPs, **H** BC-BioF and **I** BC-BioF + Mo NPs. Statistically significant differences between treatments are indicated by stars above the mean values at $P \leq 0.05$

volume (103.17%) and the number of root tips (23.42%). In contrast, Mo NPs alone led to a smaller increase in root volume (30.92%) and the number of root tips (2.80%) compared to CK.

3.3 Ultrastructural characterization of leaf mesophyll cells and wheat phenotypical appearance

Figure 3 illustrates the ultrastructural changes in wheat mesophyll cells and their phenotypical appearance under various treatments in a Cd-stressed environment. Specifically, the chloroplast ultrastructure in control plants exposed solely to Cd displayed several pronounced abnormalities: disorganized grana stacking, distorted chloroplast membranes, and a disrupted thylakoid system (Fig. 3A). The treatments with only Mo NPs or the BC-BioF under Cd stress (Fig. 3B, C) displayed less developed structures compared to the combined application of the IPMM BC biofilter and Mo NPs (BC-BioF+Mo NPs under Cd stress). Plants treated with the IPMM BC biofilter and Mo NPs (BC-BioF+Mo NPs with Cd stress) exhibited typical chloroplast features, including a proper shape with a complete external envelope, perfect borders, and proper plastoglobuli (Fig. 3D). Moreover, thylakoid grana and stroma were well-built and densely packed. In the BC-BioF+Mo NPs treatment, the cracks of the

grana thylakoids were insignificant and flat, running along the axis and connecting to the stroma thylakoids to make the incessant membranes. Thylakoids were also positioned along the cell periphery for optimizing absorption of light to transfer it through the photosynthetic pathways. Extreme Cd stress also led to chloroplast swelling, resulting in a spherical structure unlike the normal spindle like structure observed in the BC-BioF+Mo NPs treatment under Cd stress (Fig. 3D). Furthermore, the thylakoid lamellae were not tightly packed, and the grana thylakoids appeared wavy while the stroma lamellae were disorganized (Fig. 3A–C). In some instances, the chloroplast envelope ruptured, causing the internal contents to spill into the surrounding cytoplasm (Fig. 3A). The IPMM BC biofilter and Mo NPs treatments exhibited varying degrees of detoxification effects. The ameliorative effects of the IPMM BC biofilter and Mo NPs treatments alone were not as pronounced, despite showing chloroplasts with visibly larger sizes (Fig. 3B, C). In contrast, the combined protective effect of the BC-BioF and Mo NPs treatment was evident. Chloroplasts were located near the cell periphery and maintained a normal spindle-like structure with perfect double membranes (Fig. 3D). Additionally, there were proper plastoglobuli deposits. The combined treatment also preserved the overall

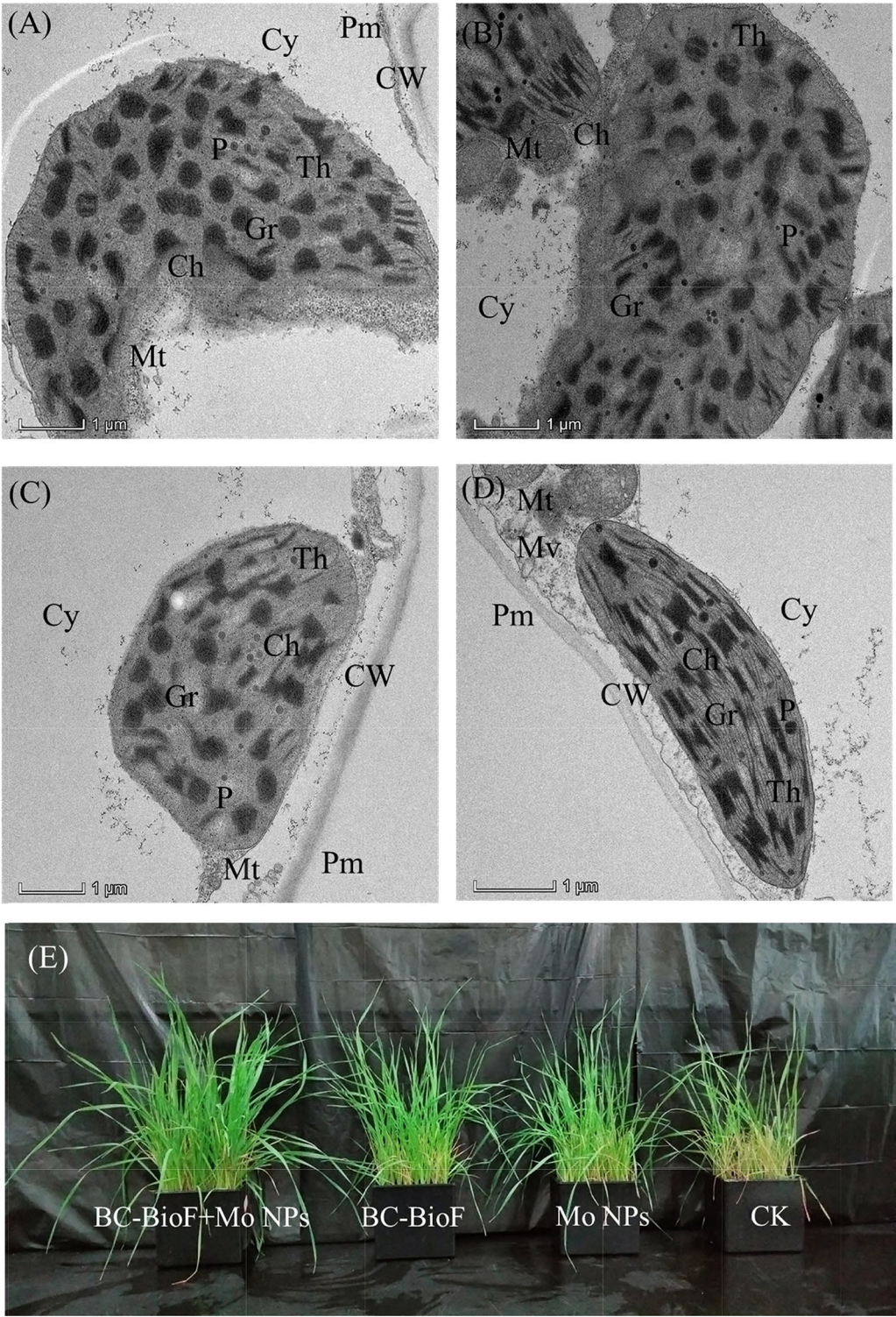


Fig. 3 Integrative effect of IPMM biochar biofilters and Mo NPs on leaf ultra-structure and plant phenotype in Cd contaminated environment. Leaf ultra-structure of **A** CK, **B** Mo NPs, **C** BC-BioF, **D** BC-BioF + Mo NPs and **E** plant phenotype. Key structures are labeled as follows: *Ch* chloroplasts, *P* plastoglobuli, *CW* cell wall, *Gr* grana, *Th* thylakoid, *Mt* mitochondria, *Pm* plasma membrane, *Cy* cytoplasm. Scale bars = 1 μm

Table 2 Co-exposure influence of IPMM biochar biofilters and Mo NPs supply on elemental profile of wheat under Cd stressed environment

Treatment	Primary element (%)			Secondary element (mg g ⁻¹)			Micro element (μg g ⁻¹)				Heavy metal (μg g ⁻¹)	
	N	P	K	Mg	Ca	Mn	Zn	Cu	Fe	Mo	Cd	
Root	CK	0.65 ± 0.011c	0.61 ± 0.257b	0.55 ± 0.023d	1.70 ± 0.013c	7.25 ± 0.147a	55.15 ± 2.89b	48.37 ± 2.98a	16.11 ± 2.06a	60.40 ± 2.64d	–	398.22 ± 9.08a
	Mo NPs	0.73 ± 0.026b	0.89 ± 0.094ab	0.68 ± 0.005c	1.89 ± 0.080bc	8.12 ± 1.241a	56.72 ± 2.10b	44.45 ± 2.32a	11.63 ± 1.57ab	71.84 ± 2.07c	3.36 ± 0.015b	375.49 ± 8.55a
	BC-BioF	1.04 ± 0.029a	1.01 ± 0.044ab	0.77 ± 0.027b	2.01 ± 0.023ab	9.22 ± 1.413a	60.35 ± 2.80ab	52.73 ± 3.31a	10.54 ± 1.65b	89.56 ± 1.50b	–	303.63 ± 12.07b
	BC-BioF + Mo NPs	1.11 ± 0.021a	1.15 ± 0.026a	0.99 ± 0.017a	2.25 ± 0.130a	8.68 ± 0.051a	67.56 ± 2.51ab	54.28 ± 0.96a	8.34 ± 0.77b	113.88 ± 1.69a	3.75 ± 0.058a	276.59 ± 2.53b
Shoot	CK	0.19 ± 0.018c	0.13 ± 0.023b	0.22 ± 0.023c	0.98 ± 0.024b	4.23 ± 0.057b	30.45 ± 1.90b	31.25 ± 2.31a	5.21 ± 0.05ab	29.99 ± 2.98c	–	89.44 ± 3.04a
	Mo NPs	0.29 ± 0.025b	0.24 ± 0.025b	0.46 ± 0.008b	1.20 ± 0.018c	4.35 ± 0.021b	35.02 ± 2.03ab	24.9 ± 2.11b	5.86 ± 0.98a	35.18 ± 2.85bc	2.22 ± 0.504b	72.77 ± 1.93b
	BC-BioF	0.35 ± 0.014b	0.41 ± 0.034a	0.51 ± 0.017b	1.45 ± 0.051b	4.55 ± 0.034b	38.25 ± 3.15ab	34.22 ± 2.67ab	4.1 ± 0.06bc	40.16 ± 2.10ab	–	56.49 ± 3.40c
	BC-BioF + Mo NPs	0.60 ± 0.023a	0.48 ± 0.054a	0.69 ± 0.027a	1.82 ± 0.043a	5.17 ± 0.210a	42.59 ± 1.63ab	39.30 ± 0.57a	3.21 ± 0.03c	49.35 ± 3.32a	2.78 ± 0.047a	41.51 ± 3.11d
Root + Shoot concentration	CK	0.84 ± 0.020d	0.746 ± 0.270c	0.77 ± 0.046d	2.68 ± 0.037d	11.49 ± 0.101a	85.60 ± 3.42b	79.62 ± 1.13c	21.32 ± 2.10b	90.39 ± 5.53d	–	487.67 ± 7.72a
	Mo NPs	1.02 ± 0.005c	1.13 ± 0.078bc	1.14 ± 0.013c	3.09 ± 0.061c	12.48 ± 1.226a	91.74 ± 2.95b	69.35 ± 3.74b	17.49 ± 2.48b	107.02 ± 2.52b	5.58 ± 0.060b	448.27 ± 9.48a
	BC-BioF	1.39 ± 0.015b	1.42 ± 0.031bc	1.29 ± 0.032b	3.46 ± 0.073b	13.77 ± 1.380a	98.61 ± 6.18ab	86.95 ± 1.68ab	14.64 ± 1.71ab	129.73 ± 1.25b	–	360.12 ± 12.23b
	BC-BioF + Mo NPs	1.71 ± 0.042a	1.63 ± 0.060a	1.69 ± 0.032a	4.07 ± 0.120a	13.85 ± 0.227a	98.61 ± 4.19a	93.59 ± 1.50a	11.56 ± 0.75a	163.24 ± 2.33a	6.53 ± 0.021a	318.10 ± 3.36c

The data are the average of three replicates ± SE from different independent treatments. Different lowercase (a, b) represent significant differences by the LSD test ($P \leq 0.05$) among different treatments

shape, quantity, and characteristic structure of the mitochondria.

3.4 Co-exposure influence of IPMM biochar biofilters and Mo NPs supply on elemental profile, Cd absorption and translocation in wheat

Exogenous supplementation of Cd led to diverse changes in primary, secondary, and micro-nutrient concentrations in wheat plants (Table 2). For instance, in roots, N, P, Ca, and Mg contents illustrated modest increases in the Mo NPs-supplemented treatment, approximately 12.30%, 46.19%, 11.98%, and 11.15% higher, respectively, compared to plants under pure Cd stress. Similar trends were observed for these macro elements in the roots with the BC-BioF+Mo NPs treatments under Cd stress. The highest K content in shoots was found in combined treatment of IPMM BC biofilter and Mo NPs (211.94%), followed by BC-BioF (131.43%) and Mo NPs (108.95%). Control plants exhibited the highest Cu concentration in roots ($16.11 \mu\text{g g}^{-1}$), followed by Mo NPs ($11.63 \mu\text{g g}^{-1}$) and BC-BioF ($10.54 \mu\text{g g}^{-1}$), while the BC-BioF+Mo NPs plants had the lowest Cu concentration ($8.34 \mu\text{g g}^{-1}$). Mo NPs treatment reduced Zn content in shoots by 20.32% compared to the sole Cd-contaminated treatment (CK). However, the combination of BC-BioF and Mo NPs resulted in a synergistic effect, increasing the Zn concentration by 25.77%. Furthermore, the accumulation of these elements was generally lower in shoots than in roots across most treatments. BC-BioF+Mo NPs treated plants exhibited the highest Fe concentration in both roots ($113.88 \mu\text{g g}^{-1}$) and shoots ($49.35 \mu\text{g g}^{-1}$), followed by BC-BioF ($89.56 \mu\text{g g}^{-1}$ and $40.16 \mu\text{g g}^{-1}$), Mo NPs ($71.84 \mu\text{g g}^{-1}$ and $35.18 \mu\text{g g}^{-1}$), and CK ($60.40 \mu\text{g g}^{-1}$ and $29.99 \mu\text{g g}^{-1}$). The Mn concentrations were higher in the roots of the BC-BioF+Mo NPs treatment compared to other treatments, and the shoot demonstrated the highest increase in Mn concentration by 39.86% compared to

plants under sole Cd stress conditions. Additionally, the IPMM BC biofilter facilitated greater translocation of Mo in both roots and shoots compared to the sole Mo treatment under Cd stress. Cadmium content varied significantly among experimental treatments, with the highest concentration observed in sole Cd stressed plants ($487.67 \mu\text{g g}^{-1}$). The IPMM BC biofilter reduced Cd concentration by approximately 26.15% and 34.77% in BC-BioF and BC-BioF+Mo NPs treatments, respectively.

3.5 Chlorophyll and carotenoids concentration in wheat leaves in response to IPMM biochar biofilters and Mo NPs

Significant reductions in chlorophyll pigments and carotenoid concentrations were observed following exposure to sole Cd stress compared to other treatments; however, the IPMM BC biofilter and Mo NPs mitigated this adverse effect compared to sole Cd stress and Mo NPs treatment (Table 3). Total chlorophyll content improved by 23.17%, 49.11%, and 111.08% in the Mo NPs, BC-BioF, and BC-BioF+Mo NPs treatments, respectively, compared to the sole Cd stress condition. Additionally, the chlorophyll a/b ratio decreased from 4.12 in CK to 3.28 and 3.09 in BC-BioF and BC-BioF+Mo NPs treatments, respectively, while it increased slightly by 7.29% following Mo NPs treatment. The synergistic effect of BC-BioF+Mo NPs was particularly evident in carotenoid concentrations. The highest increase (161.35%) in carotenoids was observed with the combined application of IPMM BC biofilters and Mo NPs. Whereas, Mo NPs supply alone in the Cd-contaminated environment showed a minimal increase of 12.70%. These findings underscore the heightened sensitivity of chlorophyll and carotenoid contents to Cd stress, while demonstrating that the IPMM BC biofilter in combination with Mo NPs supply mitigated the negative impact of Cd on these characteristics.

Table 3 Alterations in plant chlorophyll and carotenoids in Cd contaminated environment co-exposure with IPMM biochar biofilters and Mo NPs

Treatment	Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)	Chlorophyll a:b	Chlorophyll ab	Carotenoid (mg g ⁻¹ FW)
CK	1.06 ± 0.07d	0.26 ± 0.02c	4.12 ± 0.32a	1.32 ± 0.08d	0.09 ± 0.020b
Mo NPs	1.31 ± 0.03c	0.32 ± 0.06c	4.42 ± 0.95a	1.63 ± 0.06c	0.10 ± 0.018b
BC-BioF	1.51 ± 0.02b	0.46 ± 0.03b	3.28 ± 0.96a	1.97 ± 0.03b	0.21 ± 0.021a
BC-BioF+Mo NPs	2.11 ± 0.09a	0.68 ± 0.03a	3.09 ± 0.97a	2.79 ± 0.11a	0.22 ± 0.019a

The data are the average of three replicates ± SE from different independent treatments. Different lowercase (a, b) represent significant differences by the LSD test ($P \leq 0.05$, $n = 3$) among different treatments

3.6 IPMM biochar biofilter and Mo NPs induced effect on gas exchange parameters, photosynthetic pigments and photosynthetic efficiency in Cd contaminated environment

Under Cd stress, wheat experienced a significant decrease in gas exchange parameters compared to other treatments. However, the use of IPMM BC biofilters with Mo NPs supply led to notable ($P \leq 0.05$) increases in Pn, Tr, Gs and CO_2 concentration compared to sole Cd stressed treatment. The BC-BioF+Mo NPs treatment under Cd stress demonstrated the most significant improvements, with the Pn, Tr, Gs and CO_2 of wheat increasing by 294.42%, 95.36%, 118.86%, and 21.19%, respectively, compared to CK treatments (Fig. 4). Moreover, significant changes in stomatal conductance were observed with the combined supply of IPMM BC biofilters and Mo NPs compared to other treatments. In contrast, the results for CK, Mo NPs, and BC-BioF alone were not significant (Fig. 4). The transpiration rate was lower in BC-BioF treatments ($2.63 \text{ mol m}^{-2} \text{ s}^{-1}$) compared to Mo

NPs treatments ($3.09 \text{ mol m}^{-2} \text{ s}^{-1}$), but their combination produced a favourable effect (Fig. 4B). These findings highlight that the combined application of IPMM BC biofilters and Mo NPs may have a more complementary effect on wheat under Cd stress conditions.

3.7 Influence of IPMM biochar biofilter and Mo NPs supply on osmoregulation under Cd toxicity

The combined supply of Mo NPs, BC-BioF, and Cd significantly altered soluble protein and proline levels in wheat leaf tissues (Fig. 5A, B). IPMM BC biofilters and Mo NPs supply notably increased soluble protein concentration under Cd toxicity compared to treatment with Cd alone. Specifically, under Cd stress, Mo NPs, BC-BioF, and BC-BioF+Mo NPs supply increased soluble protein content by 55.32%, 92.92%, and 185.69%, respectively, compared to sole Cd stress treatment, indicating that IPMM BC biofilters and Mo NPs effectively mitigated the reduction in soluble proteins ($P \leq 0.05$). The mutual effect of IPMM BC biofilters and Mo NPs was particularly pronounced in

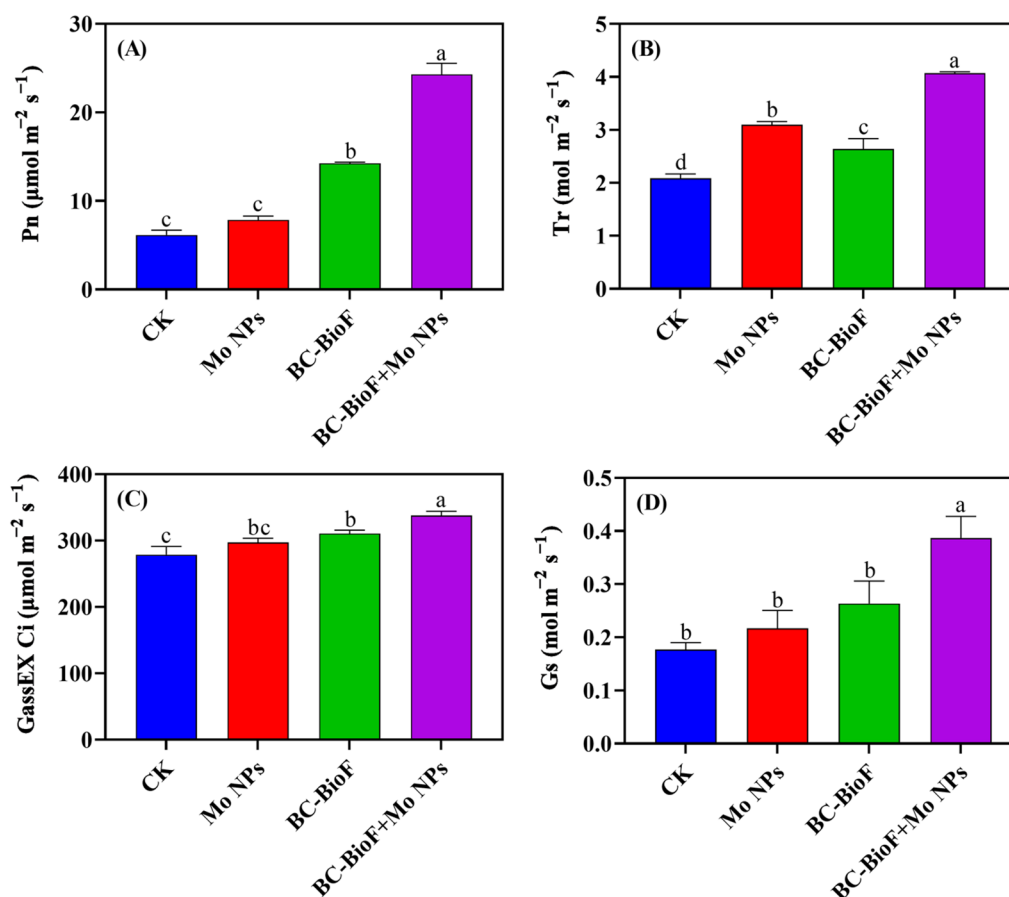


Fig. 4 Integrative effect of IPMM biochar biofilters and Mo NPs on **A** photosynthetic, **B** transpiration rate, **C** CO_2 intercellular leaf and **D** stomatal conductance in Cd contaminated environment. The bars above the means represent the standard error (S.E., $n=3$). Different lowercase letters indicate statistically significant differences among treatments, as determined by the LSD test at $P \leq 0.05$

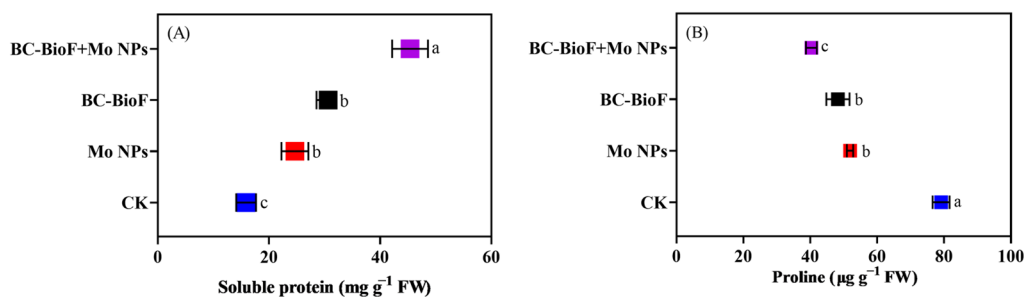


Fig. 5 Integrative effect of IPMM biochar biofilters and Mo NPs on biochemical analysis of **A** soluble protein and **B** proline in Cd contaminated environment. The bars above the mean values represent the standard error (S.E., $n=3$). Different lowercase letters indicate statistically significant differences among treatments, as determined by the LSD test at $P \leq 0.05$

alleviating the deleterious effect Cd on soluble proteins under Cd stressed environments. As compared to soluble proteins, proline contents were significantly higher under sole Cd stress (Fig. 5B). Under Cd stress, proline contents followed this order: CK ($79.12 \mu\text{g g}^{-1} \text{FW}$) > Mo NPs

($51.91 \mu\text{g g}^{-1} \text{FW}$) > BC-BioF ($48.28 \mu\text{g g}^{-1} \text{FW}$) > BC-BioF+Mo NPs ($40.35 \mu\text{g g}^{-1} \text{FW}$). The BC-BioF+Mo NPs treatment under Cd stress reduced proline concentration by 48.99% compared to CK. These findings suggest that under Cd toxicity, IPMM BC biofilters and Mo

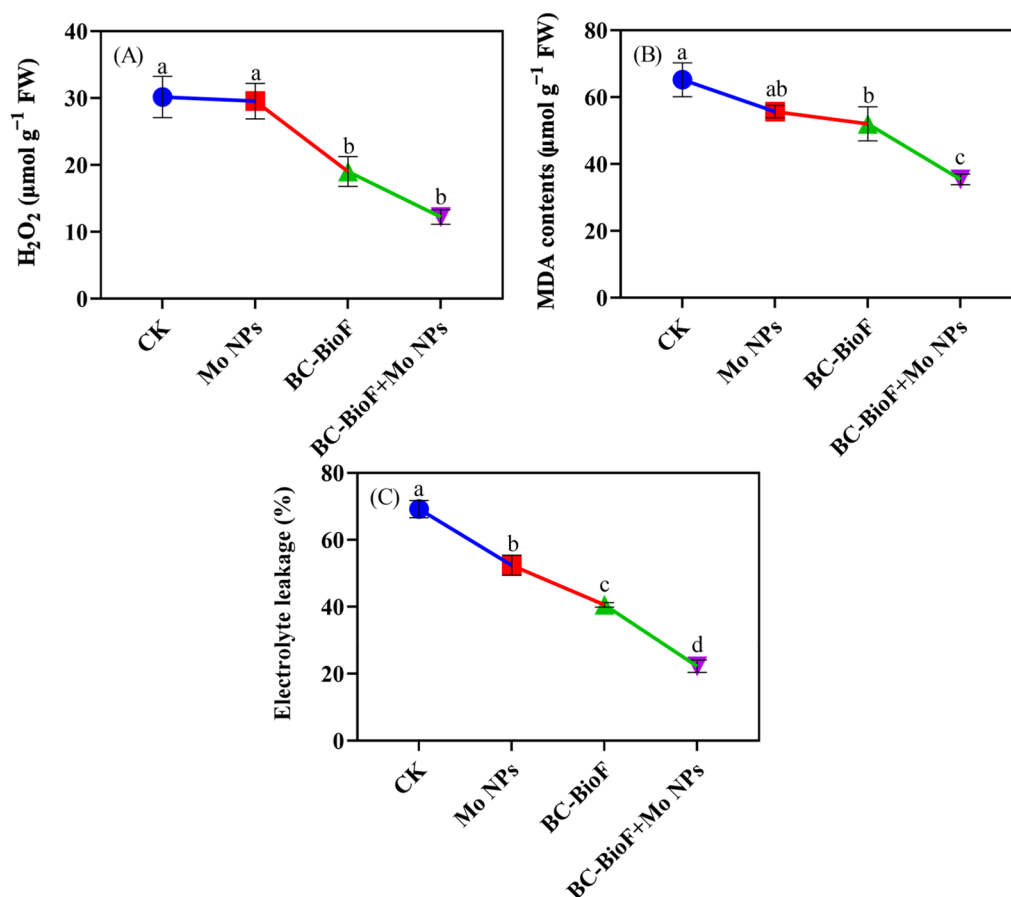


Fig. 6 Integrative effect of IPMM biochar biofilters and Mo NPs on biochemical analysis of **A** hydrogen peroxide, **B** malondialdehyde and **C** electrolyte leakage in cadmium-contaminated environment. The bars above the mean values represent the standard error (S.E., $n=3$). Different lowercase letters indicate statistically significant differences among treatments, as determined by the LSD test at $P \leq 0.05$

NPs supply could play a crucial role in the osmoregulation process of wheat, thereby mitigating the negative effects of Cd.

3.8 Membrane integrity in response to IPMM biochar biofilter and Mo NPs supply

Membrane integrity in wheat, associated with H_2O_2 accumulation, EL, and MDA generation, was significantly impacted by Cd stress, as evidenced by increased levels of H_2O_2 , MDA, and EL. However, the application of IPMM BC biofilter and Mo NPs supply notably ($P \leq 0.05$) reduced these contents by 59.41%, 45.70%, and 67.89%, respectively, under Cd stress conditions (Fig. 6A–C). In contrast, Mo NPs supply alone showed minimal reductions in H_2O_2 , MDA, and EL by 2.03%, 14.63%, and 24.29%, respectively. These results underscore that IPMM BC biofilter in conjunction with Mo NPs supply effectively maintained membrane integrity in wheat under Cd toxicity, indicating a significant role in mitigating Cd toxicity by reducing oxidative damage.

3.9 Influence of IPMM biochar biofilter and Mo NPs supply on enzymatic and non-enzymatic oxidative defense system under Cd toxicity

Activities of numerous antioxidant enzyme were assessed to understand the involvement of IPMM BC biofilter and Mo NPs supply in the oxidative defence systems under Cd toxicity. The findings demonstrated that Cd stress significantly decreased the activities of POD, SOD, CAT, and APX in wheat compared to the sole Cd stress treatment when IPMM BC biofilter and Mo NPs were not supplied. Interestingly, IPMM BC biofilter mitigated the effects of Cd toxicity more effectively when combined with Mo NPs treatment, and significantly increased their enzymatic activities. Specifically, the SOD activity increased from $55.0 \text{ U g}^{-1} \text{ FW}$ (CK) to $290.60 \text{ U g}^{-1} \text{ FW}$, POD 39.56 to $55.12 \text{ U s}^{-1} \text{ g}^{-1} \text{ FW}$, CAT 15.61 to $43.22 \text{ U min}^{-1} \text{ g}^{-1} \text{ FW}$, and APX 2.11 to $5.07 \text{ } \mu\text{mol min}^{-1} \text{ g}^{-1} \text{ FW}$ in BC-BioF + Mo NPs (Fig. 7). In contrast, BC-BioF treatment showed a minimal increase (2.32%) in POD activity compared to CK.

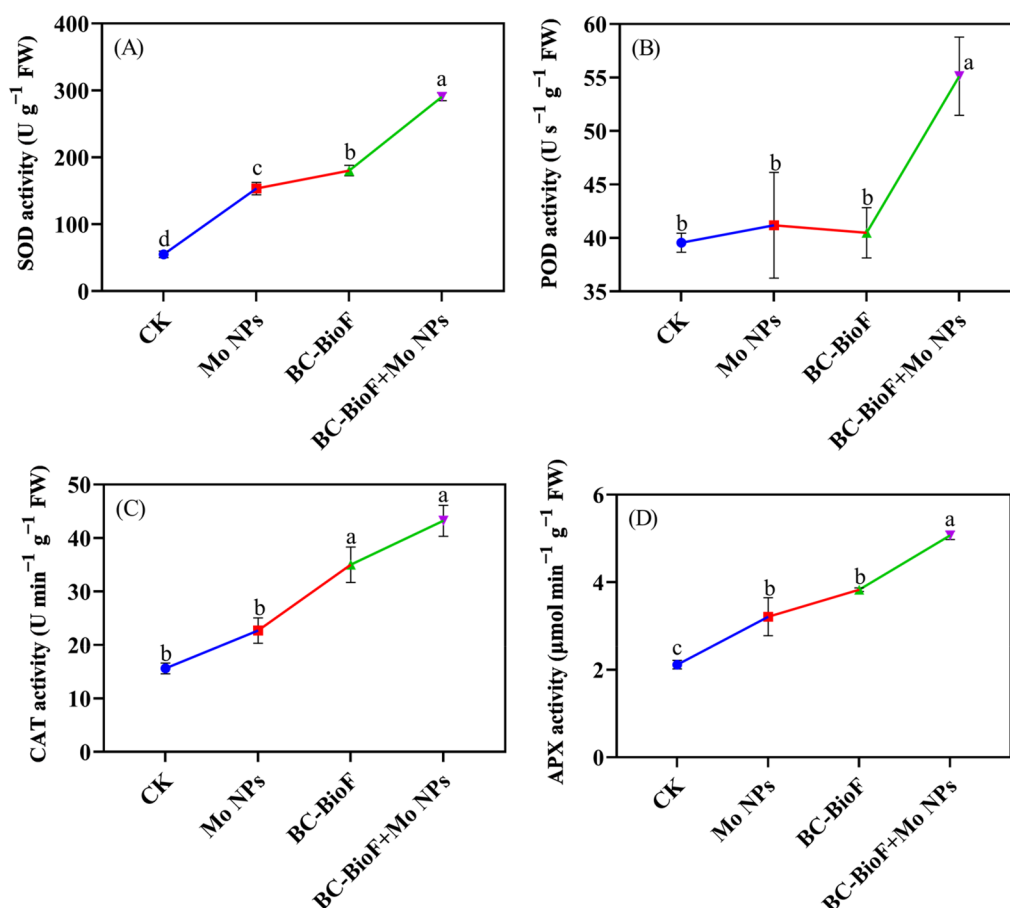


Fig. 7 Oxidative stress response of wheat plant under integrative supply of IPMM biochar biofilters and Mo NPs. **A** superoxide dismutase (SOD), **B** peroxidase (POD), **C** catalase (CAT), and **D** ascorbate peroxidase (APX). The bars above the mean values represent the standard error (S.E., $n=3$). Different lowercase letters indicate statistically significant differences among treatments, as determined by the LSD test at $P \leq 0.05$

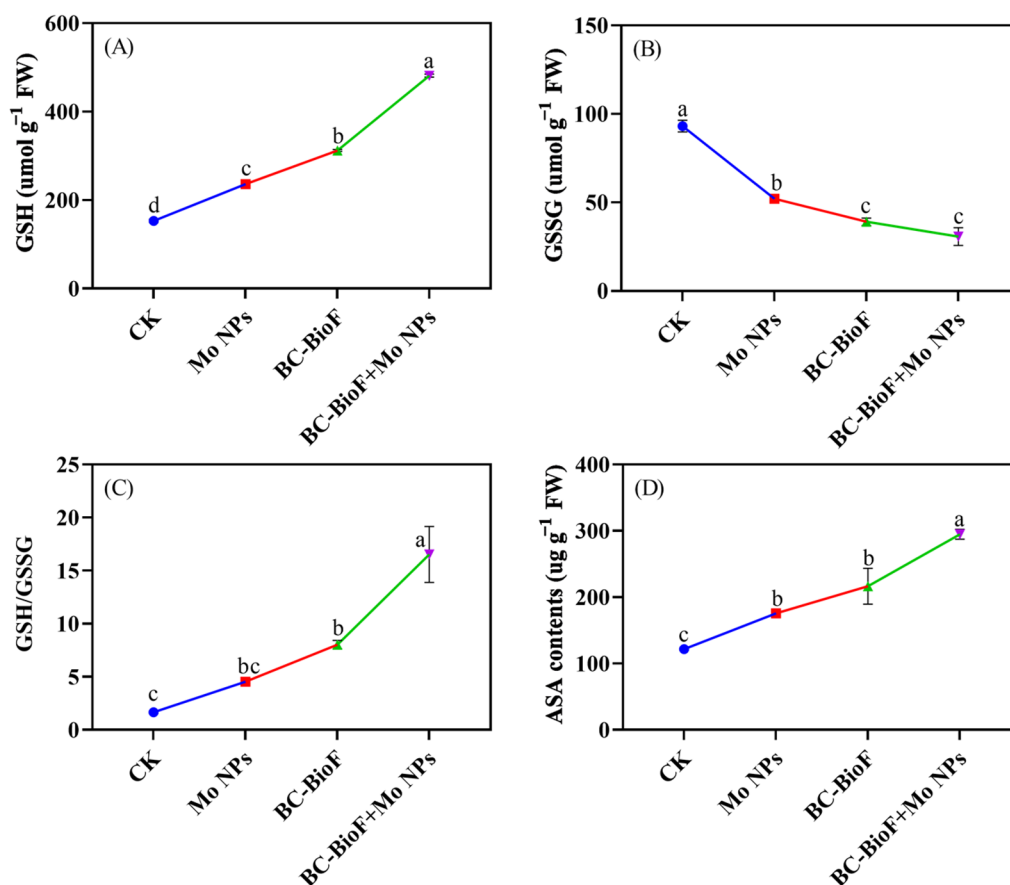


Fig. 8 Non-enzymatic oxidative stress response of wheat plant under IPMM biochar biofilters and Mo NPs. **A** GSH, **B** GSSG, **C** GSH/GSSG and, **D** ASA contents. The bars above the mean values represent the standard error (S.E., $n=3$). Different lowercase letters indicate statistically significant differences among treatments, as determined by the LSD test at $P \leq 0.05$

Additionally, glutathione contents were reduced significantly under sole Cd contamination. Whereas oxidized glutathione contents were significantly higher compared to other treatments (Fig. 8). Under Cd stress, Mo, BC-BioF, and BC-BioF + Mo NPs applications increased GSH content by 54.42%, 104.127%, and 214.59%, respectively, while decreasing GSSG by 43.99%, 57.96%, and 67.03% in wheat leaves. Moreover, in comparison to CK treatment, BC-BioF and Mo NPs supply increased the GSH to GSSG ratio in wheat leaves under Cd stress (Fig. 8C), although the results were not significant for sole BC-BioF and Mo NPs supply alone. These findings highlight the role of IPMM BC biofilter and Mo NPs in enhancing antioxidant defense mechanisms and maintaining redox homeostasis in wheat plants exposed to Cd stress.

3.10 Oxidative defense genetic expression alteration in response to IPMMM biochar biofilters and Mo NPs under Cd toxicity

Figure 9 illustrates how the presence of an IPMM BC biofilter and Mo NPs supply affected the genetic expression

of oxidative defense system in wheat under Cd toxicity. The expression of genes encoding antioxidants (SOD, POD, CAT and APX) was lower in the absence of Mo NPs and under IPMM BC biofilter conditions compared to the sole Cd stressed treatment (CK). Nevertheless, the impact of Cd stress varied depending on the presence of Mo NPs, BC-BioF, and BC-BioF + Mo NPs (Fig. 9). In the presence of Cd toxicity, supplementation with Mo NPs, BC-BioF, and BC-BioF + Mo upregulated the expression of CAT, SOD, APX and POD genes. The fold change ranged from 0.19 to 0.65 for SOD, 0.31 to 1.39 for POD, 0.56 to 4.39 for CAT, and 0.45 to 1.49 for APX across different treatments, while the highest change was observed in the Mo NPs, BC-BioF, and BC-BioF + Mo treatments (Fig. 9). Interestingly, the expression of the MDA-encoding gene in wheat was lower in treatments where Mo NPs, BC-BioF, or BC-BioF + Mo NPs were present under Cd toxicity compared to CK. Under Cd stress, the MDA gene was down-regulated by 56.54%, 72.89%, and 87.61% in Mo NPs, BC-BioF, and BC-BioF + Mo treatments, respectively. A heat map demonstrated clear differences

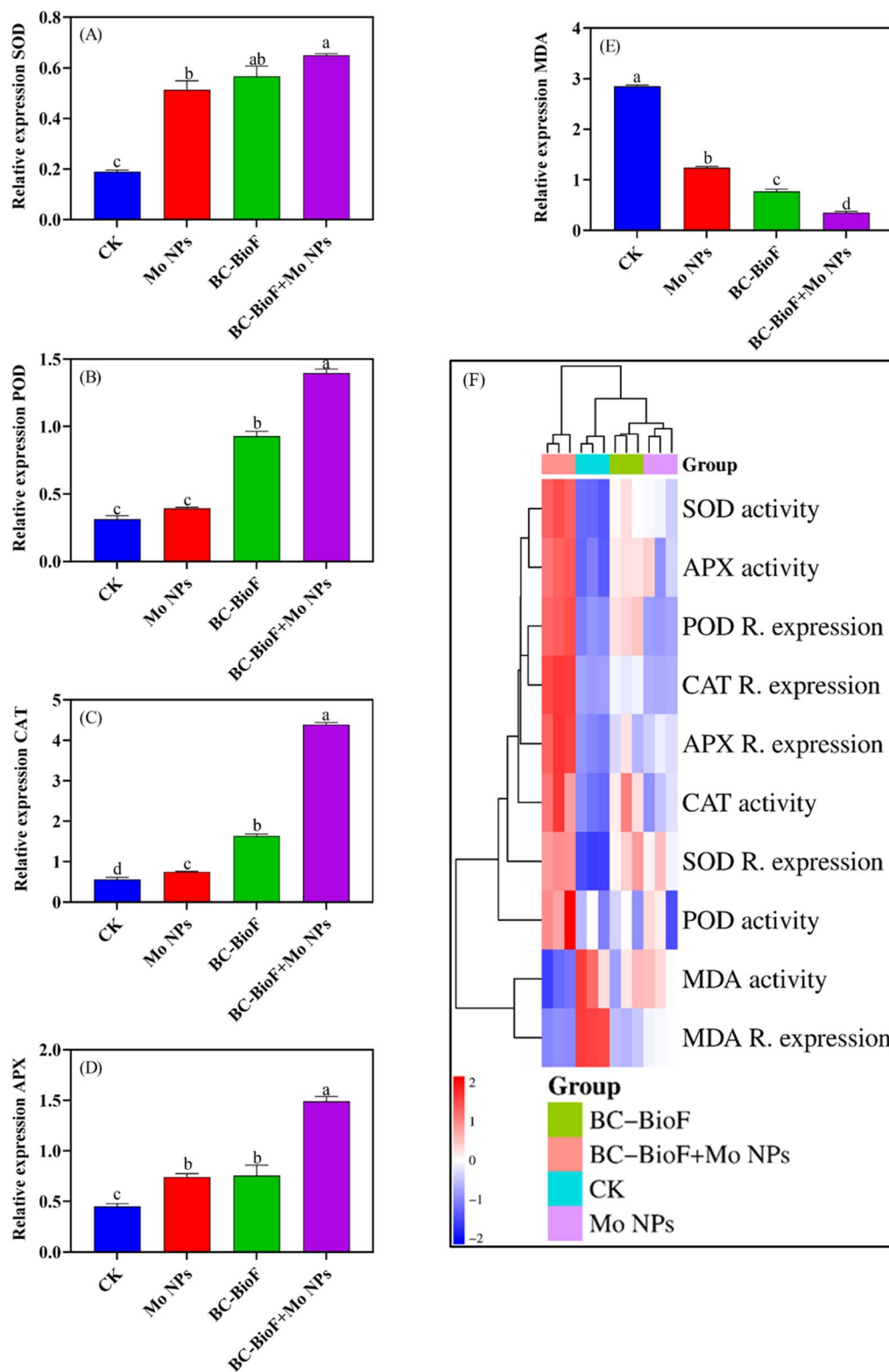


Fig. 9 Integrative effect of IPMM biochar biofilters and Mo NPs on qRT-PCR analysis of oxidative stress genes in a Cd-contaminated environment. **A** superoxide dismutase (SOD), **B** peroxidase (POD), **C** catalase (CAT), and **D** ascorbate peroxidase (APX), and **E** malondialdehyde (MDA). The heatmap showing the impact of different treatments on the activities of oxidative stress enzymes and their relative gene expressions (**F**). The bars above the mean values represent the standard error (S.E., $n=3$). Different lowercase letters indicate statistically significant differences among treatments, as determined by the LSD test at $P \leq 0.05$

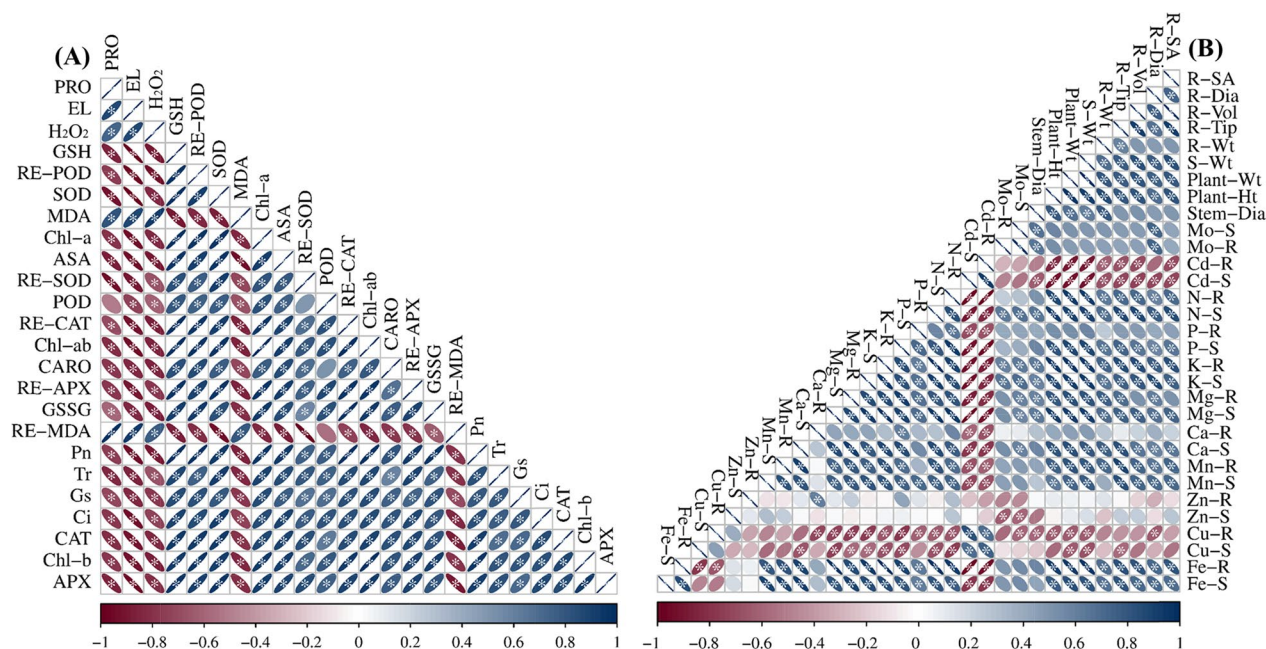


Fig. 10 Integrative effect of IPMM biochar biofilters and Mo NPs on correlation analyses between chemical, biochemical and molecular indicators affected by the cadmium, molybdenum and biochar. *Chl* chlorophyll, *EL* electrolyte leakage, *CARO* carotenoids, *RE* relative expression, *R* root, *S* shoot, *SA* surface area, *Dia* diameter, *Vol* volume, *Wt* weight, *Ht* height. "RE," "S," and "R" placed before or after different indices represent relative expression, shoot, and root, respectively

in the expression of oxidative genes across treatments (Fig. 9F).

3.11 Associations between the combined application of IPMM biochar filters and Mo NPs on different growth and biochemical attributes under Cd toxicity

Pearson's correlation analysis was conducted to investigate interrelationship between the combined application of IPMM BC biofilters and Mo NPs on various growth and biochemical characteristics of wheat under Cd toxicity (Fig. 10A, B). The concentration of Cd positively correlated with H_2O_2 , MDA levels, EL, and proline content. Conversely, it showed negative correlations with plant growth parameters such as biomass accumulation, plant height, photosynthetic characteristics, and soluble protein content. However, a weak relationship was noted between antioxidant enzyme activities and transcript abundance. As compared to Cd, the elemental profiles of primary, secondary, and micronutrients in roots and shoots positively correlated with plant height, biomass increase, root volume, diameter, length, and number of root tips. The contents of Tr, Gs, Pn, Ci, S-protein and oxidative responses in wheat plants indicated negative correlations with proline, H_2O_2 , EL, and MDA production. Chlorophyll content exhibited a strong correlation with photosynthetic pigments and oxidative stress attributes. Overall, this analysis highlights a strong

constructive interaction between plant physiological attributes and Mo NPs concentrations, while revealing destructive associations under Cd toxicity in wheat.

3.12 The impact size of IPMM biochar biofilters and Mo NPs under Cd toxicity

The scatter plot analysis, with a fold change cutoff 2 (Fig. 11A), revealed that out of 513 recorded observations, 123 were upregulated, 368 remained unchanged, and only 22 were downregulated. Similarly, using a cutoff at 1 with log2, the analysis showed 410 observations were upregulated and 103 were downregulated (Fig. 11B). These results indicate significant impacts of the treatments on wheat plants. The score and loading plots from principal component analysis (PCA), evaluating the effects of IPMM BC filter and Mo NPs treatments on various attributes of wheat plants, are depicted in Fig. 11C. The first two components, PC1 and PC2, contributed significantly, explaining 81.1% of the total variance in the dataset. Specifically, PC1 accounted for 74.2% and PC2 for 6.9%. All four treatments were clearly differentiated by the first two principal components, indicating that the combined application of IPMM BC biofilter and Mo NPs under Cd stress had a substantial ameliorative effect on the studied attributes of wheat compared to the control. The BC-BioF and BC-BioF+MoNPs treatments under Cd stress were notably separated from treatments such

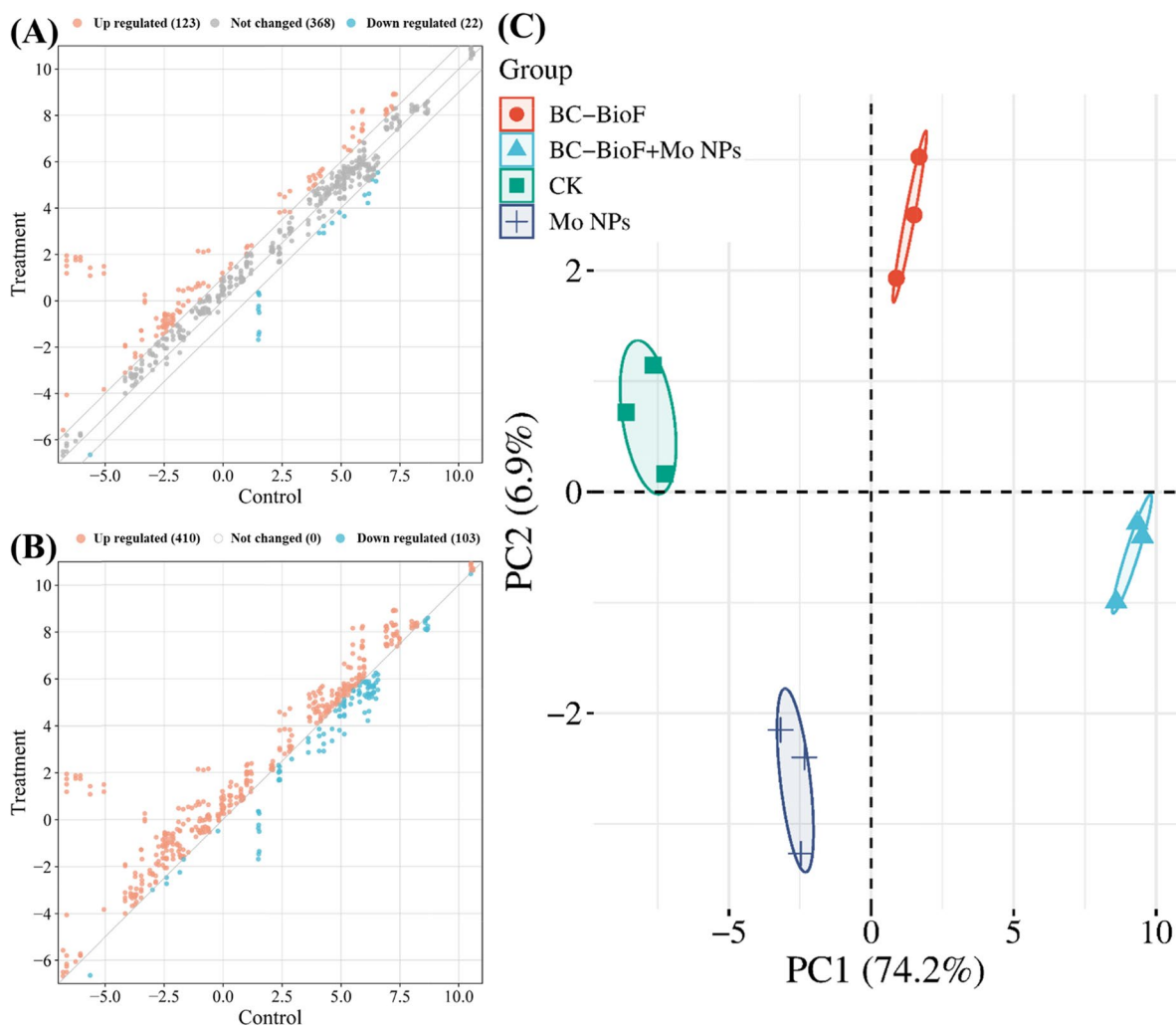


Fig. 11 Principal component (PCA) of physiological, biochemical and molecular characteristics studies in the current study across CK, Mo NPs, BC-BioF, BC-BioF + Mo NPs treatments. **A** Scatter plot with fold change cut-off 2, **B** Scatter plot at cut-off 1 with log2 representing the cumulative integrative effect of control vs treatment among 513 repeated observations in CK, Mo NPs, BC-BioF, BC-BioF + Mo NPs treatment. **C** PCA analysis showing the variation among different groups regarding the computed indicators

as the control and Mo NPs, suggesting that Cd stress had detrimental impacts on the studied attributes of wheat.

4 Discussion

4.1 Effect of IPMM biochar biofilter and Mo NPs on plant physiological characteristics and elemental profile in Cd contaminated environment

The findings of the study indicated that the BC biofilter derived from the IPMM and Mo NPs efficiently alleviated Cd-induced toxicity in wheat plants. This was evidenced by enhanced chlorophyll contents, improved gas exchange characteristics, optimized elemental profiles, and reduced Cd contents in plants (Fig. 4; Tables 2 and 3). Plant height and dry weight, usually used as growth indicators particularly under abiotic stress, notably

increased with the application of Mo NPs compared to the control (Table 1). Conversely, higher Cd levels in the control plants likely contributed to their reduced biomass (Table 1). Previous studies have consistently shown that Cd adversely affects plant growth under various experimental conditions (Bayçu et al. 2018; Shi et al. 2016; Wang et al. 2024a). This reduction in growth is associated with elevated levels of MDA, H_2O_2 , and EL, which disrupt critical plant processes such as chlorophyll biosynthesis, water balance, and cellular ultrastructure (Ali et al. 2013). The increased shoot length and biomass observed with Mo NPs clearly indicate their role in promoting wheat growth by enhancing Cd tolerance in wheat plants (Table 1). These findings align with previous studies that have demonstrated the ability of NPs to improve

plant growth under metal stress conditions (Sharifan et al. 2020; Zhang et al. 2019). Specifically, studies have reported that NPs can increase shoot length and biomass in plants exposed to Cd and Pb stress (Venkatachalam et al. 2017; Zhou et al. 2020). The enhanced wheat biomass observed with Mo NPs may be linked to their ability to alleviate oxidative stress in plants, as demonstrated in previous studies with *Leucaena leucocephala* seedlings treated with ZnO NPs under Cd and Pb stress (Venkatachalam et al. 2017). Additionally, the results illustrate that the BC biofilter derived from the IPMM, combined with Mo NPs, promoted wheat development in Cd-contaminated environments (Table 1). Biochar is well-known for its capability to improve plant growth under Cd and other metal stresses by enhancing nutrient availability (e.g., Zn and Mn) and reducing toxic metal levels in plants (Jing et al. 2020; Xu et al. 2016). This enhancement may be attributed to increased competition for metals and nutrients at root surfaces, as well as the immobilization of metals in the soil (O'Connor et al. 2018). The increased biomass observed in the combined treatments could be because of the dual impact of these amendments on plants (Phares et al. 2022), including enhanced levels of primary, secondary, and micronutrients, as well as increased Mo contents in plants treated with Mo NPs (Fig. 5). Furthermore, the application of IPMM BC biofilter reduced the bioavailable Cd in the contaminated environment (Table 2), leading to decreased Cd levels in wheat tissues (Table 2).

4.2 IPMM biochar biofilter and Mo NPs induced effect on root architecture in Cd contaminated environment

Consistently, root morphological factors such as cumulative root length, number of root tips, root volume, and surface area all highlight the beneficial role of IPMM BC biofilters and Mo NPs (Fig. 2). The combined application of IPMM BC biofilter and Mo NPs significantly improved wheat plant root architecture compared to treatments exposed solely to Cd stress, demonstrating a more pronounced effect than that observed in biomass weight and root morphology alone. Root architecture plays a crucial role in determining the ability of a plant to absorb water and nutrients, as well as its overall root longevity (Rewald and Meinen 2013). Previous research has presented that BC in combination with other amendments positively influences root shape and architecture (Yue et al. 2019). Therefore, the use of IPMM BC biofilter in conjunction with Mo NPs appears to mitigate Cd phytotoxicity to the roots by reducing Cd translocation to cell organelles. This mechanism helps in alleviating the adverse effects of HMs on root morphology and architecture, contributing to improved plant health and growth under Cd-contaminated conditions.

4.3 IPMM biochar biofilter and Mo NPs induced effect on photosynthetic efficiency and chlorophyll in Cd contaminated environment

The literature consistently demonstrates that photosynthetic pigments in plant leaves serve as critical indicators of oxidative stress induced by HMs (Pullagurala et al. 2018; Xia et al. 2015). In the current study, the IPMM BC biofilter and Mo NPs were found to increase chlorophyll contents and improve gas exchange characteristics in wheat (Fig. 4). The combined action of IPMM BC biofilter and Mo NPs alleviated the toxic effects of Cd, preventing the typical decline in pigment concentrations often linked to reduced plant growth and productivity. Our results showed that co-exposure of IPMM BC biofilter and Mo NPs resulted in higher photosynthetic pigment concentrations compared to control plants (Table 3), suggesting that IPMM BC biofilter and Mo NPs together helped in mitigating the Cd-induced disruptions to photosynthesis. Decreased pigment concentrations are often a primary factor leading to reduced plant growth and productivity. Our results indicated that control plants had lower photosynthetic pigment concentrations compared to treated plants (Table 3). This reduction in pigment concentrations can be attributed to elevated Cd levels in plants (Fig. 5), which disrupts plant photosynthetic capability and leads to Cd-induced decreases in photosynthesis (Chen et al. 2023). According to existing literature, BC and NPs have varying effects on plant photosynthesis, influenced by dosage and plant species. Micronutrient NPs, for instance, have been revealed to increase chlorophyll concentrations in plants stressed by metals (Pullagurala et al. 2018; Venkatachalam et al. 2017). Similarly, the application of SiO₂ NPs improved photosynthetic pigments in pea leaves under Cr stress. Changes in mesophyll cell and chloroplast structure under Cd stress are known to contribute to reduced photosynthetic pigments in plants (Hafeez et al. 2023; Sharma and Dietz 2009). In this study, the combination of IPMM BC and Mo NPs not only protected the mesophyll cell and chloroplast structures from Cd-induced damage but also promoted an increase in chlorophyll concentrations and improved photosynthetic parameters (Fig. 4). This enhancement in photosynthetic pigments can be attributed to reduced oxidative stress in wheat plants facilitated by the amendments, consistent with findings from previous studies utilizing various types of amended materials (Pullagurala et al. 2018; Venkatachalam et al. 2017).

4.4 Oxidative defense system and membrane integrity in response to IPMM biochar biofilter and Mo NPs supply under Cd toxicity

In metal-stressed conditions, plants struggle to detoxify ROS, leading to oxidative stress characterized by elevated

levels of MDA, EL, and H_2O_2 (Eskander and Saleh 2020; Srivastava et al. 2014; Yue et al. 2019). Electrolyte leakage is a reliable indicator of plant damage, often exacerbated by HMs such as Cd. Cadmium stress in plants initiates the production of excessive ROS as a primary response (Chamseddine et al. 2009). However, the synergistic application of Mo NPs and IPMM BC biofilters significantly enhanced the ability of plants to cope with this stress. Together, these two materials created a powerful defense system that boosted photosynthesis, improved osmolyte accumulation, and enhanced the antioxidant activities, ultimately leading to better growth and yield of wheat under Cd stress. Similarly, higher levels of cellular ROS are typically observed under abiotic stress compared to controlled conditions (Caverzan et al. 2016). The combination of Mo NPs and IPMM BC biofilter worked in synergy to trigger the metabolic processes, which in turn strengthened the oxidative defense system. This synergism reduced ROS levels by optimizing cell development and metabolic activities, alleviating the negative effects of Cd through regulation of physiological and biochemical processes. This includes improving root growth, maintaining osmotic balance and cell turgor pressure, and ensuring a steady balance between antioxidant enzymes and ROS (Ahanger and Agarwal 2017). Similarly, Alwutayd et al. (2024) found that BC treatment increased oxygen functional groups and porous structure, strengthening the antioxidant defence system against ROS.

Moreover, the Cd stress downregulated the genetic expression of antioxidant gene, leading to higher production of ROS. In contrast, the supply of IPMM biochar biofilters along with Mo NPs significantly upregulated the expressions of genes involved in oxidative stress response. This synergistic interaction not only upregulated the activity of antioxidant enzymes and their genetic expressions but also helped in maintaining the ROS levels. This increased resilience was attributed to intensified activities of crucial antioxidant enzymes, indicating a robust detoxification mechanism against ROS (Rahman et al. 2024). Additionally, the strategic upregulation of antioxidant enzyme activities and their genetic expressions within chloroplasts, key sites of photosynthesis and oxidative stress vulnerability, played a critical role in mitigating the oxidative damage.

The application of Mo NPs and IPMM BC biofilter, either individually or in combination, increased the activities of CAT, SOD, APX and POD in wheat while reducing oxidative stress under Cd stress (Fig. 4). Irshad et al. (2020) and Zhang et al. (2016) reported similar findings, indicating that BC improved the plant defense system in rice. The reduction in ROS and enhancement in antioxidant enzyme activity may contribute to maintaining the integrity of plant cell membranes (Rizwan et al. 2017).

The synergistic effect of IPMM BC biofilter and Mo NPs amplified the plant resistance to Cd toxicity, enforcing prime importance of their combined use in alleviating the oxidative damage and preserving the plant health.

4.5 Ultrastructural characterization of leaf mesophyll cells in response to IPMM BC biofilters and Mo NPs under Cd contamination

The negative effects of Cd on cellular organization are crucial for understanding its negative impact on plant physiological systems. Cadmium phytotoxic effects on chloroplast ultrastructure are associated with various biochemical abnormalities in lipids, proteins, and cellular connections. Transmission electron microscopy micrographs showed that wheat plants treated with both IPMM BC biofilters and Mo NPs exhibited well-organized chloroplast structures with properly arranged thylakoids, in contrast to those exposed solely to Cd stress, which resulted in amoeboid-shaped chloroplasts with damaged thylakoid membranes. The synergy between IPMM BC biofilters and Mo NPs becomes mainly evident when alleviating Cd induced damage to chloroplast structure, while IPMM BC biofilters alone reduced Cd translocation and detoxified the cellular environment. The addition of Mo NPs boosted this protective shielding effect through further stabilizing chloroplast structure and preserving the integrity of cellular membranes. On the other hand, under sole Cd stress, chloroplasts exhibited amoeboid shapes with extensive damage to the thylakoid membranes, as prominently observed in Fig. 3A. Cadmium stress can disrupt glucose transport through the phloem outside chloroplasts, leading to distorted mesophyll cells and chloroplast structures (Gratão et al. 2009; Mosa et al. 2016), which was more pronounced in plants treated solely with Cd (Fig. 3A) compared to those treated with IPMM BC biofilter and Mo NPs. Cadmium phytotoxic effects on cell organelles also lead to significant alterations in mitochondrial morphology as a detoxification approach against Cd harmful effects. In this defence mechanism, mature mitochondria incline to sequester phyto-available Cd to protect themselves from damage and reduce Cd potentially harmful effects on other mitochondria, thereby helping to maintain normal physiological functions (Ding et al. 2016). The observed changes in mitochondrial size and shape could be attributed to ROS generation, which induces enlargement, and breach of the outer membrane (Li and Xing 2011). However, the co-application of IPMM BC biofilter and Mo NPs helped in preserving the mitochondrial size and shape, prevented Cd-induced stress and supported the cellular detoxification processes. Furthermore, Cd induced phytotoxicity causes molecular disorder in thylakoids owing to biochemical deteriorations such as

protein heat denaturation and disruption of protein-lipid ratios by disrupting membrane protein complexes (El-Banna et al. 2018; Maleki et al. 2017). The ultrastructural analysis revealed that the co-exposure of IPMM BC biofilters and Mo NPs treatments countered the other changes in mesophyll leaf cells, including increased cell wall thickness, enhanced vacuolization, and vesicle-like structures with plasma membrane invaginations appearing in a wavy shape under Cd stress (Fig. 3A). The protective impact of IPMM BC biofilters, whether used alone or in combination with Mo NPs, mitigated these dysfunctions in mesophyll leaf cells (Fig. 3B, C, D). These findings underscore the potential of IPMM BC biofilter and Mo NPs to alleviate Cd-induced structural and functional disruptions in plant cells, highlighting their role in enhancing plant resilience under HMs stress conditions.

4.6 The Co-exposure influence of IPMM biochar biofilters and Mo NPs supply on Cd absorption and translocation in wheat

The concentrations of Cd in wheat tissues were notably lower in plants treated with the IPMM BC biofilter and Mo NPs compared to untreated plants (Table 2). The combined effect of the IPMM BC biofilter and Mo NPs was particularly significant in reducing Cd concentrations in wheat (Table 2). This substantial decrease in Cd content with the combined amendments can be attributed to their double action in Cd-stressed environments at both growth medium and plant levels. Biochar has been shown to reduce metal bioavailability (Wang et al. 2021), a phenomenon supported by our current investigation (Table 2). The decrease in bioavailable Cd in plants treated with IPMM BC biofilter and Mo NPs could be due to a shift in Cd fractionation within the growth medium, as BC has been demonstrated to decrease the bioavailable form of Cd and other metals in growth medium. Similar findings have been reported in previous studies, showing reduced Cd concentrations in the edible parts of plants after BC application (Munir et al. 2020; Tan et al. 2023). This reduction is attributed to decreased Cd transport from growth medium to roots and altered translocation capacity from roots to shoots (Liu et al. 2021; Wang et al. 2022). Our results align with earlier research indicating that high doses of BC can reduce Cd translocation from roots to shoots, consequently reducing Cd accumulation in crops such as rice (Jing et al. 2020; Meng et al. 2019). The translocation of HMs from roots to shoots is crucial in determining their accumulation in plants. Studies have also shown that the application of ZnO NPs can reduce Cd levels in wheat (Hussain et al. 2018; Venkatachalam et al. 2017). The decreased Cd levels in these plants may be partially due to increased nutrient levels competing with Cd, as documented in

previous studies (Wu et al. 2018; Wang et al. 2020). Our study also indicates a negative correlation between Cd levels and the levels of primary, secondary, and micronutrients in wheat tissues (Fig. 9). The improved elemental nutritional status achieved with the IPMM BC biofilter and Mo NPs may have contributed to reduced expression of Cd transporters in roots, thereby reducing Cd accumulation, as observed in Table 2. In contrast, studies have shown that Cd and Pb contents in plants like *L. leucocephala* increased under supply of ZnO NPs in hydroponic (Venkatachalam et al. 2017). Variations in metal build-up in plants under NPs supply can be attributed to factors such as NPs size, shape, crop species, and method of NPs application. Overall, the dual impact of the IPMM BC biofilter and Mo NPs was more effective in reducing Cd levels in wheat compared to sole Mo NPs under Cd toxicity. This greater reduction in Cd levels in wheat tissues can be attributed to the combined effects of these amendments on Cd accumulation, including enhanced Cd absorption by the IPMM BC biofilter and improved elemental translocation in roots and shoots facilitated by Mo NPs in Cd-contaminated environments. Studies have suggested that the redistribution of Cd within the plant system by applied amendments, including BC, plays a crucial role in controlling Cd availability to crops like wheat (Li et al. 2018). The use of an IPMM BC biofilter as a precautionary measure mitigates the negative effects of Cd on plants growing in Cd-stressed conditions, and potentially may reduce Cd levels in crops and minimizing Cd entry into the food chain. Thus, the combined use of the IPMM BC biofilter and Mo NPs presents a promising approach for reducing Cd contamination in agricultural systems and safeguarding food safety.

4.7 The Co-exposure influence of IPMM biochar biofilters and Mo NPs supply on elemental profile

In a Cd-contaminated environment, wheat treated with an IPMM BC biofilter and Mo NPs exhibited significantly higher concentrations of Mo in wheat tissues compared to untreated plants (Table 2). Mo NPs demonstrated synergistic effects with N, P, K, Mn, Ca, Mg, Fe, and Zn, while revealing a detrimental effect on Cd uptake. This is consistent with findings of Dimkpa et al. (2017), who demonstrated that Zn NPs enhanced sorghum production and grain nutritional quality under various conditions. Studies have also indicated that Mo (Imran et al. 2020) and BC (Li et al. 2019; Wang et al. 2019) can reduce Cd levels in rice grains while increasing nutrient concentrations. Similarly, the application of the IPMM BC biofilter and Mo NPs reduced Cd levels in wheat while enhancing the elemental profile in wheat plants. Previous research has shown that separate applications of Mo (Imran et al. 2020) and BC (Li et al. 2019; Wang et al.

2019) can limit Cd accumulation in plants. At the surface of root, Mo can contest with Cd, potentially limiting Cd uptake. Higher Mo NPs concentrations in roots (Table 2) may hinder Cd uptake by wheat, as Cd and Mo may be utilizing the similar transport channels during root uptake and translocation to shoots. These channels could become less available in the presence of elevated Mo NPs concentrations in roots. The current experiment demonstrated that the presence of an IPMM BC biofilter increased Mo concentrations in plants compared to the same Mo NPs treatment without an IPMM BC biofilter (Table 2). The elevated levels of N, P, K, Mn, Ca, Mg, Fe, and Zn in wheat plants treated with the IPMM BC filter and Mo NPs could be attributed to Cd sorption on IPMM BC, which creates a more favourable growth environment for plants, facilitating increased nutrient translocation to roots and shoots. As Zn concentrations increased due to Mo NPs and IPMM BC biofilters, Zn might reduce Cd levels in plants through antagonistic interactions, as observed in previous studies (Jiang et al. 2023). Moreover, the literature suggests that a reduction in Cd concentration in plants may be associated with an increase in Zn concentration. The application of organic amendments in combination with other treatments has been shown to reduce Cd uptake by wheat plants (ur Rehman et al. 2018). The varying effects of the IPMM BC biofilter and Mo NPs on plant nutrient levels and Cd accumulation could be attributed to differences in trial duration, growing conditions, BC sources, and crop species, among other factors. These complexities highlight the need for further research to optimize the use of IPMM BC biofilters and Mo NPs for the sustainable management of HMs-contaminated soils and crops.

5 Conclusion

Cadmium contamination remains a major environmental issue that poses a serious risk to human health. This research illustrates that the wise use of native resources, such as BC biofilters derived from the IPMM, combined with the application of Mo NPs, can ensure robust wheat growth in Cd-contaminated environments while simultaneously mitigating the adverse impacts of HMs and invasive plants. In this study, IPMM BC biofilters, with or without Mo NPs, improved osmoregulation, root architecture, and the oxidative defence system of wheat by regulating the genetic expressions while reducing the bioavailability of Cd. These amendments increased the root and shoot biomass of wheat and reduced the Cd contents in these parts by improving the elemental profile. The combined application of IPMM BC biofilters and Mo NPs was particularly more effective in reducing Cd contents in wheat roots and preserving mesophyll cell structure

and proper functioning. So, the combined use of IPMM BC biofilters and Mo NPs could be used to address Cd contamination in the environment. However, before recommending the application of IPMM BC biofilters and Mo NPs on a broader scale, further investigation is needed to validate these findings in various soil contamination levels, soil types, plant species, invasive species, and diverse climatic conditions.

Supplementary Information

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Supplementary material 1.

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Author contributions

Muhammad Shoaib Rana: Conceptualization, Writing an original draft, Methodology, Data curation, Software, Writing review & editing. Hong-Yu Chen: Conceptualization, Data curation, Software. Shi-Wen Deng: Data curation, Software. Muhammad Imran: Visualization, Data curation. Yousif Abdelrahman Yousif Abdellah: Visualization, Software. Wan-Ting Li: Conceptualization, Software. Jia-Yu Lin: Visualization, Investigation. Jia-Xi Li: Data curation, Software. Rui-Long Wang: Supervision, Writing, review & editing, Project administration, Funding acquisition.

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Availability of data and materials

The authors declare that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

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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