Environmental Technology & Innovation 36 (2024) 103811

Contents lists available at ScienceDirect

Environmental Technology & Innovation

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

Effects of titanium oxide nanoparticles and 24-epibrassinosteroid to mitigate the toxicity of cadmium (Cd) and improve physio-morphological traits of soybean (*Glycine max* L.) cultivated under Cd-contaminated soil

Fasih Ullah Haider [a,b,*], Usman Zulfiqar [c], Noor-ul- Ain [d], Tariq Mehmood [e], Babar Shahzad [f], Cai Liqun [a,**], Jean Wan Hong Yong [g,**], Manal Abdulaziz Binobead [h]

[a] *College of Resources and Environmental Sciences, Gansu Agricultural University, Lanzhou, China*
[b] *Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510650, China*
[c] *Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur, Pakistan*
[d] *Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, China*
[e] *Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Department Sensors and Modeling, Max-Eyth-Allee 100, Potsdam 14469, Germany*
[f] *School of Agriculture, Biomedine, and Environment La Trobe University Melbourne, Australia*
[g] *Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Alnarp, Sweden*
[h] *Department of Food Science and Nutrition, College of Agriculture Food Science, King Saud University, Riyadh, Saudi Arabia*

ARTICLE INFO

Keywords:
Cd stress
Plant physiology
TiO_2-NPs
24-epiBRs
Crop productivity
Nanoparticles
Heavy metals
Hormones
Brassinosteriod

ABSTRACT

Cadmium (Cd) toxicity is a serious environmental threat to living organisms. Nanoparticles (NPs) and plant growth regulators are able to mitigate Cd toxicity and restore crop growth in heavy metals-contaminated soils. However, the synergistic potential of combining 24-epibrassinosteroid (24-epiBRs) and titanium oxide nanoparticles (TiO_2-NPs) to alleviate Cd toxicity and restore soybean (*Glycine max* L.) production remains unexplored. Thus, a pot-based experimental trial was conducted to assess the effects of applying TiO_2-NPs (15 mg L^{-1}) and 24-epiBRs (10^{-7} M), individually and in combination, on soybean growth in soil cultivated with 30 ppm of Cd. The study revealed that Cd toxicity significantly inhibited soybean root length (11.0 %), root dry biomass (63.5 %), root fresh biomass (84.9 %), shoot length (11.7 %), shoot dry biomass (49.0 %), and shoot fresh biomass (27.3 %), compared to the control. Additionally, the toxicity of Cd enhanced the oxidative stress and lowered the photosynthetic efficiency, gas exchange characteristics, and antioxidant defense system of soybeans. Interestingly, the combined application of TiO_2-NPs and 24-epiBRs ameliorated the Cd toxic effects and improved the agronomic traits, photosynthesis efficiency, and antioxidant activity in soybeans by lowering oxidative stress. Specifically, the dual application of 24-epiBRs and TiO_2-NPs effectively lowered the Cd levels in roots, shoots, and leaves of soybean plants by 62.5, 162.7, and 87.1 %, respectively,

---

* Corresponding author at: College of Resources and Environmental Sciences, Gansu Agricultural University, Lanzhou, China.
** Corresponding authors.
E-mail addresses: haider281@scbg.ac.cn (F.U. Haider), cailq@gsau.edu.cn (C. Liqun), jean.yong@slu.se (J.W.H. Yong).

https://doi.org/10.1016/j.eti.2024.103811

relative to the control soybean plants grown under Cd stress. Overall, the combined treatment of TiO_2-NPs and 24-epiBRs synergistically reduced Cd uptake and restored soybean physiology in Cd-contaminated soils. Moving forward, further research should include field trials to assess the effectiveness and economic viability of this novel method.

---

1. Introduction

Heavy metals (HMs), despite having no essential requirement for plants, animals, and humans, pose serious consequences subject to exposure beyond their minimum threshold levels (Jiang et al., 2023; Shen et al., 2022). Within the spectrum of HMs, cadmium (Cd) is well known for its ubiquitous presence, and is classified as a non-essential and highly toxic element (Li et al., 2022; Pietrini et al., 2010; Song et al., 2019). Its detrimental effects on plant biology have made it a focal point of environmental and agricultural research (Lang et al., 2024; Liu et al., 2012; Nejad et al., 2017; Shaghaleh et al., 2024; Shen et al., 2022; Song et al., 2017; Yu et al., 2021). Environmental Cd contamination stems from natural processes and human activities, encompassing mineral mining, agricultural fertilizer application, and the release of untreated wastewater into ecosystems (Zong et al., 2022). Like other HMs, Cd is taken up by plant roots, distributed to various plant tissues (Kaya et al., 2019; Song et al., 2017; Song et al., 2019), and ultimately enters the food chain (Liu et al., 2012; Shahzad et al., 2018a). As a non-essential nutrient, Cd competes antagonistically with other essential nutrients by binding at the receptor sites, causing nutrient deficiency and toxicity, and ultimately affecting plant growth and development (Chen et al., 2019; de Bang et al., 2021). Elevated Cd levels will induce excessive reactive oxygen species (ROS) in plant tissues; triggering a phenomenon known as 'oxidative burst ' (Ghassemi-Golezani and Farhangi-Abriz, 2023). This rapid and transient ROS production causes damage to cellular organelles, disrupting vital biochemical processes (Abbas et al., 2020), damaging membrane integrity, and ultimately perturbing the redox equilibrium, and ultimately leading to cell death (Shah et al., 2023). However, plants have managed to evolve an efficient redox detoxification system known as antioxidant production that helps scavenge ROS and stabilizes the redox balance. To what extent plants can tolerate excessive Cd contents without compromising growth and development remains unanswered.

The urgency of developing and implementing effective, long-term soil reclamation strategies cannot be overstated (Shen et al., 2022; van der Ent et al., 2013; Zong et al., 2022). These strategies are crucial for extracting HMs, especially Cd, from agricultural soil and ecosystem (Jiang et al., 2023; Li et al., 2024; Liu et al., 2012; Nejad et al., 2017; Shen et al., 2022; van der Ent et al., 2013). They can be classified into biological, chemical, or physical, and their goal is either phytoremediating the contaminated soil or mitigating the environmental risks posed by soil pollutants (Haider et al., 2022; Shen et al., 2018, Shen et al., 2022). Another good method is to use the stabilization/solidification technique, which involves adding things like coal fly ash, lime, or cement to make sure that contaminants in polluted soil or water stay stable over time (Moon et al., 2009; Alpaslan and Yukselen, 2002). Stabilization changes contaminants into less toxic, mobile, and soluble forms (Shen et al., 2018). Solidification, conversely, makes the wastes solid by enclosing them in a stable, strong, and unified structure (Haider et al., 2022). Some other methods include using barium acetate to clump together the large-sized contaminants, using hydroxide compounds (like $Ca(OH)_2$, $Mg(OH)_2$, and NaOH) to remove the harmful metal ions, and using phosphorous extracts, aqueous nitrogen-donor extracts, and Cyanex-30 to remove toxic pollutants (Nejad et al., 2017). However, these methods may not be viable for small-scale metal contamination; may pose groundwater pollution hazards under certain circumstances (Haider et al., 2022). Therefore, it is crucial to harness cost-efficient, feasible, effective, and ecologically-compatible restoration methods. These adopted strategies should aim at reducing the negative effects of Cd-linked toxicity on plants, minimize adverse environmental effects, and supporting sustainable agricultural practices.

Researchers have explored various strategies to mitigate the adverse effects of abiotic stresses. One of the most promising and popular approaches is the application of nanotechnology to address HMs stress in plants (Azarin et al., 2024; Chen et al., 2023; Haider et al., 2024; Sani et al., 2023; Zhou et al., 2020; Jalil et al., 2023). Nanotechnology, specifically the use of chemical or organically synthesized nanoparticles (NPs) smaller than 100 nm, has shown significant potential in restoring plant growth under HMs stress (Azarin et al., 2024; Sardar et al., 2022; Sunny et al., 2022; Jalil et al., 2023). For instance, the application of titanium dioxide nanoparticles ($gTiO_2$-NPs) under chromium (Cr) stress conditions resulted in the improved growth of sunflowers (*Helianthus annuus*; Kumar et al., 2024). The biological improvement was linked to better foliar photosynthetic efficacy and decreased oxidative stress (Kumar et al., 2024). The application of TiO_2-NPs has also been successful in ameliorating the negative impacts of Cd toxicity in different crops such as rice (*Oryza sativa*), chestnut wine (*Tetrastigma hemsleyanum*), wheat (*Triticum aestivum*), cow-pea (*Vigna unguiculata*) and ramie (*Boehmeria nivea*; Ogunkunle et al., 2020; Chen et al., 2023; Deng et al., 2023; Lai et al., 2023; Huang et al., 2024). Research findings indicated that TiO_2-NPs ameliorated the adverse effects of HMs stress on plant development through various mechanisms, including modulating the expression of metal transport genes, lowering ROS production, and improving antioxidant activity by creating metal-binding proteins and chelating agents (Deng et al., 2023; Lai et al., 2023; Kumar et al., 2024). Thus, the potential of nanotechnology in agriculture is indeed promising, offering hope for restoring plant productivity under HMs stress conditions.

In addition to NPs, phytohormones (auxins, cytokinins, gibberellins, karrikins, abscisic acid, salicylic acid) and synthetic growth modulators, are crucial in regulating many growth processes and enabling the plants to cope with environmental stressors (Ahammed et al., 2023; Betti et al., 2021; Khripach et al., 2000; Pan et al., 2017; Peng et al., 2021; Tarkowski et al., 2009; Wong et al., 2015; Yu et al., 2004; Sabagh et al., 2022; Jameson, 2023). Within these growth substances and modulators, the brassinosteroids (BRs)are steroidal phytohormones with interesting biological functionality (Haubrick and Assmann, 2006; Shahzad et al., 2018b); these growth substances have garnered significant attention in recent years due to their capacity to ameliorate biotic and abiotic stresses in plants (Rehman et al., 2021a; Suzuki et al., 2014; Wong et al., 2015). The application of 24-epibrassinolide (24-EBL) has shown remarkable properties in regulating biotic and abiotic stressors (Soares et al., 2020; Shahzad et al., 2018b). Anuradha and Rao (2007) reported that radish (*Raphanus sativus*) exposed to exogenously applied 24-EBL showed reduced adverse effects of Cd toxicity. This was achieved by increasing the production of free proline and antioxidants enzymes, such as ascorbate peroxidase (APX), guaiacol peroxidase (GPOD), catalase (CAT), superoxide dismutase (SOD; Kaya et al., 2020). Similarly, 24-EBL applied to Cd-stressed mustard (*Brassica juncea* L.) plants improved carbonic anhydrase activity, photosynthetic pigment content, photosynthesis, and osmotic regulation (Huang et al., 2024). Moreover, the enhanced production of non-enzymatic and enzymatic antioxidants facilitated the neutralization of ROS, thereby improving plant resilience under harsh environmental conditions through the mitigation of oxidative stress (Jakubowska and Janicka, 2017; Rehman et al., 2021b). The role of 24-EBL is well established and utilized in mitigating the negative effects of elevated Cd in various crops such as rice (Sun et al., 2024), tomato (*Solanum lycopersicum*; Hayat et al., 2012), and mustard (Soares et al., 2020).

The individual use of TiO_2-NPs and 24-epiBRs was proven effective in mitigating the negative effects of Cd and other HMs, leading to improvements in the yields of crops cultivated in contaminated soils (Rehman et al., 2021a; Sunny et al., 2022; Zhou et al., 2020; Zulfiqar et al., 2022). Interestingly, recent literature reported that the purposeful combinations of two or more dissimilar substances may interact synergistically, and enhance growth and resilience over a single substance (Chowdhury et al., 2024; Sani et al., 2020; Sani and Yong, 2022). In view of these research developments, more comprehensive research needs to be conducted on the effects of applying these two treatments together, particularly in legume plants like soybean (*Glycine max*), to ameliorate Cd toxicity, and commitantly restoring crop productivity. Therefore, this research was performed to examine the effectiveness of applying TiO_2-NPs and 24-epiBRs alone; and in combination, to synergistically reduce the negative effects of Cd while improving the growth of soybean plants in Cd-polluted soil. In addition, the role of TiO_2-NPs and 24-epiBRs was also examined in relation to the oxidative and anti-oxidant defense systems during high Cd exposures. It was hypothesized that TiO_2-NPs and 24-epiBRs would effectively minimize Cd concentration in soybean plants by regulating their oxidative and anti-oxidative defense systems, thereby improving soybean growth and development in Cd-contaminated soils.

2. Materials and methods

2.1. Collection of soil samples and experimental design

The soil samples for this experiment was collected from the uppermost 20 cm layer of an agricultural field at South China Agricultural University (SCAU) in Guangzhou, China. The soil was then sieved through a 2 mm mesh to ensure uniformity. Key soil properties, including soil pH, total and available essential nutrients (N, K, and P), and organic matter, were assessed using standard procedures. Table S1 presents data on the physicochemical properties of soil used in this study. As a continuation of our research, based on our previous findings, we added cadmium chloride ($CdCl_2$) to the soil at a concentration of 30 parts per million (ppm). Based on our previous research, $CdCl_2$ was added to the soil at a concentration of 30 ppm, and the Cd-spiked soil was left to stabilize for four weeks at ambient temperature, with periodic mixing to ensure uniform Cd distribution in the agricultural soil.

The TiO_2-NPs used in this study were obtained from Shanghai Chaowei Nanotechnology Co., Ltd., with a purity level of 99.9 %. The 24-epiBRs were acquired from Shanghai Acmec Biochemical Co. Ltd., Shanghai, China. Figure S1 illustrates the structural characteristics of TiO_2-NPs. The soybean (*Glycine max* (L.) Merr.) cultivar "Longhuang 3" used in this experiment was sourced from the laboratory of Gansu Agricultural University in Lanzhou, China. The pot experiments were carried out in a wire house at SCAU from late April to mid-July 2023. The wire house was enclosed with a polythene sheet to shield the plants from rain, and the temperature was consistently maintained at 25°C to provide suitable growth conditions for the soybean plants. Every pot in the experiment contained 3 kg of soil, and six soybean seeds were sown per pot. The experiment was conducted using a completely randomized design, with eight treatments arranged factorially and having three replications. The experimental treatments consisted of different levels of two Cd (Cd_0 control = 0 ppm Cd and Cd_1 = 30 ppm Cd) treatments, two TiO_2NPs (NP_0 control = 0 mg L^{-1} TiO_2-NPs and NP_1= 15 mg L^{-1} TiO_2-NPs) treatments, and two 24-epiBRs (BRs_0 control = 0 M 24-epiBRs and BRs_1 =10^{-7} M 24-epiBRs) treatments. The control groups were used as references for comparison. The doses of TiO_2-NPs and 24-epiBRs were standardized by conducting pre-experimental trials, and the application of TiO_2-NPs and 24-epiBRs to the leaves of soybean plants was carried out twice, specifically at 15 and 30 days after the emergence of the plants. Every experimental pot was given 0.217 g of urea, 0.0946 g of di-ammonium phosphate (DAP), and 0.05 g of potassium oxide. Distilled water was regularly applied to each experimental pot to maintain the optimum moisture levels throughout the experimental duration. Manual weeding was performed to maintain a weed-free environment, and the remaining agronomic practices were kept constant throughout the experiments. All the experiments were done in compliance with relevant institutional, national, and international guidelines and legislations. High research standards were maintained throughout the experiments and following the various established scientific protocols (Cornelissen et al., 2003; Pang et al., 2023; Yong et al., 2014).

2.2. Soybean harvesting and biomass observation

The soybeans were harvested after 60 days of soybean emergence. The shoot and root lengths of the soybean were recorded using a ruler. The plants were then divided into aerial and subterranean portions, with the fresh biomass determined for each using a weight balance. The weight balance was used to determine the fresh biomass of both groups. Subsequently, these sections were oven-dried for several days to obtain dry weight measurements. The dried plant material was preserved for later Cd content analysis.

2.3. Assessment of gas exchange and photosynthetic pigments

Following 60 days of soybean emergence, measurements were taken to assess the levels of photosynthetic pigments. We performed gas exchange measurements on soybean plants using the methodology outlined by Chen et al. (2023) and Yong et al., (2010). The SPAD chlorophyll index of fully mature leaves were selected from the top section; was assessed non-destructively using a SPAD meter (SPAD 502, Konica Minolta, Osaka, Japan)(Yong et al., 2010).. The gas exchange properties of soybean leaves were evaluated using a portable and open system gas exchange instrument (model LI-6800, LI-COR, Lincoln, NE, USA). This assessment included measuring the intercellular carbon dioxide concentration (Ci), transpiration rate (Tr), photosynthetic rate (A), stomatal conductance (gsw), and chlorophyll fluorescence (Fv/Fm); these gas exchange calculations were in accordance to Farquhar et al.(1980). The gas exchange characteristics were assessed by measuring the fully inflated penultimate leaves between 10:00 and 12:00 h, using the methods outlined by Yong et al., (2010).

2.4. Measurement of MDA, H_2O_2, and EL content

After 60 days of soybean emergence, one soybean plant was harvested to observe soybean leaves' oxidant activity (MDA, H_2O_2, and EL). The concentration of malondialdehyde (MDA) was determined using the method outlined by Heath and Packer (1968). In brief, a 0.25 g sample of a recently collected leaf was extracted using a solution containing 1 % trichloroacetic acid (TCA). After centrifugation, we mixed the liquid component of the sample with a solution containing 0.5 % thiobarbituric acid and 20 % trichloroacetic acid (TCA). MDA was measured using an extinction coefficient of 155 mM^{-1} cm^{-1}. The quantification of MDA was carried out using an extinction coefficient of 155 mM^{-1} cm^{-1}. The H_2O_2 from soybean leaves was extracted using a specific method, and its absorbance was measured at a particular wavelength, following a methodology described in a previous study. Afterward, finely chopped soybean leaves weighing 0.25 g were added to a vertical test tube containing 20 mL of distilled water to measure electrolyte leakage (EL). We measured the initial electrical conductivity (EC) of the solution. The tubes were then placed in an autoclave and heated to 100 °C for 30 minutes. The filtrate's final electrical conductivity (EC) was higher when the samples were cooled following autoclaving, suggesting a state of stress. The EL of soybeans was determined according to the protocol by Dionisio-Sese and Tobita (1998).

2.5. Measurements of SOD, POD, and CAT content

A spectrophotometer was utilized to quantify the enzyme activity of the antioxidant enzymes: SOD, POD, and CAT. Subsequently, the samples were pulverized using liquid nitrogen. Soybean sample standardization was conducted using a 0.05 M phosphate buffer with a pH of 7.8. The standardized and filtered samples underwent centrifugation for 10 minutes at a speed of 12,000 g at a temperature of 4°C. The resulting supernatant from the centrifugation was utilized to measure antioxidant enzymes. Catalase activity was assessed using enzyme assays according to the protocol described by Aebi (1984). For sample preparation, 100 μL of 300 mM H_2O_2 and 100 μL of enzyme extract were mixed with 2.8 mL of 50 mM phosphate buffer that had two mM CA in it (pH 7.0). The mix had a final volume of 3 mL. The catalase (CAT) activity measurement was determined by measuring the change in absorbance at 240 nm, an indicator of CAT activity in this stage. Leaf samples were crushed in a pre-cooled pestle and mortar, and then a pH 7.8 phosphate buffer was added. The sample was then placed in test tubes to be examined by SOD and POD activities. After being prepared at four °C, the sample was centrifuged for 20 minutes at 12,000 rpm. Utilizing Zhang's (1992) methodology, the SOD and POD activities were evaluated.

2.6. Cadmium levels in soybean leaves, shoots, roots, and soil

The soybean leaves, shoots, roots, and soil were oven-dried and then ground into a fine powder. Subsequently, following the methodology outlined by Ryan et al. (2001) where 0.25 g of the appropriate samples were introduced into a solution containing nitric acid and perchloric acid HNO_3:$HClO_4$ (6 mL) at a ratio of 5:1 (v/v). The mixture was allowed to undergo digestion overnight until only 1 mL of liquid remained. The liquid's initial volume of 1 mL was then diluted with distilled water to achieve the intended final volume of 50 mL. Once the liquid had cooled, the concentration of Cd^{2+} was determined using an atomic absorption spectrophotometer (Model 3200-C, Heinz Walz GmbH, Effecltrich, Germany).

2.7. Statistical analysis

The data were analyzed using the SPSS statistical software, utilizing the LSD test to ascertain significant distinctions. The results were reported as the average values and the standard error, and a significance level of $P < 0.05$ was set. Each treatment was reproduced three times, and the standard deviation (SD) and mean results were considered. In addition, a thorough study of multivariate data patterns was performed using R software for principal component analysis (PCA) and correlation analysis. The significance level between treatments was evaluated using the least significant difference (LSD) test with a 95 % confidence level. Additionally, a three-way ANOVA was conducted to assess the interaction effects between the treatments. The graphs were generated using SigmaPlot (version 15).

3. Results

3.1. Morphological traits of soybean

As shown in Table 1, adding TiO_2-NPs and 24-epiBRs had a significant ($p \leq 0.05$) impact on the root and shoot shapes of soybean growth when high levels of Cd were present. The study found that Cd phytotoxicity greatly decreased the soybean roots length (RL), dry root biomass (DRB) and fresh root biomass (FRB), shoots length (SL), and dry shoot biomass (DSB) and fresh shoot biomass (FSB) of soybean plant by 11.0, 63.5, 84.9, 11.7, 49.0, and 27.3 %, respectively, when compared to the control group. However, using TiO_2-NPs alone or with 24-epiBRs greatly reduced Cd's harmful effects on the morphological traits of soybeans (Fig. 1). Interestingly, it was observed that the applying TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs considerably ($p \leq 0.05$) improved the RL, DRB, and FRB by (5.1, 11.9, and 32.0 %), (20.9, 25.1, and 40.8 %), and (20.9, 25.1, and 40.8 %), respectively, compared to control soybean plants grown under non-contaminated Cd soil. Applying TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs also made the RL, DRB, and FRB much better than in control soybean plants grown under Cd stress, by (12.0, 12.1, and 26.3 %), (18.6, 26.21, and 52.2 %), and (33.0, 36.3, and 54.7 %), respectively. Similarly, using TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs greatly increased the SL, DSB, and FSB by (10.1, 9.4, and 25.5 %), (10.2, 12.5, and 30.3 %) and (10.2, 12.8, and 32.0 %), respectively, higher as compared to control soybean plants grown under non-contaminated Cd soil. Furthermore, application of TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs significantly ($p \leq 0.05$) improved the SL, DSB, and FSB by (3.8, 5.8, and 25.7 %), (21.3, 23.8, and 35.7 %), and (20.4, 25.3, and 34.4 %), respectively, as compared to control soybean plants grown under Cd stress.

3.2. Photosynthesis activity of soybeans

According to the ANOVA results (Table 1), adding TiO_2-NPs and 24-epiBRs significantly ($p \leq 0.05$) altered soybean foliar gas exchange growing under Cd-contaminated soil. The findings revealed that Cd was phytotoxic to soybean leaves, reducing the SPAD value, intercellular carbon dioxide concentration (Ci), transpiration rate (Tr), photosynthesis rate (A), stomatal conductance (gsw), and water use efficiency (Fv/Fm) by 43.9, 46.7, 48.3, 45.8, 39.9, and 30.3 %, respectively, compared to the control. Also, using TiO_2-NPs alone or with 24-epiBRs greatly decreased the harmful effects of Cd on the photosynthesis activity and gas exchange traits of soybeans (Fig. 2). Applying TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs significantly ($p \leq 0.05$) improved the SPAD value, Ci, and Tr of soybean leaves by (4.1, 7., and 13.6 %), (8.1, 11.3, and 29.4 %), and (11.1, 12.9, and 25.7 %), respectively, as compared to control soybean plants grown under non-contaminated Cd soil. When growing soybean plants under Cd stress, adding TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs significantly ($p \leq 0.05$) increased the leaves' SPAD value, Ci, and Tr. The increases were 13.9 %, 14.1 %, and 26.7 % for SPAD value; 20.8 %, 22.2 %, and 41.1 % for Ci; and 14.9 %, 17.8 %, and 29.1 % for Tr. Additionally, using TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs greatly enhanced the A, gsw, and Fv/Fm of soybean plants by 10.3, 13.2, and 24.6 %

Table 1
The factorial ANOVA results highlighted the effects of Titanium oxide nanoparticles (TiO_2-NPs), 24-epibrassinosteroid (24-epiBRs) amendment and cadmium (Cd) on the various parameters of soybean (*Glycine max* L.) plants.

Variables	Cd	Ti-NPs	24-epiBRs	Cd × TiO_2-NPs	Cd × 24-epiBRs	TiO_2-NPs × 24-epiBRs	Cd × TiO_2-NPs × 24-epiBRs
Root length	0.0006	**<0.001**	**<0.001**	0.0592	0.5416	0.0064	0.0847
Root dry biomass	**<0.001**	**<0.001**	**<0.001**	0.9286	0.8921	0.0603	0.2811
Root fresh biomass	**<0.001**	**<0.001**	**<0.001**	0.1275	0.8591	0.1353	0.5110
Shoot length	0.0001	**<0.001**	**<0.001**	0.9605	0.5567	0.0053	0.3982
shoot dry biomass	**<0.001**	**<0.001**	**<0.001**	0.6001	0.6265	0.2039	0.1230
shoot fresh biomass	**<0.001**	**<0.001**	**<0.001**	0.8342	0.4711	0.2198	0.0454
SPAD value	**<0.001**	0.0006	0.0023	0.5342	0.2284	0.5261	0.9093
Ci	**<0.001**	**<0.001**	**<0.001**	0.4669	0.0888	<0.001	0.0485
Transpiration rate	**<0.001**	0.0003	0.0012	0.5841	0.5347	0.4869	0.5997
A	**<0.001**	**<0.001**	**<0.001**	0.6623	0.9919	0.2257	0.8936
Stomatal conductance	**<0.001**	**<0.001**	**<0.001**	0.9555	0.8866	0.0579	0.8615
Fv/Fm	**<0.001**	0.0004	0.0019	0.9298	0.8426	0.1429	0.9533
MDA	**<0.001**	**<0.001**	0.0033	0.0197	0.0886	0.0807	0.6737
H_2O_2	**<0.001**	**<0.001**	**<0.001**	0.1914	0.1338	0.0537	0.7828
EL	**<0.001**	0.0019	0.0115	0.2134	0.2293	0.7304	0.2334
SOD	**<0.001**	**<0.001**	**<0.001**	0.0012	0.0024	0.0033	0.0484
POD	**<0.001**	<0.001	0.0001	0.0210	0.0272	0.3992	0.4633
CAT	**<0.001**	**<0.001**	**<0.001**	0.9559	0.3832	0.0006	0.2767
Cd leaves	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.1116	0.1246
Cd shoot	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.3113	0.4392
Cd root	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.0001	0.1503	0.1314
Cd soil	**<0.001**	0.0001	0.0001	0.0001	0.0001	0.0290	0.0273

Note: Bold fonts highlighting $P < 0.001$. chlorophyll content = SPAD value; Intercellular carbon dioxide concentration = Ci; transpiration rate = Tr; photosynthetic rate = A; stomatal conductance = gsw; chlorophyll fluorescence = Fv/Fm; malondialdehyde = MDA; hydrogen peroxide = H_2O_2; electrolyte leakage = EL; Superoxide dismutase = SOD; peroxidase = POD; catalase = CAT; cadmium = Cd.

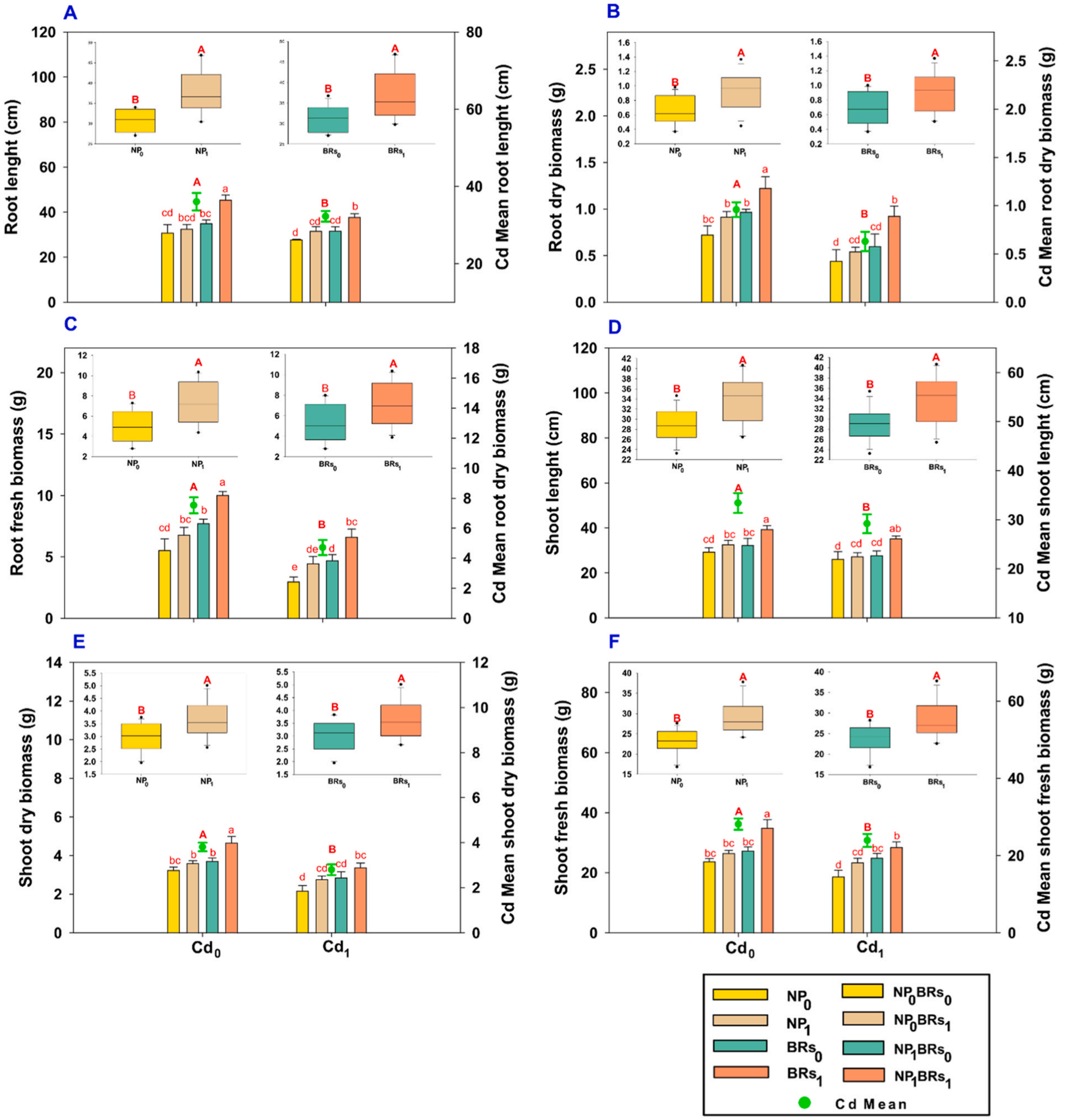

Fig. 1. Effects of sole and combined application of titanium oxide nanoparticles (TiO_2-NPs) and 24-epibrassinosteroid (24-epiBRs) on morphological traits **(A)** root length, **(B)** root dry biomass, **(C)** root fresh biomass, **(D)** shoot length, **(E)** shoot dry biomass, and **(F)** shoot fresh biomass of soybean plants grown under cadmium (Cd)-contaminated agricultural soil. The various letters indicated that there are notable differences in the values, as determined by the Least Significant Difference Test (LSD) and three-way ANOVA analysis in the R statistical package. The means (based on three replications) are reported along with the standard error (SE). Note: Cd_0 (Control; 0 ppm Cd); Cd_1 (30 ppm Cd); BRs_0 (0 M 24-epiBRs); BRs_1 (10^{-7} M 24-epiBRs); NP_0 (0 mg L^{-1} TiO_2-NPs); and NP_1 (15 mg L^{-1} TiO_2-NPs).

compared to control soybean plants grown in Cd-free soil; 15.8, 16.7, and 22.3 % compared to 5.1, 7.2, and 17.9 % compared to control soybean plants. Adding TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs to soybean plants under Cd stress made A, gsw, and Fv/Fm much better than in control plants by 14.92, 20.5, and 33.4 %, 19.4, 21.5, and 28.4 %, and 8.1, 10.1, and 23.4 %, respectively.

3.3. Oxidant and antioxidant defense system of soybeans

The ANOVA results demonstrated a significant ($p \leq 0.05$) impact on soybean growth's oxidative and anti-oxidative defense systems

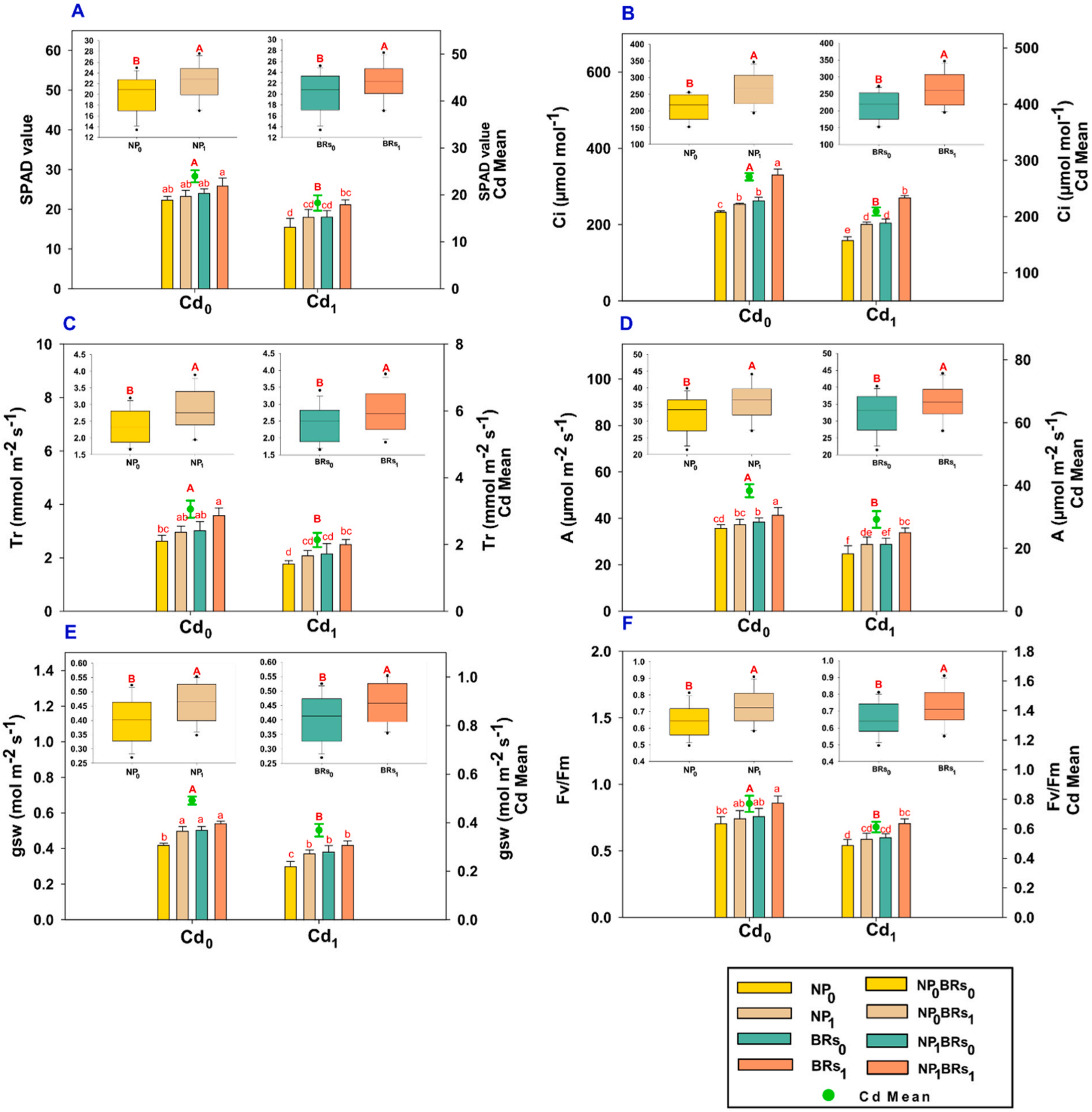

Fig. 2. Effects of sole and combined application of titanium oxide nanoparticles (TiO_2-NPs) and 24-epibrassinosteroid (24-epiBRs) on photosynthesis parameter **(A)** chlorophyll content (SPAD value), **(B)** intercellular carbon dioxide concentration (Ci), **(C)** transpiration rate (Tr), **(D)** photosynthetic rate (A); **(E)** stomatal conductance (gsw); and **(F)** chlorophyll fluorescence (Fv/Fm) of soybean plants grown under cadmium (Cd)-contaminated agricultural soil. The various letters indicated that there are notable differences in the values, as determined by the Least Significant Difference Test (LSD) and three-way ANOVA analysis in the R statistical package. The means (based on three replications) are reported along with the standard error (SE). Note: Cd_0 (Control; 0 ppm Cd); Cd_1 (30 ppm Cd); BRs_0 (0 M 24-epiBRs); BRs_1 (10^{-7} M 24-epiBRs); NP_0 (0 mg L^{-1} TiO_2-NPs); and NP_1 (15 mg L^{-1} TiO_2-NPs).

when exposed to Cd (Table 1). Moreover, Cd toxicity significantly enhanced oxidative stress and significantly, ($p \leq 0.05$) increased the concentration of MDA, H_2O_2, and EL in soybean leaves by 32.1, 19.4, and 26.7 %, respectively, compared to the control. In fact, both single and combined usage of TiO_2-NPs and 24-epiBRs greatly lowered the effects of oxidative stress on soybean leaves (Fig. 3A–C). When soybean plants were grown in soil without Cd, the levels of MDA, H_2O_2, and EL were 14.7 %, 23.2 %, and 24.8 % higher, 8.4 %, 10.1 %, and 31.1 % lower, and 0.2 %, 3.4 %, and 17.7 % lower, respectively, when TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs were used. Applying TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs reduced the levels of MDA, H_2O_2, and EL in soybean leaves compared to control soybean plants grown under Cd stress. The amounts decreased by (26.9, 42.3, 61.6 %,), (11.4, 12.0, and 39.1 %), as well as (15.5, 19.0, and 32.7 %), respectively.

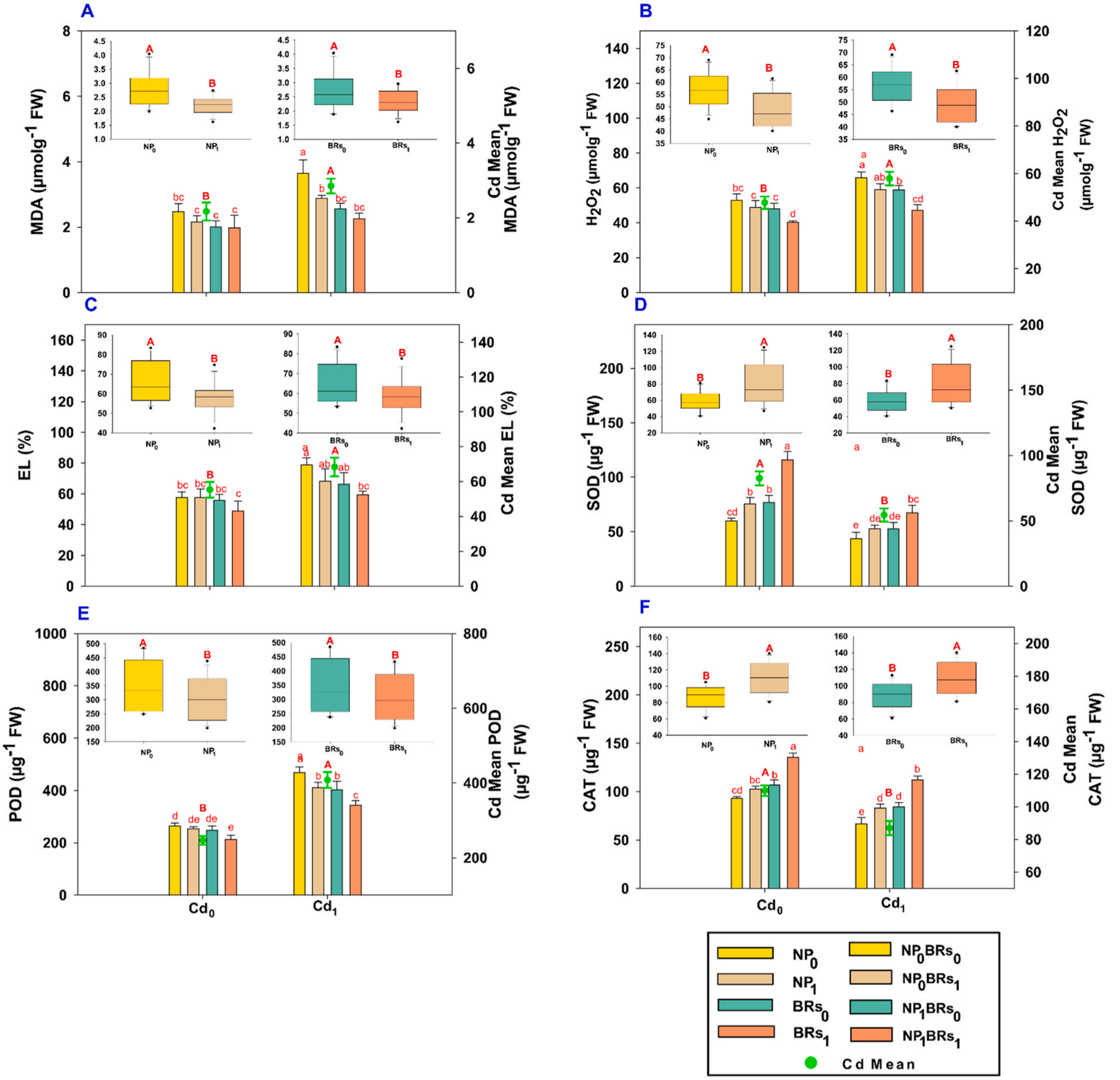

Fig. 3. Effects of sole and combined application of titanium oxide nanoparticles (TiO_2-NPs) and 24-epibrassinosteroid (24-epiBRs) on oxidative stress **(A)** malondialdehyde, **(B)** hydrogen peroxide, **(C)** electrolyte leakage, and antioxidant **(D)** superoxide dismutase, **(E)** peroxidase, and **(F)** catalase activities of soybean plants grown under cadmium (Cd)-contaminated agricultural soil. The various letters suggest that there are notable differences in the values, as determined by the Least Significant Difference Test (LSD) and three-way ANOVA analysis in the R statistical package. The means (based on three replications) are reported along with the standard error (SE). Note: Cd_0 (Control; 0 ppm Cd); Cd_1 (30 ppm Cd); BRs_0 (0 M 24-epiBRs); BRs_1 (10^{-7} M 24-epiBRs); NP_0 (0 mg L^{-1} TiO_2-NPs); and NP_1 (15 mg L^{-1} TiO_2-NPs).

Specifically, the phytotoxicity of Cd affected the antioxidative defense system of the soybean plants. It significantly ($p \leq 0.05$) reduced the concentration of SOD and CAT in soybean leaves by 37.5 and 39.5 %, respectively, compared to the control. Moreover, the concentration of POD in soybean leaves increased by 43.4 % compared to the control. Using either TiO_2-NPs and 24-epiBRs alone, or in combination, significantly ($p \leq 0.05$) improved the antioxidative defense system of soybean leaves (Fig. 3D-F). Adding TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs greatly increased the amounts of SOD and CAT in soybean leaves by (20.4, 21.9, and 48.1 %) and (9.2, 12.7, and 31.1 %), respectively, compared to control soybean plants grown in Cd-free soil. Additionally, the application of TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs significantly ($p \leq 0.05$) boosted the levels of SOD and CAT in soybean leaves by 17.2 %, 17.1 %, and 35.2 %, as well as 19.7 %, 20.9 %, and 40.4 %, respectively, when compared to legume plants not under Cd stress. Additionally, applying TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs significantly ($p \leq 0.05$) reduced the concentration of POD in soybean leaves by (4.2, 6.8, and 23.8 %) lower as compared to control soybean plants grown under non-contaminated Cd soil and

(13.9, 16.2, and 36.1 %) lower as compared to control soybean plants grown under Cd stress.

3.4. Cd concentration in leaves, shoots, roots, and soil

The ANOVA results showed that adding TiO_2-NPs and 24-epiBRs affected the levels of Cd in the leaves, shoots, and roots of soybean plants grown in agricultural soil that was spiked with Cd (Table 1; Fig. 4). Soybean plants grown under Cd stress without applying TiO_2-NPs and 24-epiBRs treatments contained higher concentrations of Cd in their leaves, shoots, and roots, which were 95.7, 95.1, and 96.00 %, respectively, higher than the control. The amount of Cd in soybean plants' leaves, shoots, and roots dropped by (21.3, 24.7, and 87.1 %), (35.7, 39.4, and 162.7 %), and (14.8, 18.1, and 62.5 %), respectively, when TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs were used compared to control soybean plants grown under Cd stress. Correspondingly, applying TiO_2-NPs, 24-epiBRs, and TiO_2-NPs + 24-epiBRs significantly ($p \leq 0.05$) reduced the concentration of Cd in soil by (13.2, 11.6, and 65.7 %), respectively, as compared to control soybean plants grown under Cd stress.

3.5. Principal component analysis (PCA) and correlation analysis

Based on the correlational analyses among the various physio-morphological traits of soybeans, the pattern of change in various treatments was also studied using PCA to evaluate the salienttrends associated with the various treatments The soil properties and physio-morphological traits of soybeans were subjected to PCA; Two PCs have eigenvalues greater than 1. The rest of the PCs (eigenvalue < 1) were not worth further interpretation. The PC values explained all the characters, influencing about 91.4 % of the treatment differences that accounted for the first two components (Table S1, Fig. 5). The PCA-biplot revealed that PC1 accounted for about 83.5 % of the total variability, primarily explained by MDA, EL, SOD, and transpiration rate (Table S1, Fig. 5). The second PC accounted for about 7.9 % of the total variation, mainly driven by root length, stomatal conductance, root Cd, and Cd leaves. The PCA biplot revealed a significant difference between Cd_0 and Cd_1 (Fig. 5b). NP_1BR_1 was significantly different from NP_0BR_0, NP_0BR_1, and NP_1BR_0, while NP_0BR_0, NP_0BR_1, and NP_1BR_0 were relatively similar (Fig. 5a).

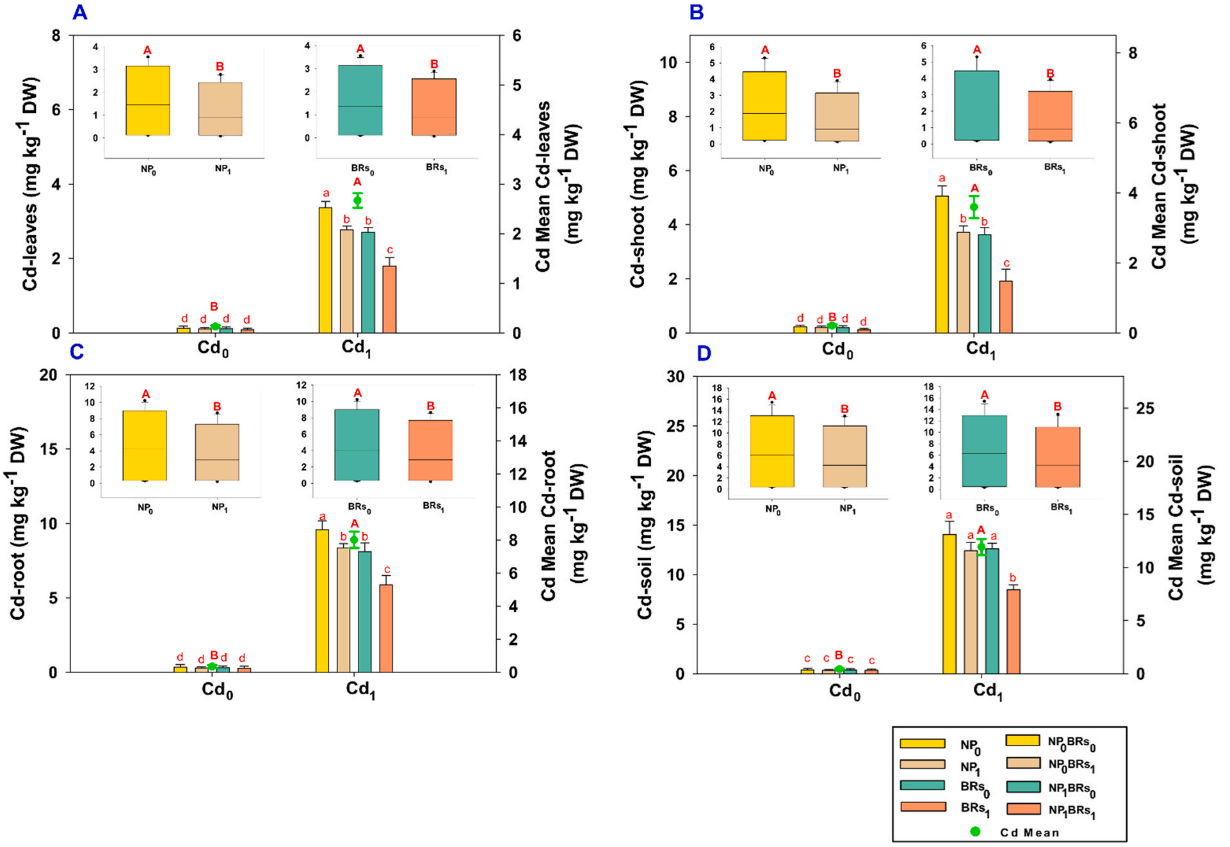

Fig. 4. Effects of sole and combined application of titanium oxide nanoparticles (TiO_2-NPs) and 24-epibrassinosteroid (24-epiBRs) on cadmium (Cd) contamination in **(A)** leaves **(B)** shoots **(C)** roots, and **(D)** soil of soybean plants grown under cadmium (Cd)-contaminated agricultural soil. The various letters suggest that there are notable differences in the values, as determined by the Least Significant Difference Test (LSD) and three-way ANOVA analysis in the R statistical package. The means (based on three replications) are reported along with the standard error (SE). Note: Cd_0 (Control; 0 ppm Cd); Cd_1 (30 ppm Cd); BRs_0 (0 M 24-epiBRs); BRs_1 (10^{-7} M 24-epiBRs); NP_0 (0 mg L^{-1} TiO_2-NPs); and NP_1 (15 mg L^{-1} TiO_2-NPs).

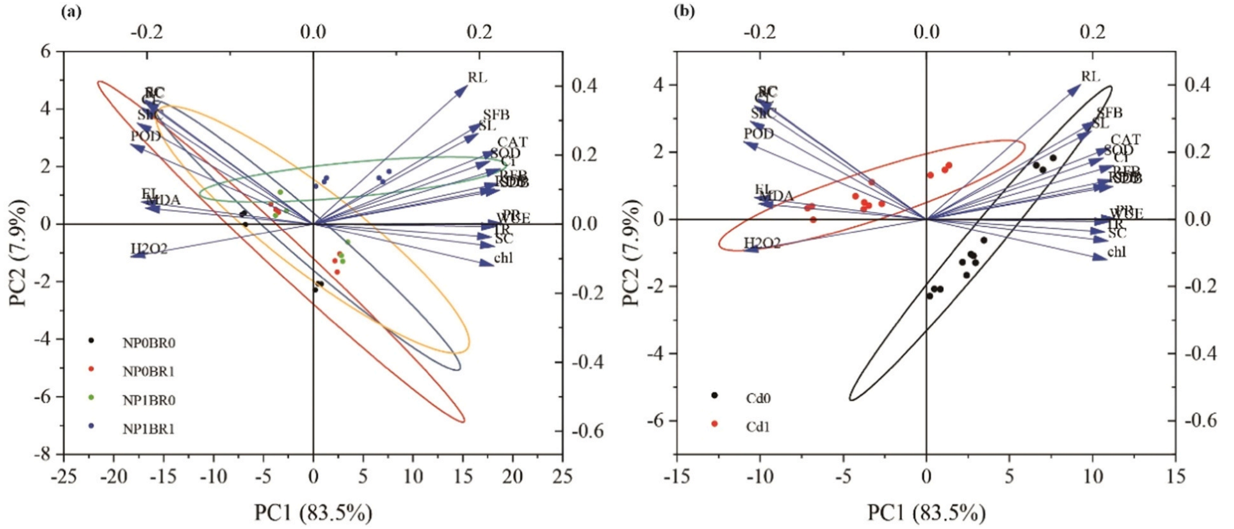

Fig. 5. The principal component analysis (PCA-biplot) of the various physio-morphological traits of soybean (*Glycine max* L.) plants grown under cadmium (Cd)-contaminated soil. Note: RL= Root length; RFB= Root Fresh biomass; RDB= Root dry biomass; SL= shoot length; SFB= shoot fresh biomass; SDB= shoot dry biomass; Chl= chlorophyll; TR= transpiration rate; WUE= water use efficiency; StC= Stomatal conductance; PR= photosynthesis rate; Ci= intercellular carbon dioxide concentration (Ci); SC= Soil Cd; RC= Root Cd; ShC= Shoot Cd; CL= Cd leaves; MDA = malondialdehyde; H_2O_2 = hydrogen peroxide; EL= electrolyte leakage; SOD = superoxide dismutase; POD = peroxidase; and CAT= catalase.

Fig. 6 illustrates the relationship among the various physio-morphological traits of soybeans. The study found that CAT, SPAD value, tr, Fv/Fm, gsw, A, and C_i significantly positively correlated with RL, RFB, RDB, SL, SFB, SDB, and SOD. In contrast, EL, MDA, H_2O_2, POD, soil Cd, root Cd, shoot Cd, and Cd leaves, were negatively correlated. Specifically, EL, MDA, H_2O_2, and POD were significantly positively correlated with soil Cd, root Cd, shoot Cd, and Cd leaves; but significantly negatively correlated with CAT, SPAD value, tr, Fv/Fm, gsw, A, and C_i. The Soil Cd, root Cd, shoot Cd, and Cd leaves were significantly negatively correlated with CAT, SPAD value, tr, Fv/Fm, gsw, A, and C_i.

4. Discussion

The present research demonstrated the biological potential of harnessing exogenous TiO_2-NPs and 24-epiBRs to restore the growth of soybean plants under Cd stress. By applying these substances to the leaves of soybean plants, the levels of Cd accumulation were reduced. The TiO_2-NPs, with their unique characteristics, can reduce oxidative stress in plants by scavenging ROS and increasing the activity of antioxidant enzymes (Deng et al., 2023; Kumar et al., 2024; Lai et al., 2023; Ogunkunle et al., 2020). Similarly, the steriodal phytohormone 24-epiBRs were able to increase the production of antioxidant enzymes andstrengthening the plant's defense against oxidative stress brought upon by the high Cd levels. The favourable growth results in this study implied that it would be possible for soybeans to grow in soils containing high in Cd. . Increasing anthropogenic activities have generally increased cadmium and the other HMs discharges into the environment, contributing additional toxicity-linked stress to plants, animals, and humans (Askari et al., 2021; Shaghaleh et al., 2024; Song et al., 2017; van der Ent et al., 2013; Zulfiqar et al., 2022). Stress-relief agents and substances may provide effective solutions to this problem by enabling plants to withstand better stress caused by HMs and other abiotic factors (Dumon and Ernst, 1988; Hussain et al., 2021; Liang et al., 2015; Paramo et al., 2020; Seleiman et al., 2020; Suzuki et al., 2014; Zhou et al., 2020). BRs, a polyhydroxysteroid were initially developed as plant growth regulators to help plants to improve their eco-physiological resilience to environmental stress (Ahammed et al., 2020, 2023; Kaya et al., 2020; Khripach et al., 2000). Moreover, recent literature has highlighted the potential of nanotechnology in various crops, particularly in enhancing tolerance to environmental stress (Paramo et al., 2020; Zhou et al., 2020).

The current study showed that high levels of Cd negatively affected the growth of soybeans (Fig. 1). In general, elevated levels of Cd cause molecular, biochemical, and cellular disruptions that affect plant biology (Shanmugaraj et al., 2013), resulting in deformities in growth and development (Song et al., 2017, Song et al., 2019). Many perturbations in plant metabolism are associated with Cd-induced retardation and anomalies in biomass and overall plant growth (Sebastian and Prasad 2015a). These biological disruptions slow down photosynthesis activity, nitrogen uptake, and assimilation (Sebastian and Prasad 2015b), lowering the turnover of proteins and carbohydrates and eventually slowing down plant growth. Interestingly, applying TiO_2-NPs and 24-epiBRs significantly improved the morphological traits of soybeans. TiO_2-NPs, by reducing oxidative stress, promote normal cell division and elongation, leading to better root and shoot morphological traits (Chen et al., 2023). 24-epiBRs, as plant growth regulators, stimulate the development of lateral roots and enhance root system development, thereby improving the plant's ability to absorb optimum water and nutrients. The use of BRs affects the formation of lateral and crown roots, which may help counteract the negative effects of high Cd on roots (Betti et al., 2021). In this study, applying BRs to soybeans notably reduced the inhibitory effects of Cd on plant growth. Working in

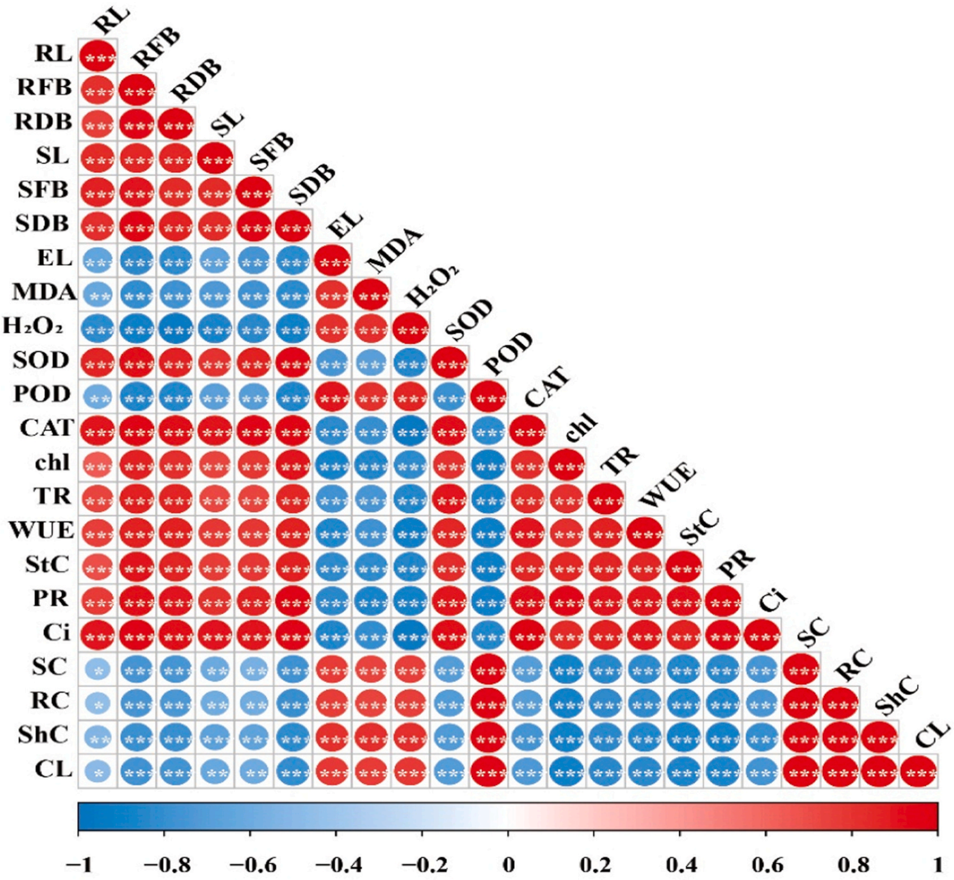

Fig. 6. Correlation analysis of physio-morphological traits of soybean (*Glycine max* L.) grown under cadmium (Cd)-contaminated soil. Note: *, ** and ***in the Figure respectively indicate $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, RL= Root length; RFB= Root Fresh biomass; RDB= Root dry biomass; SL= shoot length; SFB= shoot fresh biomass; SDB= shoot dry biomass; Chl= chlorophyll; TR= transpiration rate; WUE= water use efficiency; StC= Stomatal conductance; PR= photosynthesis rate; Ci= intercellular carbon dioxide concentration (Ci); SC= Soil Cd; RC= Root Cd; ShC= Shoot Cd; CL= Cd leaves;; MDA = malondialdehyde; H_2O_2 = hydrogen peroxide; EL= electrolyte leakage; SOD = superoxide dismutase; POD = peroxidase; and CAT= catalase.

partnership with the other phytohormones, BRs generally promote cell division and elongation by triggering enzymes that loosen the cell wall, increasing the synthesis of cell wall and cell membrane components (Khripach et al., 2000; Shahzad et al., 2018b; Sabagh et al., 2022). H^+-ATPases activate these enzymes by acidifying the apoplasts.

Consequently, BRs may enhance seedling growth by activating H^+-ATPases (Haubrick and Assmann, 2006). Regarding TiO_2-NPs, numerous mechanisms have been proposed regarding the positive function of titanium (Ti) in plant physiology (Bacilieri et al., 2017; Dumon and Ernst, 1988; Lyu et al., 2017). This includes its role in improving plant metabolism and its function by increasing nutrient uptake such as iron (Fe), magnesium (Mg) and calcium (Ca; Bacilieri et al., 2017). Ti also promotes photosynthetic and enzymatic activities, leading to hormesis (Carvajal et al., 1995). Moreover, it was reported that Cd inhibited growth by disrupting the translocation of Fe from roots to shoots, leading to Fe deficiency in the shoots. Conversely, Ti enhanced Fe uptake by activating genes related to Fe acquisition, thereby potentially mitigating the negative effects of Cd on plant growth (Lyu et al., 2017).

Faizan et al. (2021) used several photosynthetic processes and pigment levelsas biomarkers of Cd stress. The current research highlighted that Cd stress negatively affected various gas exchange parameters, photosynthetic pigements and processes (e.g. photosystems) (Fig. 2; Ma et al., 2021; Yong et al., 2010). This was similar to the observations reported by various researchers (Pietrini et al., 2010; Sacramento et al., 2018; Song et al., 2019). Our research showed that applying TiO_2-NPs to the soybean leaves improved the gas exchange parameters. These included Ci, Tr, A, gsw, and Fv/Fm. It was plausible that the TiO_2-NPs increased the activity and quantity of RuBisCO activase, an enzyme involved in the Calvin cycle, thereby enhancing photosynthesis (Gao et al., 2013). In another study, Faizan et al. (2018) reported that zinc oxide (ZnO)-NPs improved the gas exchange attributes such as net photosynthetic activity, transpiration rate, and stomatal conductivity in metal-stressed plants. Similarly, TiO_2-NPs can potentially boost chlorophyll synthesis, enhance plant quantum yield, and elevate the chemical energy within the photosynthetic system. Synergistically, the use of BRs improved the photosynthetic traits; more enzymes involved in photosynthetic activity, such as RuBisCO, were produced after phytohormonal applications (Yu et al., 2004).

Elevated levels of ROS result in a lowered antioxidant system (Abbas et al., 2017), and may lead to disruption in redox balance, contributing to heightened oxidative stress in soybeans and other species (Gutsch et al., 2019; Younis et al., 2016; Fig. 3). . Researchers have discovered that applying TiO_2-NPs reduces lipid peroxidation levels under Cd exposure (Hussain et al., 2021). Our study confirmed the earlier findings of Sardar et al. (2022), who reported that applying TiO_2-NPs significantly decreased the concentration of MDA in coriander (*Coriandrum sativum*) plants grown under Cd-contaminated soil. Specifically, the applied TiO_2-NPs lowered the oxidative stress and lipid peroxidation in plants treated with Cd stress by neutralizing ROS and boosting the activity of antioxidant enzymes (Lai et al., 2023). Raghib et al. (2020) observed that TiO_2-NPs protected the membranes from damage caused by HMs in wheat by reducing oxidative stress, as evidenced by lower lipid peroxidation levels. Similarly, Mostofa et al. (2019) observed that high Cd lowered the plants' ability to defend against oxidative stress; causing ROS and MDA levels to rise. Plants have adopted various ways to alleviate the generation of ROS, and these anti-oxidative enzymes are very important for eliminating the extra ROS (Hossain et al., 2012; Jakubowska and Janicka, 2017; Kaya et al., 2019; Kohli et al., 2017; Noman and Aqeel, 2017). The scheme shows that the Cd-linked toxicity diminishes the functionationality of enzymes responsible for antioxidant defense systems (Fig. 3). However, the positive and ameliorative effects of BRs were attributed to the production of more antioxidant enzymes, which decreased the production of H_2O_2 in plants subjected to Cd phytotoxicity (Kohli et al., 2017). Similarly, applications of TiO_2-NPs improved the antioxidant systems and lowered the EL and MDA levels; demonstrating their effectiveness in reducing tissue Cd levels in plants.

Plant tissues showed increased Cd concentrations (Fig. 4), which could lead to cellular structural damage and the loss of functionality (Sardar et al., 2022). In this study, combining TiO_2-NPs and BRs was effective for ameliorating Cd toxicity in soybeans grown in Cd-contaminated soil. This approach leveraged synergistically the unique properties of two dissimilar substances to reduce Cd uptake while concomitantly strengthening the planadaptive responses. Specifically, BRs are known to down regulate some genes that make HM-ATPases (HMAs) and phytochelatins; HMAs and phytochelatins are responsible for mobilizing Cd within plant tissues (Soares et al., 2020; Sun et al., 2024). The specific regulation of Cd mobility will restrict Cd entry and translocation, thereby reducing overall Cd accumulation. According to Kumar et al. (2024), TiO_2-NPs inhibited Cd entry to the stems and leaves (Chen et al., 2023) by immobilizing them in the root cell walls. BRs also have the ability to mobilize important minerals like Fe, manganese (Mn), and zinc (Zn) into the cells. These minerals collectively prevented the excessive buildup of Cd; by competing antagonistically against Cd for the various transporters and binding sites (Shahzad et al., 2018). Additionally, it also facilitated the uptake of more silicon (Si), forming a complex with Cd at the root surfaces (Yu et al., 2021). In general, the applications of TiO_2-NPs improve the overall physiological metabolism , which includedoptimized tissue ion balance, photosynthesis and nutrient uptake (Huang et al., 2024; Lai et al., 2023) Interestingly, BRs also alter the different stress-response pathways, like the glyoxalase system and methylglyoxal detoxification pathways; improving the resilience of plants during stress (Shahzad et al., 2018). Mechanistically, the TiO_2-NPs are known to enhance the activity of genes that regulate the removal of Cd from the tissues, such as those producing phytochelatins and metallothioneins; thereby facilitating the cellular storage of Cd in vacuoles ("safe sequestration") and effectively mitigating its toxicity effects. The synergistic applications of BRs and TiO_2-NPs significantly reduced Cd uptake, and mitigated its phytotoxicity through various complementary mechanisms (Kumar et al., 2024; Fig. 7).

One pertinent limitation in this studywas the usage of a pot-based experimental approach. Further detailed analysis and scale-up experiments (e.g. field trials) are needed to evaluate the effectiveness of this approach under natural conditions and for large-scale implementation. Another limitation of this study was the lack of specific Cd levels in the beans, which would be useful for assessing the HM impact on food safety. Nevertheless, it could be inferred that TiO_2-NPs and 24-epiBRs effectively lowered Cd concentrations in organs like shoots and leaves; thus, it was reasonable to expect lower Cd levels in the beans grown under these similar conditions. Future studies should assess the Cd concentrations of the beans and their pods, in order to address this uncertainty comprehensively. With respect to the potential toxicity risks associated with Ti, some consideration for further research should also be given to determining the optimal concentration of TiO_2-NPs; in order to optimize their beneficial effects while minimizing their potential toxicity.

5. Conclusion

This research demonstrated that applying TiO_2-NPs and 24-epiBRs to the leaves of soybean plants in Cd-contaminated soil ameliorated the negative effects of high Cd levels. In general, the Cd-linked toxicity decreased root length, root dry and fresh biomass, shoot length, and shoot dry and fresh biomass by 11.0 %, 63.5 %, 84.9 %, 11.7 %, 49.0 %, and 27.3 %, compared to plants that were not exposed to the HM. Interestingly, the combined application of TiO_2-NPs and 24-epiBRs significantly improved these biological attributes. It lowered the Cd concentrations in leaves, shoots, and roots by 87.1 %, 162.7 %, and 62.5 %, respectively, compared to control plants under Cd stress. Specifically, the combined treatment of TiO_2-NPs and 24-epiBRs enhanced the antioxidant enzyme activities and concomitantly reduced oxidative stress markers like MDA, H_2O_2, and EL. These results indicated that TiO_2-NPs and 24-epiBRs were proven effective in minimizing the negative effects of high Cd and restoring soybean growth in polluted soils. Moving beyond pot-based experiments, further detailed analyses and extensive field trials are needed to verify the reported ameliorative efficacy under natural conditions and to assess practical and large-scale implementation. Future research should focus on determining the optimal concentration of TiO_2-NPs to balance their beneficial effects with the potential concomitant toxicity risks. Moving forward, studies should also assess the long-term impacts and scalability of this approach for broader agricultural applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

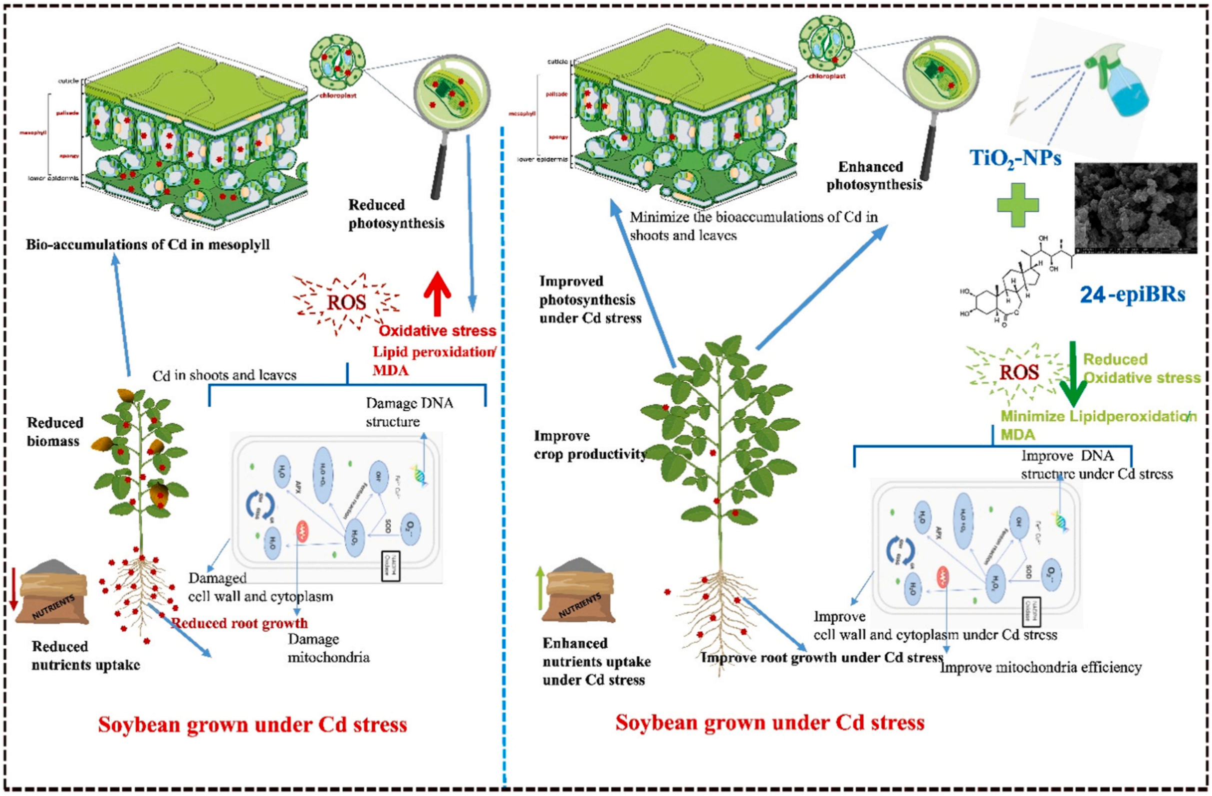

Fig. 7. The schematic diagram illustrating how titanium oxide nanoparticles (TiO_2-NPs) and 24-epibrassinosteroid (24-epiBRs) minimize oxidative stress and improve soybean (*Glycine max* L.) growth under Cd-contaminated conditions. On the left side, it was observed that Cd stress significantly reduces photosynthesis, biomass, root growth, and nutrient uptake in soybeans. Cd accumulation in shoots and leaves causes oxidative stress, lipid peroxidation, and damage to DNA, cell walls, cytoplasm, and mitochondria. Similarly, on the right side, TiO_2-NPs and 24-epiBRs, alone or combined, significantly reduce Cd stress effects. These two dissimilar substances collectively improve photosynthesis, biomass, root growth, and nutrient uptake, better tissue ion balance, and reduce Cd bioaccumulation, oxidative stress, lipid peroxidation, and DNA damage, thereby restoring physiological functionality and sustainaing crop productivity during unfavaourable growth conditions.

influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgement

This work was supported by the National Key R&D Program of China (2021YFD1900700). The authors are grateful for additional funding by the Researchers Supporting Project, number (RSPD2024R637), King Saud University, Riyadh, Saudi Arabia.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2024.103811.

References

Abbas, S., Javed, M.T., Shahid, M., Hussain, I., Haider, M.Z., Chaudhary, H.J., Tanwir, K., Maqsood, A., 2020. *Acinetobacter* sp. SG-5 inoculation alleviates cadmium toxicity in differentially Cd tolerant maize cultivars as deciphered by improved physio-biochemical attributes, antioxidants and nutrient physiology. Plant Physiol. Biochem. 155, 815–827. https://doi.org/10.1016/j.plaphy.2020.08.024.

Abbas, T., Rizwan, M., Ali, S., Adrees, M., Zia-ur-Rehman, M., Qayyum, M.F., Ok, Y.S., Murtaza, G., 2017. Effect of biochar on alleviation of cadmium toxicity in wheat (*Triticum aestivum* L.) grown on Cd-contaminated saline soil. Environ. Sci. Pollut. Res 25, 25668–25680. https://doi.org/10.1007/s11356-017-8987-4.

Aebi, H., 1984. Catalase *in vitro*. Meth. Enzym. 105 (C), 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Ahammed, G.J., Li, X., Yu, J., 2023. Introduction to plant hormones and climate change. In Plant Hormones and Climate Change. Springer Nature Singapore, Singapore, pp. 1–16.

Ahammed, G.J., Li, X., Liu, A., Chen, S., 2020. Brassinosteroids in plant tolerance to abiotic stress. J. Plant Growth Regul. 39 (4), 1451–1464.

Alpaslan, B., Yukselen, M.A., 2002. Remediation of lead contaminated soils by stabilization/solidification. Water Air Soil Pollut. 133, 253–263. https://doi.org/10.1023/A:1012977829536.

Anuradha, S., Rao, S.S.R., 2007. Effect of brassinosteroids on radish (*Raphanus sativus* L.) seedlings growing under cadmium stress. Plant Soil Environ. 53, 465–472.

Askari, S.H., Ashraf, M.A., Ali, S., Rizwan, M., Rasheed, R., 2021. Menadione sodium bisulfite alleviated chromium effects on wheat by regulating oxidative defense, chromium speciation, and ion homeostasis. Environ. Sci. Pollut. Res. 28, 36205–36225.

Azarin, K., Usatov, A., Minkina, T., Duplii, N., Fedorenko, A., Plotnikov, A., Mandzhieva, S., Kumar, R., Yong, J.W.H., Sehar, S., Rajput, V.D., 2024. Evaluating the phytotoxicological effects of bulk and nano forms of zinc oxide on cellular respiration-related indices and differential gene expression in *Hordeum vulgare* L. Ecotoxicology and Environmental Safety 282, 116670. https://doi.org/10.1016/j.ecoenv.2024.116670.

Bacilieri, F.S., Pereira de Vasconcelos, A.C., Quintao Lana, R.M., Mageste, J.G., Torres, J.L.R., 2017. Titanium (Ti) in plant nutrition - A review. Aust. J. Crop Sci. 11 (4), 382–386.

Betti, C., Rovere, F.Della, Piacentini, D., Fattorini, L., Falasca, G., Altamura, M.M., 2021. Jasmonates, ethylene and brassinosteroids control adventitious and lateral rooting as stress avoidance responses to heavy metals and metalloids. Biomolecules 11 (1), 77.

Carvajal, M., Martínez-Sanchez, F., Pastor, J.J., Alcaraz, C.F., 1995. Leaf spray with Ti (IV) ascorbate improves the iron uptake and iron activity in *Capsicum annuum* L. plants. In Iron Nutrition in Soils and Plants: Proceedings of the Seventh International Symposium on Iron Nutrition and Interactions in Plants. Springer Netherlands, Zaragoza, Spain, pp. 1–5. June 27–July 2, 1993.

Chen, D., Chen, D., Xue, R., Long, J., Lin, X., Lin, Y., Jia, L., Zeng, R., Song, Y., 2019. Effects of boron, silicon and their interactions on cadmium accumulation and toxicity in rice plants. J. Hazard. Mat. 367, 447–455. https://doi.org/10.1016/j.jhazmat.2018.12.111.

Chen, F., Li, Y., Irshad, M.A., Hussain, A., Nawaz, R., Qayyum, M.F., Ma, J., Zia-ur-Rehman, M., Rizwan, M., Ali, S., 2023. Effect of titanium dioxide nanoparticles and co-composted biochar on growth and Cd uptake by wheat plants: A field study. Environ. Res. 231, 116057 https://doi.org/10.1016/j.envres.2023.116057.

Chowdhury, M.S.N., Sani, M.N.H., Siddique, A.B., Hossain, M.S., Yong, J.W.H., 2024. Synergistic effects of biochar and potassium co-application on growth, physiological attributes, and antioxidant defense mechanisms of wheat under water deficit conditions. Plant Stress 12, 100452. https://doi.org/10.1016/j.stress.2024.100452.

Cornelissen, J.H.C., et al., 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Australian J. Bot. 51, 335–380. https://www.publish.csiro.au/BT/pdf/BT02124.

de Bang, T.C., 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, H., Lei, H., Luo, Y., Huan, C., Li, J., Li, H., He, F., Zhang, B., Yi, K., Sun, A., 2023. The effects of titanium dioxide nanoparticles on cadmium bioaccumulation in ramie and its application in remediation of cadmium-contaminated soil. Alex. Eng. J. 86, 663–668. https://doi.org/10.1016/j.aej.2023.12.019.

Dionisio-Sese, M.L., Tobita, S., 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135 (1), 1–9.

Dumon, J.C., Ernst, W.H.O., 1988. Titanium in plants. J. Plant Physiol. 133 (2), 203–209. https://doi.org/10.1016/S0176-1617(88)80138-X.

EL Sabagh, A., Islam, M.S., Hossain, A., Iqbal, M.A., Mubeen, M., Waleed, M., Reginato, M., Battaglia, M., Ahmed, S., Rehman, A., Arif, M., Athar, H., Ratnasekera, D., Danish, S., Raza, M.A., Rajendran, K., Mushtaq, M., Skalicky, M., Brestic, M., Soufan, W., Fahad, S., Pandey, S., Kamran, M., Datta, R., Abdelhamid, M.T., 2022. Phytohormones as growth regulators during abiotic stress tolerance in plants. Front Agron. 4, 765068 https://doi.org/10.3389/fagro.2022.765068.

Faizan, M., Faraz, A., Yusuf, M., Khan, S.T., Hayat, S., 2018. Zinc oxide nanoparticle-mediated changes in photosynthetic efficiency and antioxidant system of tomato plants. Photosynthetica 56, 678–686.

Faizan, M., Rajput, V.D., Al-Khuraif, A.A., Arshad, M., Minkina, T., Sushkova, S., Yu, F., 2021. Effect of foliar fertigation of chitosan nanoparticles on cadmium accumulation and toxicity in *Solanum lycopersicum*. Biology 10 (7), 666.

Farquhar, G.D., von Caemmerer, S., Berry, J.A., 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149, 78–90. https://doi.org/10.1007/BF00386231.

Gao, J., Xu, G., Qian, H., Liu, P., Zhao, P., Hu, Y., 2013. Effects of nano-TiO_2 on photosynthetic characteristics of *Ulmus elongata* seedlings. Environ. Pollut. 176, 63–70.

Ghassemi-Golezani, K., Farhangi-Abriz, S., 2023. Solid and modified biochars mitigate root cell lignification and improve nutrients uptake in mint plants under fluoride and cadmium stresses. Plant Physiol. Biochem. 200, 107757 https://doi.org/10.1016/j.plaphy.2023.107757.

Gutsch, A., Sergeant, K., Keunen, E., Prinsen, E., Guerriero, G., Renaut, J., Hausman, J.F., Cuypers, A., 2019. Does long-term cadmium expo- sure influence the composition of pectic polysaccharides in the cell wall of *Medicago sativa* stems? BMC Plant Biol. 19, 271. https://doi.org/10.1186/s12870-019-1859-y.

Haider, F.U., Wang, X., Farooq, M., Hussain, S., Cheema, S.A., Ain, N.U., Virk, A.L., Ejaz, M., Janyshova, U., Liqun, C., 2022. Biochar application for the remediation of trace metals in contaminated soils: Implications for stress tolerance and crop production. Ecotoxicol. Environ. Saf. 230, 113165 https://doi.org/10.1016/j.ecoenv.2022.113165.

Haider, F.U., Zulfiqar, U., Ul Ain, N., Hussain, S., Maqsood, M.F., Ejaz, M., Yong, J.W.H., Li, Y., 2024. Harnessing plant extracts for eco-friendly synthesis of iron nanoparticle (Fe-NPs): Characterization and their potential applications for ameliorating environmental pollutants. Ecotoxicol. Environ. Saf. 281, 116620 https://doi.org/10.1016/j.ecoenv.2024.116620.

Haubrick, L.L., Assmann, S.M., 2006. Brassinosteroids and plant function: some clues, more puzzles. Plant Cell Environ. 29, 446–457. https://doi.org/10.1111/j.1365-, 3040.2005.01481.x.

Hayat, S., Alyemeni, M.N., Hasan, S.A., 2012. Foliar spray of brassinosteroid enhances yield and quality of *Solanum lycopersicum* under cadmium stress. Saudi J. Biol. Sci. 19 (3), 325–335. https://doi.org/10.1016/j.sjbs.2012.03.005.

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125 (1), 189–198. https://doi.org/10.1016/0003-9861(68)90654-1.

Hossain, M.A., Piyatida, P., da Silva, J.A.T., Fujita, M., 2012. Molecular mechanism of heavy metal toxicity and tolerance in plants Central role of glutathione in detoxification of Reactive Oxygen Species and methylglyoxal and in heavy metal chelation. J. Bot. https://doi.org/10.1155/2012/872875.

Huang, Y., Cai, S., Ying, W., Niu, T., Yan, J., Hu, H., Ruan, S., 2024. Exogenous titanium dioxide nanoparticles alleviate cadmium toxicity by enhancing the antioxidative capacity of *Tetrastigma hemsleyanum*. Ecotoxicol. Environ. Saf. 273, 116166 https://doi.org/10.1016/j.ecoenv.2024.116166.

Huang, Q., Zhang, Y., Liu, S., Wang, H., Gao, F., Shao, G., 2024. 24-epibrassinolide mitigates cadmium toxicity in rice plants by activating the plant detoxification system and regulating genes expression involved in Cd/Fe uptake and translocation. Plant Stress 12, 100485. https://doi.org/10.1016/j.stress.2024.100485.

Hussain, B., Umer, M.J., Li, J., Ma, Y., Abbas, Y., Ashraf, M.N., Tahir, N., Ullah, A., Gogoi, N., Farooq, M., 2021. Strategies for reducing cadmium accumulation in rice grains. J. Clean. Prod. 286, 125557.

Jakubowska, D., Janicka, M., 2017. The role of brassinosteroids in the regulation of the plasma membrane H^+-ATPase and NADPH oxidase under cadmium stress. Plant Sci. 264, 37–47. https://doi.org/10.1016/j.plantsci.2017.08.007.

Jalil, S., Nazir, M.M., Al-Huqail, A.A., Ali, B., Al-Qthanin, R.N., Asad, M.A.U., Eweda, M.A., Zulfiqar, F., Onursal, N., Masood, H.A., Yong, J.W.H., Jin, X., 2023. Silicon nanoparticles alleviate cadmium toxicity in rice by modulating the nutritional profile and triggering stress-responsive genetic mechanisms. Ecotoxicology and Environmental Safety 268, 115699. https://doi.org/10.1016/j.ecoenv.2023.115699.

Jameson, P.E., 2023. Zeatin: The 60th anniversary of its identification. Plant Physiol. 192, 34–55. https://doi.org/10.1093/plphys/kiad094.

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

Kaya, C., Ashraf, M., Alyemeni, M.N., Ahmad, P., 2020. The role of nitrate reductase in brassinosteroid-induced endogenous nitric oxide generation to improve cadmium stress tolerance of pepper plants by upregulating the ascorbate-glutathione cycle. Ecotoxicol. Environ. Saf. 196, 110483 https://doi.org/10.1016/j.ecoenv.2020.110483.

Kaya, C., Okant, M., Ugurlar, F., Alyemeni, M.N., Ashraf, M., Ahmad, P., 2019. Melatonin-mediated nitric oxide improves tolerance to cadmium toxicity by reducing oxidative stress in wheat plants. Chemosphere 225, 627–638. https://doi.org/10.1016/j.chemosphere.2019.03.026.

Khripach, V., Zhabinskii, V., De Groot, A., 2000. Twenty years of brassinosteroids: steroidal plant hormones warrant better crops for the XXI century. Ann. Bot. 86, 441–447. https://doi.org/10.1006/anbo.2000.1227.

Kohli, S.K., Handa, N., Gautam, V., Bali, S., Sharma, A., Khanna, K., Arora, S., Thukral, A.K., Ohri, P., Karpets, Y.V., Kolupaev, Y.E., 2017. ROS signaling in plants under heavy metal stress. React. Oxyg. Species Antioxid. Syst. Plant.: Role, Regul. Abiotic Stress. Springer, Singapore, pp. 185–214. https://doi.org/10.1007/978-981-10-5254-5_8.

Kumar, D., Mariyam, S., Gupta, K.J., Thiruvengadam, M., Sampatrao Ghodake, G., Xing, B., Seth, C.S., 2024. Comparative investigation on chemical and green synthesized titanium dioxide nanoparticles against chromium (VI) stress eliciting differential physiological, biochemical, and cellular attributes in *Helianthus annuus* L. Sci. Tot. Environ. 930, 172413 https://doi.org/10.1016/j.scitotenv.2024.172413.

Lai, M., Ghouri, F., Sarwar, S., Alomrani, S.O., Riaz, M., Haider, F.U., Liu, J., Imran, M., Ali, S., Liu, X., Shahid, M.Q., 2023. Modulation of metal transporters, oxidative stress and cell abnormalities by synergistic application of silicon and titanium oxide nanoparticles: A strategy for cadmium tolerance in rice. Chemosphere 345, 140439. https://doi.org/10.1016/j.chemosphere.2023.140439.

Lang, D., Hu, Y., Zhang, W., Hao, F., Zhou, R., Zhang, P., 2024. Organic matter and *Bacillus niescheri* amendments mitigate cadmium toxicity by regulating physiology and transcription of *Diospyros lotus* L., and soil bio-chemical properties. Environ. Technol. Innov. 33, 103527 https://doi.org/10.1016/j.eti.2024.103527.

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

Li, D.M., Liu, H.Y., Gao, M., Zhou, J., Zhou, J., 2022. Effects of soil amendments, foliar sprayings of silicon and selenium and their combinations on the reduction of cadmium accumulation in rice. Pedosphere 32 (4), 649–659.

Liang, Y., Nikolic, M., Bélanger, R., Gong, H., Song, A., 2015. Silicon-mediated tolerance to salt stress. In: LIANG, Y. (Ed.), Silicon in agriculture. Springer, Dordrecht., pp. 123–142. https://doi.org/10.1007/978-94-017-9978-2_6

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.

Lyu, S., Wei, X., Chen, J., Wang, C., Wang, X., Pan, D., 2017. Titanium as a beneficial element for crop production. Front. Plant Sci. 8, 237149.

Ma, M, Liu, Y., Bai, C., Yong, J.W.H., 2021. The significance of chloroplast NAD(P)H dehydrogenase complex and its dependent cyclic electron transport in photosynthesis. Front. Plant Sci. 12, 661863 https://doi.org/10.3389/fpls.2021.661863.

Moon, D.H., Grubb, D.G., Reilly, T.L., 2009. Stabilization/solidification of selenium-impacted soils using Portland cement and cement kiln dust. J. Hazard. Mat. 168 (2-3), 944–951. https://doi.org/10.1016/j.jhazmat.2009.02.125.

Mostofa, M.G., Rahman, M., Ansary, M., Uddin, M., Fujita, M., Tran, L.-S.P., 2019. Interactive effects of salicylic acid and nitric oxide in enhancing rice tolerance to cadmium stress. IJMS 20, 5798.

Nejad, Z.D., Jung, M.C., Kim, K.-H., 2017. Remediation of soils contaminated with heavy metals with an emphasis on immobilization technology. Environ. Geochem. Health 40, 927–953. https://doi.org/10.1007/s10653-017-9964-z.

Noman, A., Aqeel, M., 2017. miRNA-based heavy metal homeostasis and plant growth. Environ. Sci. Pollut. Res. 24, 10068–10082.

Ogunkunle, C.O., Odulaja, D.A., Akande, F.O., Varun, M., Vishwakarma, V., Fatoba, P.O., 2020. Cadmium toxicity in cowpea plant: effect of foliar intervention of nano-TiO_2 on tissue Cd bioaccumulation, stress enzymes and potential dietary health risk. J. Biotechnol. 310, 54–61.

Pan, C., Tan, S.N., Yong, J.W.H., Ge, L., 2017. Gibberellins: progress and development in analytical methods. J Sep Sci 40, 346–360. https://doi.org/10.1002/jssc.201600857.

Pang, J., Ryan, M.H., Wen, Z., Lambers, H., Liu, Y., Zhang, Y., Tueux, G., Jenkins, S., Mickan, B., Wong, W.S., Yong, J.W.H., Siddique, K.H.M., 2023. Enhanced nodulation and phosphorus acquisition from sparingly-soluble iron phosphate upon treatment with arbuscular mycorrhizal fungi in chickpea. Physiologia Plantatum 13873. https://doi.org/10.1111/ppl.13873.

Paramo, L.A., Feregrino-Pérez, A.A., Guevara, R., Mendoza, S., Esquivel, K., 2020. Nanoparticles in agroindustry: Applications, toxicity, challenges, and trends. Nanomaterials 10 (9), 1654.

Peng, Y., Yang, J., Li, X., Zhang, Y., 2021. Salicylic acid: biosynthesis and signaling. Annual Review of Plant Biology 72 (1), 761–791. https://doi.org/10.1146/annurev-arplant-081320-092855.

Pietrini, F., Zacchini, M., Iori, V., Pietrosanti, L., Ferretti, M., Massacci, A., 2010. Spatial distribution of cadmium in leaves and its impact on photosynthesis: examples of different strategies in willow and poplar clones. Plant Biol. 12 (2), 355–363.

Raghib, F., Naikoo, M.I., Khan, F.A., Alyemeni, M.N., Ahmad, P., 2020. Interaction of ZnO nanoparticle and AM fungi mitigates Pb toxicity in wheat by upregulating antioxidants and restricted uptake of Pb. J. Biotechnol. 323, 254–263.

Rehman, A., Shahzad, B., Haider, F.U., Ibraheem Ahmed, H.A., Lee, D., Im, S.Y., Khan, I., 2021a. An introduction to brassinosteroids: History, biosynthesis, and chemical diversity. Brassinosteroids Plant Dev. Biol. Stress Toler. 1–14. https://doi.org/10.1016/B978-0-12-813227-2.00006-0.

Rehman, A., Shahzad, B., Haider, F.U., Moeen-ud-din, M., Ullah, A., Khan, I., 2021b. Brassinosteroids in plant response to high temperature stress. Brassinosteroids Plant Dev. Biol. Stress Toler. 173–187. https://doi.org/10.1016/B978-0-12-813227-2.00014-X.

Ryan, P.R., Delhaize, E., Jones, D.L., 2001. Function and mechanism of organic anion exudation from plant roots. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 527–560. https://doi.org/10.1146/ANNUREV.ARPLANT.52.1.527.

Sacramento, B.L.D., Azevedo Neto, A.D.D., Alves, A.T., Moura, S.C., Ribas, R.F., 2018. Photosynthetic parameters as physiological indicators of tolerance to cadmium stress in sunflower genotypes. Rev. Caatinga 31, 907–916.

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., Hasan, M., Uddain, J., Subramaniam, S., 2020. Impact of application of *Trichoderma* and biochar on growth, productivity and nutritional quality of tomato under reduced NPK fertilization. Ann. Agric. Sci. 65 (1), 107–115. https://doi.org/10.1016/j.aoas.2020.06.003.

Sani, M.N.H., Yong, J.W.H., 2022. Harnessing synergistic biostimulatory processes: a plausible approach for enhanced crop growth and resilience in organic farming. Biol 11 (1), 41. https://doi.org/10.3390/biology11010041.

Sardar, R., Ahmed, S., Yasin, N.A., 2022. Titanium dioxide nanoparticles mitigate cadmium toxicity in *Coriandrum sativum* L. through modulating antioxidant system, stress markers and reducing cadmium uptake. Environ. Pollut. 292, 118373.

Sebastian, A., Prasad, M.N.V., 2015a. Operative photo assimilation associated proteome modulations are critical for iron-dependent cadmium tolerance in *Oryza sativa* L. Protoplasma 252, 1375–1386. https://doi.org/10.1007/s00709-015-0770-0.

Sebastian, A., Prasad, M.N.V., 2015b. Trace Element Management in Rice. Agron 5, 374–404. https://doi.org/10.3390/agronomy50, 30374.

Seleiman, M.F., Santanen, A., Mäkelä, P.S., 2020. Recycling sludge on cropland as fertilizer–Advantages and risks. Resour., Conserv. Recycl. 155, 104647.

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.

Shah, T., Khan, Z., Asad, M., Imran, A., Khan Niazi, M.B., Alsahli, A.A., 2023. Alleviation of cadmium toxicity in wheat by strigolactone: Regulating cadmium uptake, nitric oxide signaling, and genes encoding antioxidant defense system. Plant Physiol. Biochem. 202, 107916 https://doi.org/10.1016/j.plaphy.2023.107916.

Shahzad, B., Tanveer, M., Che, Z., Rehman, A., Cheema, S.A., Sharma, A., Song, H., Rehman, S.U., Zhaorong, D., 2018b. Role of 24-epibrassinolide (EBL) in mediating heavy metal and pesticide induced oxidative stress in plants: A review. Ecotoxicol. Environ. Saf. 147, 935–944. https://doi.org/10.1016/j.ecoenv.2017.09.066.

Shahzad, B., Tanveer, M., Rehman, A., Cheema, S.A., Fahad, S., Rehman, S., Sharma, A., 2018. Nickel: whether toxic or essential for plants and environment - A review. Plant Physiol. Biochem. 132, 641–651. https://doi.org/10.1016/j.plaphy.2018.10.014.

Shanmugaraj, B.M., Harish, M.C., Balamurugan, S., Sathishkumar, R., 2013. Cadmium induced physio-biochemical and molecular response in *Brassica juncea*. Int J. Phytoremed 15, 206–218. https://doi.org/10.1080/15226514.2012.687020.

Shen, X., Dai, M., Yang, J., Sun, L., Tan, X., Peng, C., Ali, I., Naz, I., 2022. A critical review on the phytoremediation of heavy metals from environment: performance and challenges. Chemosphere 291, 1–11. https://doi.org/10.1016/j.chemosphere.2021.132979.

Shen, Z., Li, Z., Alessi, D.S., 2018. Stabilization-based soil remediation should consider long-term challenges. Front. Environ. Sci. Eng. 12, 16. https://doi.org/10.1007/s11783-018-1028-9.

Soares, T.F.S.N., Dias, D.C.F.D.S., Oliveira, A.M.S., Ribeiro, D.M., Dias, L.A.D.S., 2020. Exogenous brassinosteroids increase lead stress tolerance in seed germination and seedling growth of *Brassica juncea* L. Ecotoxicology and Environmental Safety 193, 110296. https://doi.org/10.1016/j.ecoenv.2020.110296.

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.

Song, Y., Jin, L., Wang, X., 2017. Cadmium absorption and transportation pathways in plants. Int J. Phytoremed 19, 133–141. https://doi.org/10.1080/15226514.2016.1207598.

Song, X., Yue, X., Chen, W., Jiang, H., Han, Y., Li, X., 2019. Detection of cadmium risk to the photosynthetic performance of hybrid *Pennisetum*. Front. Plant Sci. 10, 461577.

Sun, J.Y., Guo, R., Jiang, Q., Chen, C.Z., Gao, Y.Q., Jiang, M.M., Shen, R.F., Zhu, X.F., Huang, J., 2024. Brassinosteroid decreases cadmium accumulation via regulating gibberellic acid accumulation and Cd fixation capacity of root cell wall in rice (*Oryza sativa*). J. Hazard. Mat. 469, 133862 https://doi.org/10.1016/j.jhazmat.2024.133862.

Sunny, N.E., Mathew, S.S., Chandel, N., Saravanan, P., Rajeshkannan, R., Rajasimman, M., Vasseghian, Y., Rajamohan, N., Kumar, S.V., 2022. Green synthesis of titanium dioxide nanoparticles using plant biomass and their applications- A review. Chemosphere 300, 134612. https://doi.org/10.1016/j.chemosphere.2022.134612.

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.12797.

Tarkowski, P., Ge, L., Yong, J.W.H., Tan, S.N., 2009. Analytical methods for cytokinins. Trends Anal Chem 28, 323–335. https://doi.org/10.1016/j.trac.2008.11.010.

van der Ent, A., Baker, A.J., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362, 319–334. https://doi.org/10.1007/s11104-012-1287-3.

Wong, W.S., Tan, S.N., Ge, L., Chen, X., Yong, J.W.H., 2015. The Importance of Phytohormones and Microbes in Biofertilizers. In: Maheshwari, D. (Ed.). Bacterial Metabolites in Sustainable Agroecosystem. Sustainable Development and Biodiversity. Springer, Cham, p. 12. https://doi.org/10.1007/978-3-319-24654-3_6.

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 (12), 1323–1335. https://doi.org/10.1071/FP14066.

Yong, J.W.H., Ng, Y.F., Tan, S.N., Chew, A.Y.L., 2010. Effect of fertilizer application on photosynthesis and oil yield of *Jatropha curcas* L. Photosynt 48, 208–218. https://doi.org/10.1007/s11099-010-0026-3.

Younis, U., Malik, S.A., Rizwan, M., Qayyum, M.F., Ok, Y.S., Shah, M.H.R., Rehman, R.A., Ahmad, N., 2016. Biochar enhances the cadmium tolerance in spinach (*Spinacia oleracea*) through modification of Cd uptake and physiological and biochemical attributes. Envi- ron Sci. Pollut. Res 23, 21385–21394. https://doi.org/10.1007/s11356-016-7344-3.

Yu, J., Guo, X., Luo, Z., Ding, J., Xu, B., Chen, H., Zheng, C., Li, Y., Chen, Y., Wang, G., 2021. Do brassinosteroids and iron plaque affect the accumulation of As and Cd in rice (*Oryza sativa* L.)? Environ. Technol. Innovat. 23, 101660. https://doi.org/10.1016/j.eti.2021.101660.

Yu, J.Q., Huang, L.F., Hu, W.H., Zhou, Y.H., Mao, W.H., Ye, S.F., Nogues, S., 2004. A role of brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. J. Exp. Bot. 55, 1135–1143.

Zhang, X.Z., 1992. The measurement and mechanism of lipid peroxidation and SOD POD and CAT activities in biological system. In: Zhang, X.Z. (Ed.), Research Methodology of Crop Physiology. Beijing Agriculture Press, Beijing, pp. 208–211.

Zhou, P., Adeel, M., Shakoor, N., Guo, M., Hao, Y., Azeem, I., Li, M., Liu, M., Rui, Y., 2020. Application of nanoparticles alleviates heavy metals stress and promotes plant growth: An overview. Nanomaterials 11 (1), 26.

Zong, H.Y., Guo, X.H., Song, N.N., Liu, J., Wang, F.L., Gai, L.Y., 2022. Mitigation of cadmium toxicity in edible rape (*Brassica rapa* L.) by combined application of chitosan and silicon. J. Plant Growth Regul. 42, 2857–2866.

Zulfiqar, U., Ayub, A., Hussain, S., Waraich, E.A., El-Esawi, M.A., Ishfaq, M., Ahmad, M., Ali, N., Maqsood, M.F., 2022. Cadmium toxicity in plants: Recent progress on morpho-physiological effects and remediation strategies. J. Soil Sci. Plant Nutr. 22 (1), 212–269.