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Insights into Cadmium-Induced Morphophysiological Disorders in *Althea rosea* Cavan and Its Phytoremediation through the Exogeneous Citric Acid

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Abstract: Cadmium (Cd) is taken in plants from soil and then travels through the food cycle, posing a major threat to all the units of the ecosystem. A pot experiment was conducted to understand the influence of citric acid (CA) on Cadmium (Cd) phytoextraction ability of hollyhock (*Althea rosea* Cavan.). *A. rosea* plants were exposed to Cd concentrations (100 and 200 mg·kg^{-1}), either in simultaneous administration or without adding CA (5 mM·kg^{-1} dry weight). The results revealed that exposing *A. rosea* to different levels of Cd stress, i.e., 100 and 200 mg·kg^{-1}, significantly decreased ($p < 0.05$) plant growth and biochemical attributes, such as root length (RL), shoot length (SL), fresh biomass (FW), dry biomass (DW), relative water content (RWC), and chlorophyll and carotenoid contents. Meanwhile, a net increase in MDA and REL indicated Cd-induced oxidative stress in plants. However, the application of citric acid (CA) as an organic chelator helped the plants to alleviate the phytotoxic effects of Cd stress on *A. rosea*, which is shown in terms of enhancing plant growth and biomass; that is, the root length (27.3% and 21.12%), shoot length (32.11% and 23.02%), fresh weight (39.66% and 29.8%), and dry weight (29.8% and 57.33%) under 100 and 200 mg·kg^{-1} of Cd stress, respectively, were observed. CA application also helped to alleviate the level of chlorophyll and carotenoid contents; foster high level of antioxidants, such as SOD, POD, CAT, and APX; and lower concentration of MDA and EL. In addition to enhancing plant-growth attributes, the application of CA also managed to increase the phytoextraction potential of the plants by enhancing the concentration of Cd in roots and shoots tissues. This is also demonstrated by rising levels of bioaccumulation (BAC) and translocation factors (TFs). These findings showed that CA application could be a practical strategy to apply to ornamental plants, such as *A. rosea* seedlings, cultivated in Cd-contaminated locations, opening ways to cope with Cd stress and enhanced phytoextraction.

Keywords: Cd; chelator; citric acid; phytoextraction; oxidative stress; antioxidants; *Althea rosea* Cavan

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1. Introduction

Soil is naturally the sink of heavy metals (HMs) and other waste materials. The main reason for heavy-metal addition to soil is anthropogenic activities globally [1]. The needs of the modern world have led the world to industrialization. In the past few decades, there has been a great deal of concern about the environmental degradation that toxic metal buildup in the soil and water has caused [2,3]. Metal contamination from industrial mining, fossil-fuel burning, and sewage-disposal operations is the main environmental problem it causes. These industries release waste materials, which make us face environmental issues. Hazardous materials such as heavy metals, pesticides, herbicides, and persistent organic pollutants have been found in air, water, and soil [4–6]. Among these dangerous materials, HMs pose a significant threat to human health because of their high occurrence in the form of contaminants and low solubility in the environment. Besides this, some heavy metals are classified as carcinogens and mutagens in nature [7]. Among these carcinogenic heavy

metals, Cd is a nonessential carcinogenic element [1]. The primary sources of Cd are burning fuels, Ni-Cd batteries, sewage effluents, and sludge from cities [8]. Cd is a nonessential element for plants, inducing various toxic symptoms, such as photosynthetic damage, mineral nutrition alteration, and decreased biomass output, leading to the production of reactive oxygen species (ROS), including superoxide radicals and hydrogen peroxide (H_2O_2); elevated Cd concentration causes membrane leakage, lipid peroxidation, and also the breakdown of biological macromolecules (O_2) [9,10]. From contaminated soil, Cd is taken to aerial parts by plants through roots [11]. High accumulation of Cd in plants leads to certain biochemical and physiological disorders, such as a reduction in growth and yield, nutrient uptake, effect on photosynthetic pigments, and oxidative stress [12]. Cd enters our bodies through the food chain and is the primary constraint for food safety and agriculture [13]. This is why different steps must be taken to tackle the issue of pollution and remediate Cd contamination by in situ remediation techniques.

Many plant species have been found to have fundamental HM tolerance mechanisms [14]. Extreme HM concentrations in soils and waterways are known to be intolerable for the majority of plant species. Many plant species with low levels of metal uptake and subsequent transfer to plants' aboveground tissues have a high tolerance for trace metals [15]. Such plants are called non-hyperaccumulator plants and are also used for phytoremediation purposes, although they are metal extruders [16]. The non-hyperaccumulator plants have unregulated strategies for surviving in environments with high levels of heavy metals [17,18]. Plant toxicity can substantially impair growth, nutritional balance, and important metabolic activities, including photosynthesis and respiration, ultimately resulting in mortality, whether or not the metals are engaged in biological processes [17]. Evolution led to an effective absorption of metals into their various cellular sections to sustain a viable homeostasis to counteract metal-induced cellular toxicity [14,19,20]. When a plant comes in contact with HMs, it has two strategies: the first is to avoid them, and the second is to accumulate them [21]. Hyperaccumulators are plants that can transport heavy metals and have a high potential for accumulation [22]. In contrast, tolerant plants have a lesser accumulation capacity but usually grow when exposed to heavy metals [23]. Both of these plant kinds have several different coping techniques. Enzymatic scavengers such as superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) scavenge excessively collected reactive oxygen species (ROSs) in plants; however, non-enzyme-based scavenger molecules such as ascorbate, organic acids, non-protein (NP) thiols, and total soluble proteins also have an important role in scavenging ROS molecules [9,24].

Different remediation techniques based on plants are practiced by scientists globally because they are cost-effective and environmentally friendly [25,26]. Phytoremediation, a biological approach, is used to clean up polluted soil, as it is economical and extensively applicable [27,28]. Phytoremediation has different techniques, such as volatilization, degradation, stabilization, and phytoextraction. Among these techniques, phytoextraction has achieved great recognition because it depends on plant species' use to remediate impurities and collect them in the shoot by translocating through roots. However, the ability of plants to extract HMs and accumulate them depends on specie type, environmental conditions, and soil type [29,30]. To tackle this issue of specie variation, scientists have also used the technique of inducing phytoextraction. The employment of metal chelates and high-biomass crop species to accumulate and remove heavy metals from soils is known as induced phytoextraction. Synthetic chelates are effective for phytoextraction, although they have the disadvantage of leaching and groundwater contamination. When opposed to synthetic chelating agents, organic acids, such as citric acid, have a higher degree of biodegradability and a lower risk of leaching [31,32]. Organic acids should be used instead of synthetic chelating agents since they lessen metal-leaching risks and are environmentally friendly due to their high biodegradability [33,34]. Citric acid, among organic acids, improves metal solubility and uptake by plants, as well as increases plant intake of other nutrients [1,35]. Plants' uptake of heavy metals can be significantly enhanced by using a

lower concentration of CA. Still, a higher concentration of CA can have a robust phytotoxic effect on specific plant species [36]. Because of its quick breakdown, citric acid is a cost-effective and environmentally friendly chelate for phytoextraction, with little risk of leaching or groundwater contamination [33,34]. Citric acid has been shown to help in Cd mobilization and phytoextraction by several researchers [36]. The use of CA may help to lessen phytotoxicity and environmental issues. However, because plants' physiological response to CA treatment under metal stress has seldom been examined, more research is needed to better understand and apply *B. napus* L. for remediation purposes [37].

Many plants have been discovered as remediation plants, but there has been little information regarding ornamental plants that can help with contaminated soils. In truth, decorative resources are plentiful and can be used to detect and monitor contaminants in the atmosphere [38,39]. Ornamental species have the benefit of being visually pleasing, which may pique public interest and support. As a result, this will provide a solid foundation for weeding out remedial plants. Ornamentals, particularly in metropolitan settings, can both beautify the environment and reduce heavy-metal pollution. Screening remedial plants from ornamental resources will be essential and practical [40]. China is one of the countries rich in plants capable of the most phytoremediation potential [41]. *Althaea rosea* is a perennial upright herb with gorgeous flowers commonly planted throughout Asia, from tropical to temperate zones. High biomass, easy cultivation, deep roots, excellent competitiveness, great seed production, and broad geographic dissemination are all advantages of this specie. Keeping in mind the importance of ornamental plants and their role in the phytoremediation of heavy metals, we designed this study to evaluate the role of CA in the uptake of heavy metals and its mechanistic approach to protect the plant from the harmful effects of Cd.

2. Materials and Methods

2.1. Experimental Design and Treatments

Althea rosea L. seeds were obtained from the Plant Ecology and Physiology Lab, Nankai University Tianjin, originally gathered in Tianjin, China (39_0600.7300N, 117_09044.9000E, from a clean and non-contaminated location. Seeds were first disinfected with sodium hypochlorite and 75% ethanol solution for 1 and 5 min, respectively, followed by washing three to four times through double-distilled water. Healthy seeds were sown in a plastic seed tray under natural light and temperature in the greenhouse. This experimental work's soil was collected from Nankai University Tianjin China greenhouse between 39.10 N and 117.16 E longitude. The soil was analyzed for physicochemical attributes, which are given in Table 1. Soil was sieved through a 2 mm sieve, air dried, and thoroughly mixed with sand in 3:1 to make a uniform texture. The sand was washed before mixing with soil to remove any suspended particles. We autoclaved the soil at 121 °C for 20 min to maintain a controlled environment [42]. Plastic pots (100 mm in length, 100 mm in width, and 150 mm in height) were filled with 1.5 kg soil. Total treatments were divided into five different treatments, which are given in Table 2. A total of 5 mM·kg^{-1} citric acid was added to respective treatment one week after seedlings were transferred to pots (Table 2). Seeds were sown in the germination tray. Twenty days after seeds' sowing, two seedlings of uniform size were rooted from the seed tray and were transferred to each plastic pot.

Two concentrations (100 and 200 mg·kg^{-1}) of Cd as $CdCl_2$ were added to soil in five different treatments (Table 2). Each treatment was conducted in five replicates. Once a week, plants were supplemented with modified Hoagland's solution (mmol/L^{-1}), i.e., 4 mmol $Ca(NO_3)\cdot 9H_2O$, 1.0 mmol KH_2PO_4, 2.0 mmol/L $MgSO_4$, 5 mmol KNO_3, 1.0 mmol NH_4NO_3, 1.0 mmol/L $FeSO_4\cdot 7H_2O$, 0.1 mmol/L EDTA-Na_2, 0.005 mmol/L KI, 0.1 mmol/L H_3BO_3, 0.03 mmol/L $ZnSO_4$, 0.002 mmol/L $Na_2MoO_4\cdot 2H_2O$, 0.132 mmol/L $MnSO_4\cdot H_2O$, 0.0001 mmol/L $CoCl_2\cdot 6H_2O$, and 0.0001 mmol/L $CuSO_4 5H_2O$. Fifty days post-exposure to Cd stress, plants were harvested.

Table 1. Soil physiochemical attributes.

Characteristics	Soil Analysis/kg
Soil Texture	Slightly loamy
pH	8.06
Electrical Conductivity (dS/m)	0.8
Soil Moisture Content %	1.001
Cadmium (mg/kg)	0.37
Copper (mg/kg)	0.22
Lead (mg/kg)	0.05
Zinc (mg/kg)	0.11

Table 2. Treatment design.

Treatment	Cadmium Concentration	Citric Acid
Control	0	−
T1	0	+
T2	100 mg/kg	−
T3	100 mg/kg	+
T4	200 mg/kg	−
T5	200 mg/kg	+

2.2. Evaluation of Agronomic Characteristics

The post-harvested plants were cleaned with tap water to remove debris and soil particles. After that, plant-growth attributes such as root length (RL), shoot length (SL), and fresh and dry biomass (FW and DW) were measured. Fresh weight was measured by using a weighting machine, while for the dry weight of samples, plants were kept in the oven for 48 h, at 70 °C, before weighing again.

2.3. Determination of Chlorophyll and Carotenoid Contents

Leaf chlorophyll and carotenoid contents were estimated by following the protocol of Lichtanhaler et al. [43]. Briefly, leaves from all the treatments were collected, and their homogeneous mixture was prepared in DMSO. Readings were collected by using a spectrophotometer at different wavelengths, which are 470 nm, 649 nm, and 665 nm. The final chlorophyll contents were estimated by applying the following formulae:

$$\text{Chlorophyll (a)} = 13.95A_{665} - 6.68A_{649}$$

$$\text{Chlorophyll (b)} = 24.96A_{649} - 7.32A_{665}$$

$$\text{Chlorophyll (a + b)} = 18.08A_{649} + 6.63A_{665}$$

$$\text{Carotenoids} = \frac{1000A_{470} - 2.05Ca - 114Cb}{245}$$

2.4. Leaf Relative Water Content (RWC)

After leaf harvesting, the relative water content was calculated. In brief, the fresh weight (FW) of leaves was calculated. After that, the leaves were kept submerged for 24 h in distilled water. Then the turgid weight (TW) of fully turgid leaves was measured by reweighing them. After 72 h of oven drying at 100 °C, the leaves' constant dry weight (DW) was observed. Balestri et al.'s [44] protocol was followed for calculating the water content. The RWC was estimated by using the following formula:

$$\textit{Relative water content} = \frac{FW - DW}{TW - DW} \times 100$$

2.5. Proline Content Estimation

The protocol of Bates et al. [45] was used to estimate the proline content. Briefly, 4 mL of sulfosalicylic acid (3%) was mixed with 0.5 g of fresh leaves before being left at 5 °C overnight. On the following day, the mixture was centrifuged for 5 min at 3000 rpm after which 2 mL of acid ninhydrin was added. After heating the mixture, 4 mL of toluene was used to extract it. Equation (k = 17.52 and dilution factor = 2) was used to estimate total proline after the optical density at 520 nm was observed.

2.6. Determination of Antioxidants Enzymes

Antioxidant enzymes were also estimated by spectrophotometer. Antioxidant enzymes such as CAT, SOD, POD, and APX were evaluated by following the previously described protocols of Zhang et al., Nakano et al., and Aebi et al. [46–48], respectively.

2.7. Determination of MDA and Relative Electrolyte Leakage

The MDA level was assessed to understand the lipid peroxidation level by following the thiobarbituric method previously described by Reference [49], and the relative electrolyte leakage was evaluated as previously described by Singh et al. [50]. The following formula counted the content of REL:

$$\text{Relative Electrolyte Leakage} = x = \frac{(\text{E1} - \text{E0})}{(\text{E2} - \text{E0})} \times 100\%$$

2.8. Determination of Cd Concentration

Plants after harvesting were washed with double-distilled water. Plants from each treatment were oven-dried, and the dried powder was digested in a 4:4 *v/v* solution of HNO3:HClO4, respectively, through digiprep digestion. An atomic absorption spectrophotometer (AAS 3520, Shanghai, China) was used to quantify the concentration of Cd. The protocol of Wilkins [21] and Cheraghi et al. [51] was followed. To understand the accumulation, we determined the tolerance index (TI), translocation factor (TF), biological accumulation coefficient (BAC), and biological concentration factor (BCF) by using the following formulae:

$$\text{TI} = \text{M}_{\text{Cd\_treated}}/\text{M}_{\text{control}},$$

where $\text{M}_{\text{Control}}$ is the FW of control group plants, and $\text{M}_{\text{Cd-treated}}$ is the FW of plants under the Cadmium treatment;

$$\text{TF} = \text{C}_{\text{shoot}}/\text{C}_{\text{root}}$$

where C_{shoot} and C_{root} express the concentration of Cadmium in the shoot and root, respectively;

$$\text{BAC} = \text{C}_{\text{shoot}}/\text{C}_{\text{treatment}} \times 100\%$$

where C_{shoot} and $\text{C}_{\text{treatment}}$ show the concentration of Cadmium in the shoot and respective treatment;

$$\text{BCF} = \text{C}_{\text{root}}/\text{C}_{\text{treatment}} \times 100\%$$

where C_{root} and $\text{C}_{\text{treatment}}$ show the concentration of Cadmium in the root and respective treatment.

2.9. Statistical Analysis

Statistics software (Statistix version 8.1) was used to analyze the data statistically. A one-way ANOVA was calculated by using Fisher's LSD to determine the significant value ($p < 0.05$) between the means. PCA (principal component analysis) and Pearson's correlation of all the data were performed by using the R plotting software. For PCA, built-in R function "prcomp" and "ggplot2" library was used.

3. Results

3.1. Variation of Growth

Cadmium stress significantly decreased the morphological attributes of *A. rosea*, and the reduction in growth parameters is more obvious with the increasing concentration in treatments of *A. rosea*, as shown in Figure 1. The maximum inhibition was observed in plants under 200 mg·kg^{-1} Cd stress as compared to the control for the fresh weight (53%), dry weight (77%), root length (19.41%), and shoot length (24.01%). However, CA addition enhanced the morphological parameters, such as root length (23.01%), shoot length (27.01%), fresh weight (44%), and dry weight (26.92), under non-stress conditions in T1 plants as compared to the control. The same pattern of increase in agronomic characteristics was observed in CA-assisted plants in root length (27.3% and 21.12%), shoot length (32.11% and 23.02%), fresh weight (39.66% and 29.8%), and dry weight (29.8% and 57.33%) under 100 and 200 mg·kg^{-1} of Cd stress, respectively. Figure 1c shows the RWC of the leaves under different treatments. It is obvious that the RWC in T2 plants is higher (42%) than that of the control and T3 plants under 100 mg·kg^{-1} + CA application, which do not show any significant role of CA in RWC enhancement. The RWC decreased in T4 and T5 plants, respectively, under the increasing concentration of Cd exposure.

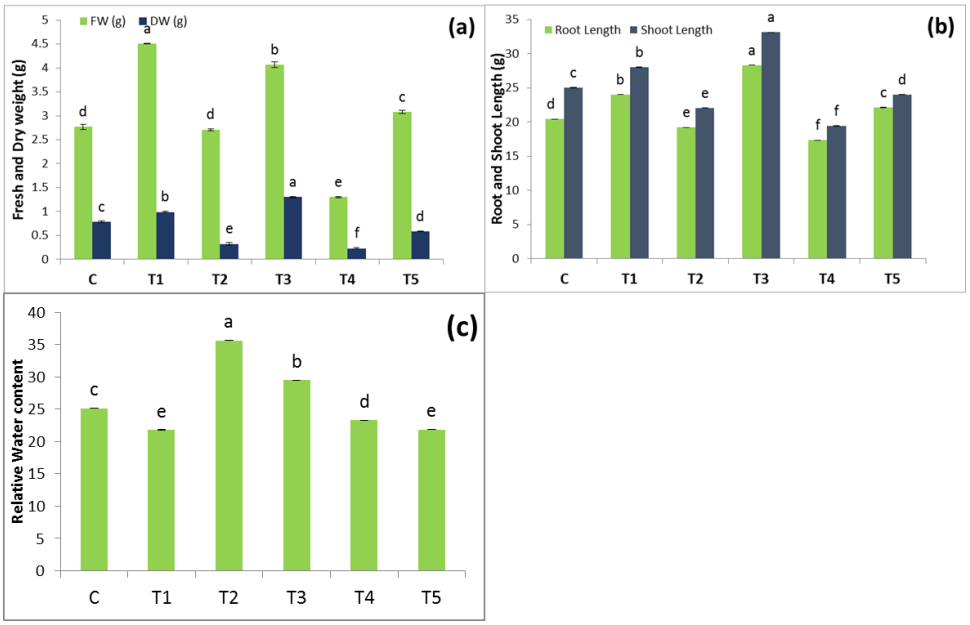

Figure 1. Fresh and dry biomass (**a**), root and shoot length (**b**), and relative water content (**c**) of *A. rosea* plants under Cd and citric acid application. The bars indicate mean values ± standard error. Where T1 = 5 mM·kg^{-1} CA, T2 = 100 mg·kg^{-1} Cd, T3 = 100 mg·kg^{-1} + 5 mM·kg^{-1}, T4 = 200 mg·kg^{-1} Cd, and T5 = 200 mg·kg^{-1} + 5 mM·kg^{-1}. The different lowercase letter on the vertical bars represent significant differences among the treatments at $p < 0.05$.

3.2. Chlorophyll and Carotenoids

The chlorophyll contents significantly decreased in Cd-treated plants with increasing concentrations, i.e., chlorophyll a (85.46 and 76.7%), chlorophyll b (22.89 and 29.11%), and carotenoids (27.83 and 93%), under exposure to 100 and 200 mg·kg^{-1} of Cd stress, respectively, as compared to the control; however, the CA application helped the plants at 100 mg·kg^{-1} to alleviate these negative effects in *A. rosea* (Table 3). Increase in photosynthetic pigments were found in Cd treatments with simultaneous administration of citric acid in the limit of chlorophyll a at (30.7%), chl b (61.71%), total chlorophyll (37.04%), and carotenoids (11.95%) under 100 mg·kg^{-1} Cd treatments, respectively, as compared to Cd-alone counter treatments. However, at 200 mg·kg^{-1}, no significant difference was found as compared to the control.

Table 3. Chlorophyll contents in *A. rosea* plants under different Cd treatments. Values indicate mean values ± standard error. Where T1 = 5 mM·kg^{-1} CA, T2 = 100 mg·kg^{-1} Cd, T3 = 100 mg·kg^{-1} + 5 mM·kg^{-1}, T4 = 200 mg·kg^{-1} Cd, and T5 = 200 mg·kg^{-1} + 5 mM·kg^{-1}. The different lowercase letters on the vertical bars represent significant differences among the treatments at $p < 0.05$.

Chlorophyll Contents (mg.g^{-1} FW)					
Treatments	**Chlorophyll a**	**Chlorophyll b**	**Total Chlorophylls**	**Chlorophyll a/b**	**Carotenoids**
C	86.46 ± 0.03 c	20.4 ± 0.03 c	106.25 ± 0.02 c	4.25 ± 0.04 a	16.03 ± 0.01 b
T1	144.6 ± 0.04 a	58.5 ± 0.00 a	201.92 ± 0.03 a	2.49 ± 0.03 bc	17.25 ± 0.00 b
T2	65.1 ± 0.01 d	16.6 ± 0.02 c	81.24 ± 0.01 e	3.93 ± 0.03 a	12.54 ± 0.04 c
T3	113.47 ± 0.03 b	32.99 ± 0.04 b	145.61 ± 0.04 b	3.45 ± 0.01 ab	34.62 ± 0.02 a
T4	48.93 ± 0.00 e	15.8 ± 0.01 c	64.34 ± 0.00 f	3.28 ± 0.05 ab	8.29 ± 0.03 d
T5	64.85 ± 0.01 d	32.48 ± 0.01 b	96.71 ± 0.03 d	2.02 ± 0.01 c	8.70 ± 0.02 d

3.3. MDA and REL Contents

By increasing the concentration of Cd, *A. rosea* plants show damage to the leaves membrane and other morphological parameters. A net increase in MDA (Figure 2A) and REL (Figure 2B) were observed in plants exposed to 100 mg·kg^{-1} (41.93 and 78.75%) and 200 mg·kg^{-1} (87.09 and 110.8%) respectively. However, plants exposed to Cd stress supplemented with CA showed a significant decrease in both MDA and REL concentrations; that is, plants exposed to 100 mg·kg^{-1} of Cd + CA showed a net decrease of 8.7 and 27.77% in MDA and REL contents, respectively. When supplemented with CA, the same pattern of MDA and REL under 200 mg·kg^{-1} stress showed a significant decrease of 11.75 and 32.23%, respectively.

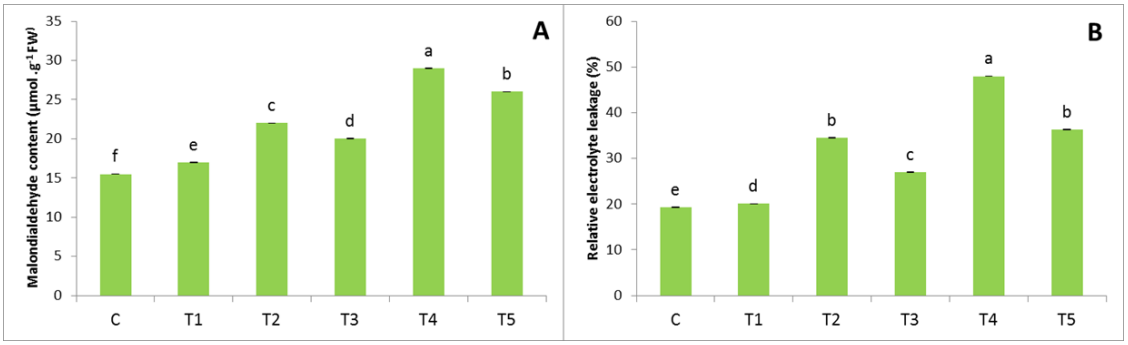

Figure 2. MDA contents (**A**) and relative electrolyte leakage (**B**) of *A. rosea* plants under Cd and citric acid application. The bars indicate mean values ± standard error. Where T1 = 5 mM·kg^{-1} CA, T2 = 100 mg·kg^{-1} Cd, T3 = 100 mg·kg^{-1} + 5 mM·kg^{-1}, T4 = 200 mg·kg^{-1} Cd, and T5 = 200 mg·kg^{-1} + 5 mM·kg^{-1}. The different alphabets on the vertical bars represent significant differences among the treatments at $p < 0.05$.

3.4. Antioxidants Enzyme

When exposed to Cd stress, antioxidant activity increased in *A. rosea* plants. As shown in the Figure 3, the activities of SOD (Figure 3a) increased in all treatments exposed to Cd stress as compared to the control. Citric acid application in T1 plants increased the SOD activity by 10.05% as compared to the control. The same pattern of SOD increase was noted in T2 and T3 plants compared to the control. However, CA enhanced the activity of SOD by 21.61% and 26.36% in T3 and T5 plants, respectively, as compared to the control. POD activity (Figure 3b) also shows increased activity with increasing Cd concentration. However, plants exposed to Cd treatments in synergy with CA (T3 and T5) showed a promising increase of 52.63% and 81.57%, respectively. CAT activity (Figure 3c) at 100 mg·kg^{-1} showed a net increase, but at 200 mg·kg^{-1} (T4 plants), there was a slight

decrease as compared to the control. However, CA induced a net increase of 24.96% and 11.21% in T3 and T5 treatments, respectively. The same pattern of results in APX activity (Figure 3d) was found. A net increase of 25.37% and 29.85% in T3 (100 mg·kg^{-1} + citric acid) and T5 (200 mg·kg^{-1} + citric acid) plants was observed, respectively, as compared to control.

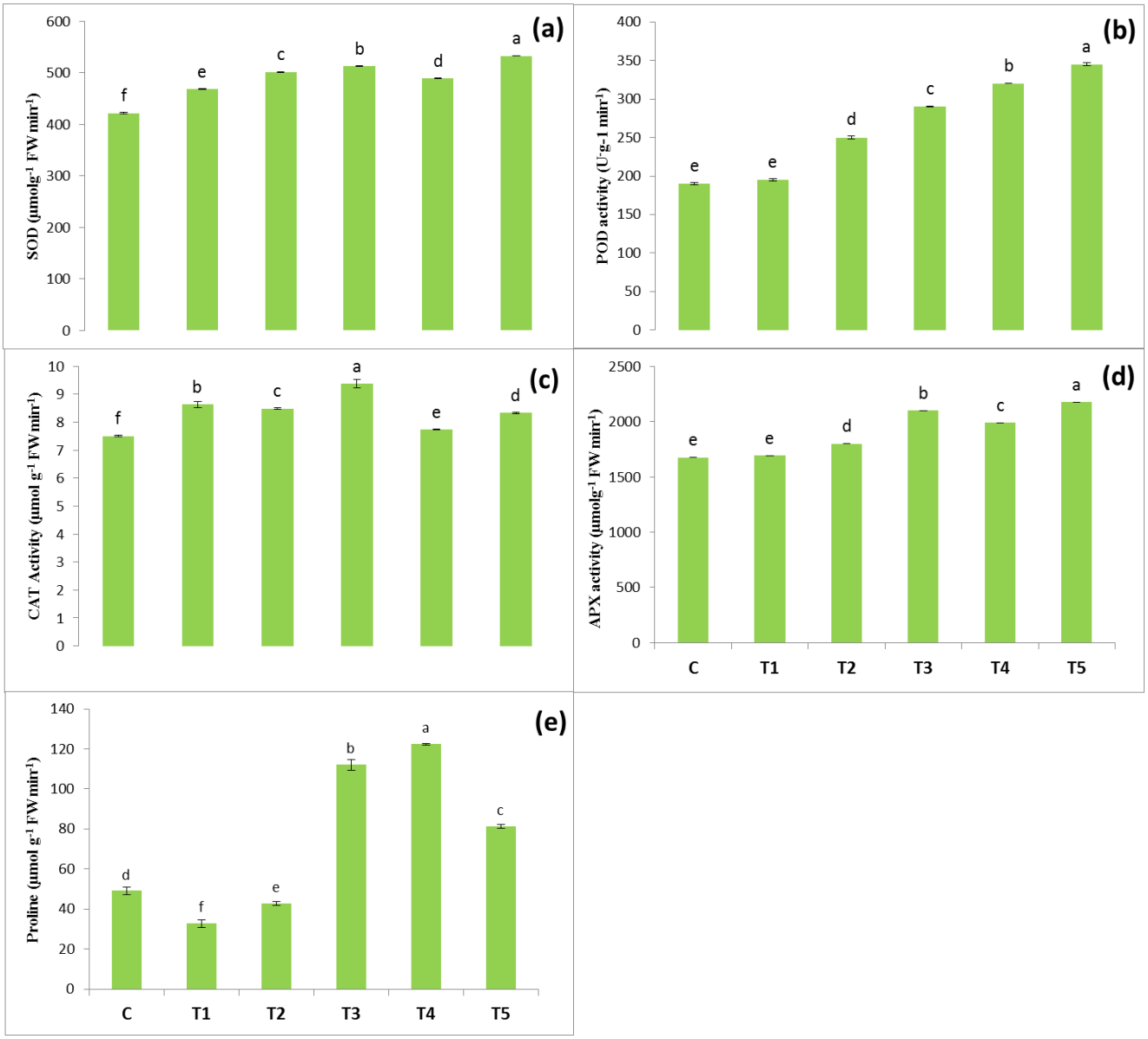

Figure 3. Activities of SOD (**a**), POD (**b**), CAT (**c**), APX (**d**), and proline (**e**) in the leaves of *A. rosea* at different Cd treatments single or in synergy with CA. Vertical bars indicate mean ± standard deviation. Where T1 = 5 mM·kg^{-1} CA, T2 = 100 mg·kg^{-1} Cd, T3 = 100 mg·kg^{-1} + 5 mM·kg^{-1}, T4 = 200 mg·kg^{-1} Cd, and T5 = 200 mg·kg^{-1} + 5 mM·kg^{-1}. Different lowercase letters represent significant differences between the treatments at $p < 0.05$.

3.5. Proline Content

The proline content in plants under Cd stress showed a net increase compared to control plants. At 200 mg·kg^{-1} of Cd, there was no significant difference in proline content in Cd + CA simultaneous administration; however, at 100 mg·kg^{-1}, Cd's net proline content is increased by 110% compared to the plants with Cd (T2) alone (Figure 3e).

3.6. Plant Biomass and Distribution of Cd

Cadmium treatment showed a significant net decrease in root and shoot biomass in *A. rosea* plants, both under 100 and 200 mg·kg^{-1}, compared to control (Table 4). A net decrease of 18.6% and 18.86% was observed in shoot and root biomass under 100 mg·kg^{-1} Cd stress, and in the same way, under 200 mg·kg^{-1} Cd concentration, the net decrease in shoot and root biomass was 82.14% and 75%, respectively. However, plants under the combinatorial application of Cd + CA showed significant increase in shoot (34.88% and 11.5%) and root biomass (38.46% and 10%) under 100 mg·kg^{-1} and 200 mg·kg^{-1}, respectively.

Table 4. Biomass, Cd concentration, tolerance index (TI), translocation factor (TF), biological accumulation coefficient (BAC), and biological concentration factor (BCF) in *A. rosea* at different Cd treatments. Where T1 = 5 mM·kg^{-1} CA, T2 = 100 mg·kg^{-1} Cd, T3 = 100 mg·kg^{-1} + 5 mM·kg^{-1}, T4 = 200 mg·kg^{-1} Cd, and T5 = 200 mg·kg^{-1} + 5 mM·kg^{-1}.

	Dry Weight (g.plant^{-1})		**Cd Concentration (mg·kg^{-1})**					
	Shoot	**Root**	**Shoot**	**Root**	**TI**	**TF**	**BAC**	**BCF**
C	0.5 ± 0.03 b	0.33 ± 0.01 d	0.01 ± 0.02 e	0.01 ± 0.01 d	N.d	N.d	N.d	N.d
T1	0.4 ± 0.04 c	0.34 ± 0.03 d	0.01 ± 0.03 e	0.01 ± 0.03 d	N.d	N.d	N.d	N.d
T2	0.43 ± 0.03 c	0.53 ± 0.05 b	1.5 ± 0.04 d	2.43 ± 0.02 c	0.979518	0.61	15%	24%
T3	0.58 ± 0.01 a	0.72 ± 0.04 a	2.1 ± 0.01 c	2.5 ± 0.02 c	1.46988	0.84	21%	25%
T4	0.13 ± 0.04 e	0.18 ± 0.04 e	2.7 ± 0.02 b	3.42 ± 0.03 b	0.46988	1.26	17%	13%
T5	0.28 ± 0.05 d	0.36 ± 0.03 c	3.4 ± 0.05 a	3.9 ± 0.04 a	1.113253	1.14	19%	17%

In the same way, when plants were exposed to Cd + CA, the accumulation of Cd increased as compared to plants treated with Cd treated along. As the results indicate, at 100 mg·kg^{-1} + CA, the accumulation of Cd was 40% higher in the shoot and 25.92% in the root compared to only Cd-treated plants. In the same way, at 200 mg·kg^{-1} + CA, the accumulation of Cd was 25.92% higher in the shoot and 14.03% in the root compared to the plants treated only with Cd. This is also evident from Figure 4A,B that uptake of Cd is enhanced both in root and shoot in CA treated plants.

The tolerance index (TI), translocation factor (TF), biological accumulation coefficient (BAC), and biological concentration factor (BCF) are given in Table 4. TI shows the highest value of 1.46 at 100 mg·kg^{-1} and 1.11 at 200 mg·kg^{-1} Cd concentration, respectively. In the same way, TF also shows the highest value of 1.26 at 100 mg·kg^{-1}. The same pattern of BAC and BCF values' increase can be seen when the Cd concentration is 100 mg, but at 200 mg, the values decrease.

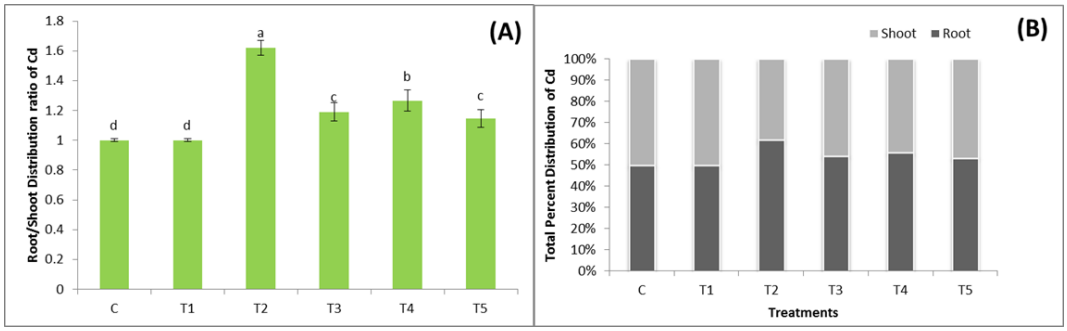

Figure 4. Total % distribution of Cd in plants (**A**) and root/shoot distribution of Cd (**B**) of *A. rosea* plants under Cd and citric acid application. The bars indicate mean values ± standard error. Where T1 = 5 mM·kg^{-1} CA, T2 = 100 mg·kg^{-1} Cd, T3 = 100 mg·kg^{-1} + 5 mM·kg^{-1}, T4 = 200 mg·kg^{-1} Cd, and T5 = 200 mg·kg^{-1} + 5 mM·kg^{-1}. The different lowercase letters on the vertical bars represent significant differences among the treatments at $p < 0.05$.

3.7. Pearson's Correlation

The results of a Pearson's correlation study between the researched parameters for *A. rosea* are displayed in Figure 5. The correlation study revealed that the levels of Cd in the roots and shoots were positively correlated, while the levels of Cd in the plant's height, FW, DW, chlorophyll (a, b, and total), and carotenoid contents were negatively correlated under stress conditions. However, Cd concentration in the roots and shoots was strongly connected when CA served as a chelator, and Cd concentration in the shoots was similarly favorably correlated with other morphological and physiological features. This correlation shows a close relationship between Cd uptake and other parameters investigated in *A. rosea* when CA was applied.

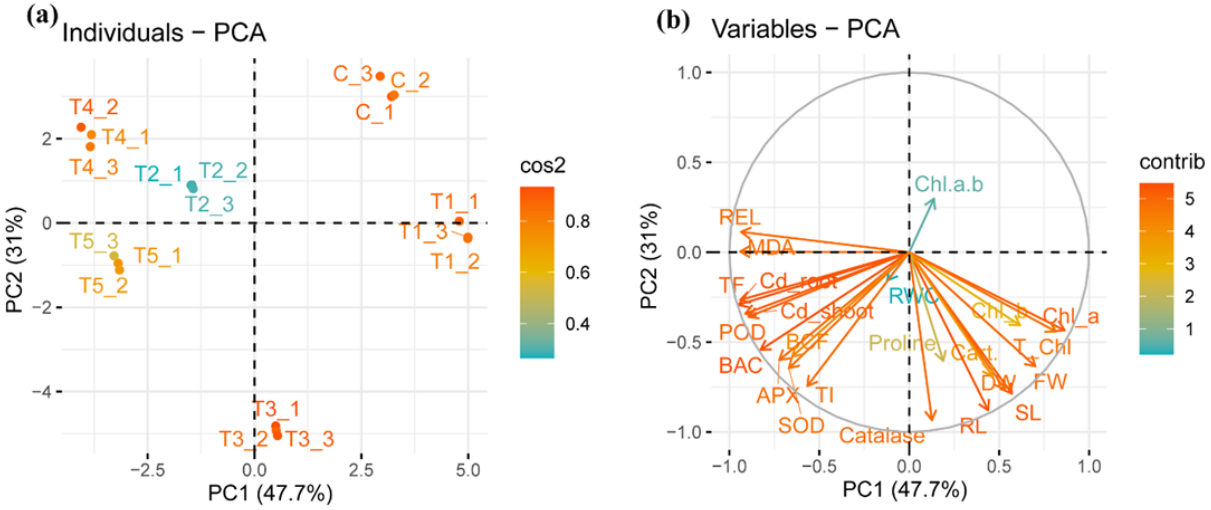

Figure 5. Score (**a**) and loading plot (**b**) of principal component analysis (PCA) on different studied attributes of *A. rosea* seedlings/plants supplemented with citric acid (CA) while grown under Cd stress. Where PC stands for pearson's correlation; REL for relative electrolyte leakage; TF for translocation factor; POD for peroxidase; APX for ascorbate peroxidase; BAC for bioaccumulation concentration; APX for ascorbate peroxidase; SOD for superoxide dismutase; RL for root length; SL for shoot length and RWC for relative water content.

3.8. Principal Component Analysis

The impact of Cd and citric acid treatment on key significant researched features of *A. rosea* plants was evaluated by using the score and loading plots of principal component analysis (PCA), which are displayed in (Figure 6). The first two components, PC1 and PC2, contributed the most out of all the components and were responsible for 77.7% of the dataset's overall variation. Among these, PC1 provided 47.7% and PC2 31%, respectively. The first two primary components successfully disseminated all five treatments (Figure 6). This arrangement of treatments demonstrated that the treatment of CA under Cd stress had a considerable ameliorative effect compared to the control on the examined properties of *A. rosea* plants. The Cd treatments without CA, i.e., T2 and T4, were more displaced from the treatments such as control, citric acid amendment under 100 mg/kg^{-1} Cd stress, and citric acid amendment under 200 mg/kg^{-1} Cd stress (Figure 6), showing that the ecophysiology and plant growth was adversely affected by Cd stress.

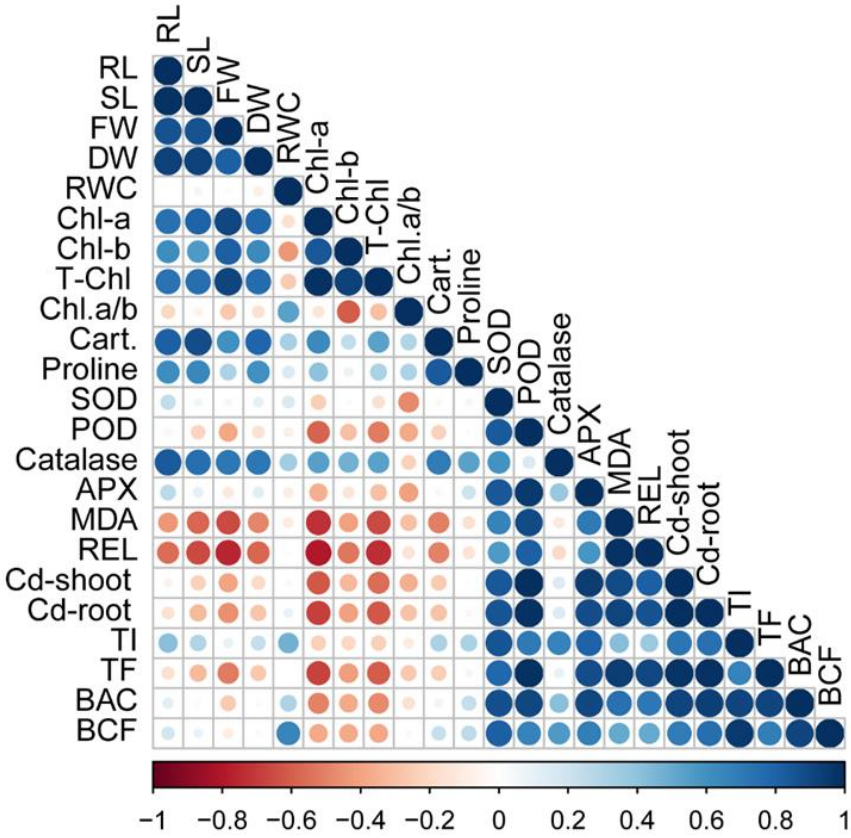

Figure 6. Pearson's correlation analysis (r-values) between different studies' parameters of *A. rosea* seedlings grown under different stress levels of Cd with and without CA application. Where PC stands for pearson's correlation; REL for relative electrolyte leakage; TF for translocation factor; POD for peroxidase; APX for ascorbate peroxidase; BAC for bioaccumulation concentration; APX for ascorbate peroxidase; SOD for superoxide dismutase; RL for root length; SL for shoot length and RWC for relative water content.

4. Discussion

The current research assessed the effects of Cd and CA alone or in simultaneous administration on *A. rosea* plants and their uptake and accumulation in shoot and root systems. Our results show that, when *A. rosea* plants were exposed to a higher concentration of Cd, i.e., 200 mg·kg^{-1}, they showed a net decline in morphological attributes, such as root length, shoot length, fresh weight, and dry weight. However, the application of 5 mmol.kg^{-1} citric acid retained the morphophysiological parameters of the plant.

4.1. Plant Biomass

Plant biomass production is a useful parameter for describing how well plants have developed when exposed to heavy-metal stress. Our results indicate that, when plants were exposed to Cd stress, they showed a significant decrease in growth parameters, such as FW, DW, RL, and SL, as compared to the control, and this decrease in growth parameters was more obvious with the increasing Cd concentration [52,53]. However, exogeneous application of citric acid enhanced both the fresh and dry weight of T3 and T5 plants as compared to the control plants and those exposed to Cd alone, as shown in (Figure 1). In a similar study that assessed the effectiveness of CA against Cd toxicity and the unfavorable changes in the plants caused by Cd, the growth and biomass of *B. juncea* were examined by Mahmud et al. [54]. Their results showed that Cd significantly hampered the biomass and development of the plants; the plant height, FW, and DW all decreased as a result of Cd exposure in a dose-dependent manner. Under mild and severe stress, respectively, plant height dropped by 18 and 24% in comparison to the control treatment, whereas the

application of CA significantly increased the plant height of the stressed plants; both FW and DW showed similar patterns, and this is in agreement with our findings. A plant biomass increase with chelator amendment indicates the preservation of harmful Cd in active non-metabolic areas, such as cell walls and vacuoles, which may be responsible for the increase in biomass. This may allow the plant to develop normally and did not affect the hormones, i.e., cytokinins that control plant growth [10,55]. It is also attributed to a chelator such as CA, which has a keen role in decreasing the soil pH; a small change in the pH leads to the enhanced uptake of nutrients, leading to the increase in growth attributes [56]. Our results are in agreement with this finding. However, plants show reduced plant biomass after exposure to a certain stress concentration. This reduction is attributed to heavy-metal accumulation in the cytoplasm and eventually affects the cell's metabolism, as previously presented by Yadav et al. [57]. Furthermore, the accumulation of heavy metals in cells leads to reduced cell turgidity and inhibition of cell growth and division, which is also a reason for biomass reduction [58–60]. Our results show that plants exposed to the high concentration of Cd, i.e., 200 mg·kg^{-1}, showed a decline in biomass, as shown in Figure 1. According to earlier research by Gill et al. [12], excessive Cd inhibited plant growth and biomass production by lowering mineral uptake and disrupting the biochemical and metabolic processes. The dry shoot and root weight also show a net decrease in biomass with increasing Cd concentration compared to the control, as shown in Table 4. The distribution and absorption of vital nutrients may be impacted by the adsorption of heavy-metal ions, which may harm plants' ability to grow normally. Our findings were in agreement with those in *Malva sinensis* and *Calendula officinalis*, as previously described by Gupta et al. (2009) and Liu et al. (2008), respectively [40,61]. Our results show that the application of citric acid as a chelator in the presence of Cd increased both the root, shoot, and net plant biomass, as shown in Figure 1 and Table 4, at 100 mg·kg^{-1} Cd concentration. These findings agree with Muhammad et al. [62], who applied CA and EDTA to artificially Cd spike soil to find the phytoextraction potential of *Typha angustifolia*. Compared to the other treatments, CA application under Cd pollution levels increased shoot and root dry weight more successfully. Numerous studies have shown that adding CA to HM-polluted soil substantially enhances plant growth in different plants, such as *Solanum nigrum, Brassica napus, Zea mays*, and *Juncus effuses*, respectively [1,63–65]. It was also estimated in our current study that RWC showed a net increase of 42% in plants exposed to 100 mg·kg^{-1}, as compared to control plants. However, at increasing concentrations of Cd, there was a net decrease in RWC. Similar results were found by Pena et al. [66] in sunflowers under Cd stress. Our results are also in agreement with the results of Aghaz et al. [67].

4.2. Cd Accumulation

Cd accumulation and uptake by *A. rosea* were consistent with elevated levels in root and shoot samples (Table 4). However, when supplemented with CA, plants under Cd exposure showed enhanced uptake in roots and shoots. As already mentioned, in T3, the accumulation of Cd was 40% higher in the shoot and 25.92% in the root compared to only Cd-treated plants. In the same way, at 200 mg·kg^{-1} + CA, the accumulation of Cd was 25.92% higher in the shoot and 14.03% in the root compared to only Cd-treated plants. CA applied exogenously improves Cd absorption in several plants [1,37]. The effectiveness of CA for phytoremediation was reported earlier by Mahmud et al. [54], who found that the lower dose (0.5 mM) of CA significantly increased Cd deposition in both the shoots and roots of *B. juncea*. Similar results of enhanced Cd uptake under CA synergy have been previously reported in *Sedum alfreddi* by Lu et al. [68]. According to Wauna et al. [34], the development of organometallic complexes in the solution and around the surface of the roots boosted their dissociation into free Cd, which may be unquestionably submerged or taken up through roots. In the current study, greater Cd concentration in plants in the presence of CA may be owing to CA's ameliorative effect on root structure and form, as shown by Najeeb et al. [37] in *J. effuses* under Cd stress with CA treatment. The chelator CA addition may have activated the root plasma membrane's

ATPases, which altered the transport of ions through the membrane and boosted Cd uptake through symplasmic or apoplasmic routes (Han et al. [69]). Metal distribution in plant tissues is a significant characteristic that can serve as a proximate signal of detoxification processes. CA application also enhanced TI, TF, BAC, and BCF, respectively, compared to the plants treated with Cd alone. Citric acid may increase the solubility and availability of Cd by reducing pH and releasing potent ligands into the soil which facilitate Cd acquisition by roots and transportation to shoots, and so a raise of BCF and BAC was observed [29].

4.3. Chlorophyll and Carotenoids

Exposure of *A. rosea* plants to Cd stress shows a net impact on photosynthetic pigments, i.e., Chl a, Chl b, Total Chlorophyll and carotenoid contents, as shown in Table 1. The Chlorophyll a, Chl b, total Chlorophyll and carotenoid contents showed a net decrease compared to control plants [70]. However, plants exposed to Cd (100 mg·kg^{-1}) supplemented with CA showed a significant increase in photosynthetic pigments, i.e., chlorophyll a at (30.7%), chl b (61.71%), total chlorophyll (37.04%), and carotenoids (11.95%) under 100 mg·kg^{-1} Cd treatments, respectively, as compared to Cd-alone counter treatments. However, at 200 mg·kg^{-1}, no significant difference was found as compared to control treatments, respectively, as compared to Cd-alone treatments. Our findings agree with Babushkina et al. [71] and Ehsan et al. [1]. Their results show similar results: Cd inhibits plants' photosynthesis and pigment levels with increasing Cd concentration. The severe distortion of the chloroplast ultrastructure, which causes a disordered shape and inflated thylakoids, may cause a decline in the photosynthesis and pigment values [70,72,73]. Another research by Mahadavian et al. [74] also agrees with our findings, as they noticed that *Calendula officinalis*, when exposed to Cd stress, showed a net decrease in chlorophyll contents and carotenoids; however, supplementation of CA enhanced the photosynthetic pigments and carotenoids. In reality, this decline in carotenoids and chlorophylls may impact the capacity of photosynthetic operations work, which might restrict the growth and development of plants [1,75,76].

4.4. Oxidative Stress Due to Cd and ROS Production

In our experiment, *A. rosea* plants exposed to Cd stress showed a net increase in MDA and REL contents (Figure 3a,b). Previously, exposure of *B. napus* and *S. lycopersicon* to Cd showed a similar increase in MDA and REL contents [77,78]. However, in our experiment, when plants were exposed to Cd + CA, they showed a decreasing trend of EL and MDA contents. These findings agree with Ehsan et al. [1], whose research previously showed a similar trend of decreasing MDA and EL in *B. napus* when exposed to Cd and CA.

Free radicals can be produced directly or indirectly during plant growth and metabolism in an unfavorable environment and can harm plants' cell membranes [79]. Our findings suggested that reactive oxygen species (ROSs) may be formed under Cd stress in *A. rosea* seedlings, resulting in lipid peroxidation. However, compared to Cd treatment alone, Cd-combined CA application dramatically reduced REL and MDA buildup, indicating a defensive role for CA in lowering the oxidative stress in *A. rosea* plants. Although Cd cannot directly cause ROS, it can bind to and interact with targets or contend for binding sites, changing target proteins' activities and causing ROS generation [80]. Plants that interact with Cd typically sustain oxidative damage [81]. Effective radical scavenging systems are required to control ROS generation in plants under cadmium stress strictly. To reduce and repair ROS damage, plants have defense mechanisms that activate antioxidant enzymes [82]. Plants contain the crucial antioxidant enzymes SOD, POD, CAT, and APX. Our current research showed increased antioxidants when plants were exposed to Cd stress. In pea leaves, Agarwal et al. [83] discovered that Cd treatment increased the activity of the enzymes CAT, SOD, and APX.

Additionally, Chen et al. [84] discovered that Cd boosted SOD and POD activity, while decreasing CAT activity within seedlings exposed to Cd. Our results show that, when plants exposed to Cd (100 mg·kg^{-1}) were treated with CA, they showed a net increase

in all the antioxidant content compared to the control plants and plants treated with Cd alone. However, at a high Cd level, no significant difference was observed. The idea that organic acids interact with heavy metal ions in soil to activate and increase bioavailability can elucidate it. Our findings agree with Ehsan et al.'s [1] research; when *B. napus* was subjected to Cd stress and 0.5 mM citric acid, it showed an upregulated production of antioxidants. Another study by Saffari et al. [85] shows the same trend of an increase in antioxidants in *Calendula officinalis* under the simultaneous administration of 100 mg·kg^{-1} Cd and 0.5 mM citric acid as a chelator.

5. Conclusions

These findings can conclude that Cd toxicity decreased plant height, fresh and dry weight, total chlorophyll and carotenoid levels, and relative water content while scavenging ROS generation. However, higher Cd concentration in the treatment causes Cd absorption and accumulation to rise in *A. rosea*. The administration of CA dramatically improved plant growth and biomass, photosynthetic pigments, RWC, and cellular organelles of the plant cell while reducing oxidative stress by lowering MDA and proline concentrations and restoring normal SOD, CAT, APX, and POD enzymatic activities. Additionally, CA treatment boosts Cd absorption in *A. rosea* plants, aiding in the effective phytoextraction of Cd. In conclusion, the results showed that CA application promoted *A. rosea* growth and biomass in both the existence and absence of Cd treatment by increasing Cd accumulation and decreasing its toxicity. As a result, *A. rosea* can be employed as a phytoextraction method for heavy metals such as Cd when CA is applied, although additional soil-based research is needed to verify these findings.

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References

1. Ehsan, S.; Ali, S.; Noureen, S.; Mahmood, K.; Farid, M.; Ishaque, W.; Shakoor, M.B.; Rizwan, M. Citric acid assisted phytoremediation of cadmium by *Brassica napus* L. *Ecotoxicol. Environ. Saf.* **2014**, *106*, 164–172. [CrossRef] [PubMed]
2. Artiola, J.; Walworth, J.; Musil, S.; Crimmins, M. Soil and land pollution. In *Environmental and Pollution Science*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 219–235.
3. Zainab, N.; Khan, A.A.; Azeem, M.A.; Ali, B.; Wang, T.; Shi, F.; Alghanem, S.M.; Hussain Munis, M.F.; Hashem, M.; Alamri, S. PGPR-mediated plant growth attributes and metal extraction ability of *Sesbania sesban* L. in industrially contaminated soils. *Agronomy* **2021**, *11*, 1820. [CrossRef]
4. Cheng, S. Heavy metal pollution in China: Origin, pattern and control. *Environ. Sci. Pollut. Res.* **2003**, *10*, 192–198. [CrossRef]
5. Rajkumar, M.; Ae, N.; Freitas, H. Endophytic bacteria and their potential to enhance heavy metal phytoextraction. *Chemosphere* **2009**, *77*, 153–160. [CrossRef]
6. Aioub, A.A.; Li, Y.; Qie, X.; Zhang, X.; Hu, Z. Reduction of soil contamination by cypermethrin residues using phytoremediation with *Plantago major* and some surfactants. *Environ. Sci. Eur.* **2019**, *31*, 26. [CrossRef]
7. Diels, L.; Van der Lelie, N.; Bastiaens, L. New developments in treatment of heavy metal contaminated soils. *Rev. Environ. Sci. Biotechnol.* **2002**, *1*, 75–82. [CrossRef]
8. Shakoor, M.B.; Ali, S.; Farid, M.; Farooq, M.A.; Tauqeer, H.M.; Iftikhar, U.; Hannan, F.; Bharwana, S.A. Heavy metal pollution, a global problem and its remediation by chemically enhanced phytoremediation: A review. *J. Biodivers. Environ. Sci.* **2013**, *3*, 12–20.
9. Mahdavian, K.; Ghaderian, S.M.; Schat, H. Pb accumulation, Pb tolerance, antioxidants, thiols, and organic acids in metallicolous and non-metallicolous *Peganum harmala* L. under Pb exposure. *Environ. Exp. Bot.* **2016**, *126*, 21–31. [CrossRef]
10. Sidhu, G.P.S.; Singh, H.P.; Batish, D.R.; Kohli, R.K. Tolerance and hyperaccumulation of cadmium by a wild, unpalatable herb *Coronopus didymus* (L.) Sm.(Brassicaceae). *Ecotoxicol. Environ. Saf.* **2017**, *135*, 209–215. [CrossRef]
11. Farid, M.; Shakoor, M.B.; Ehsan, S.; Ali, S.; Zubair, M.; Hanif, M. Morphological, physiological and biochemical responses of different plant species to Cd stress. *Int. J. Chem. Biochem. Sci.* **2013**, *3*, 53–60.

12. Gill, S.S.; Khan, N.A.; Tuteja, N. Differential cadmium stress tolerance in five Indian mustard (*Brassica juncea* L.) cultivars: An evaluation of the role of antioxidant machinery. *Plant Signal. Behav.* **2011**, *6*, 293–300. [CrossRef] [PubMed]
13. Atafar, Z.; Mesdaghinia, A.; Nouri, J.; Homaee, M.; Yunesian, M.; Ahmadimoghaddam, M.; Mahvi, A.H. Effect of fertilizer application on soil heavy metal concentration. *Environ. Monit. Assess.* **2010**, *160*, 83–89. [CrossRef] [PubMed]
14. Sytar, O.; Ghosh, S.; Malinska, H.; Zivcak, M.; Brestic, M. Physiological and molecular mechanisms of metal accumulation in hyperaccumulator plants. *Physiol. Plant.* **2021**, *173*, 148–166. [CrossRef] [PubMed]
15. Skuza, L.; Szućko-Kociuba, I.; Filip, E.; Bożek, I. Natural Molecular Mechanisms of Plant Hyperaccumulation and Hypertolerance towards Heavy Metals. *Int. J. Mol. Sci.* **2022**, *23*, 9335. [CrossRef]
16. Viehweger, K. How plants cope with heavy metals. *Bot. Stud.* **2014**, *55*, 35. [CrossRef]
17. Clemens, S. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie* **2006**, *88*, 1707–1719. [CrossRef]
18. Hunter, P. Essentially deadly: Living with toxic elements: Humans and plants have evolved various mechanisms to deal with and even adopt toxic heavy metals. *EMBO Rep.* **2015**, *16*, 1605–1608. [CrossRef]
19. Palmer, C.M.; Guerinot, M.L. Facing the challenges of Cu, Fe and Zn homeostasis in plants. *Nat. Chem. Biol.* **2009**, *5*, 333–340. [CrossRef]
20. Thomine, S.; Vert, G. Iron transport in plants: Better be safe than sorry. *Curr. Opin. Plant Biol.* **2013**, *16*, 322–327. [CrossRef]
21. Niesche, R.; Haase, M. Emotions and ethics: A Foucauldian framework for becoming an ethical educator. *Educ. Philos. Theory* **2012**, *44*, 276–288. [CrossRef]
22. Yaashikaa, P.; Kumar, P.S.; Jeevanantham, S.; Saravanan, R. A review on bioremediation approach for heavy metal detoxification and accumulation in plants. *Environ. Pollut.* **2022**, *301*, 119035. [CrossRef] [PubMed]
23. Raskin, I.; Kumar, P.N.; Dushenkov, S.; Salt, D.E. Bioconcentration of heavy metals by plants. *Curr. Opin. Biotechnol.* **1994**, *5*, 285–290. [CrossRef]
24. Krouma, A.; Drevon, J.-J.; Abdelly, C. Genotypic variation of N2-fixing common bean (*Phaseolus vulgaris* L.) in response to iron deficiency. *J. Plant Physiol.* **2006**, *163*, 1094–1100. [CrossRef] [PubMed]
25. Shah, S.S.; Mohammad, F.; Shafi, M.; Bakht, J.; Zhou, W. Effects of cadmium and salinity on growth and photosynthesis parameters of Brassica species. *Pak. J. Bot.* **2011**, *43*, 333–340.
26. Mehmood, S.; Khan, A.A.; Shi, F.; Tahir, M.; Sultan, T.; Munis, M.F.H.; Kaushik, P.; Alyemeni, M.N.; Chaudhary, H.J. Alleviation of salt stress in wheat seedlings via multifunctional *Bacillus aryabhattai* PM34: An in-vitro study. *Sustainability* **2021**, *13*, 8030. [CrossRef]
27. Aioub, A.A.; Zuo, Y.; Aioub, A.A.; Hu, Z. Biochemical and phytoremediation of *Plantago major* L. to protect tomato plants from the contamination of cypermethrin pesticide. *Environ. Sci. Pollut. Res.* **2021**, *28*, 43992–44001. [CrossRef]
28. Zainab, N.; Din, B.U.; Javed, M.T.; Afridi, M.S.; Mukhtar, T.; Kamran, M.A.; Khan, A.A.; Ali, J.; Jatoi, W.N.; Munis, M.F.H. Deciphering metal toxicity responses of flax (*Linum usitatissimum* L.) with exopolysaccharide and ACC-deaminase producing bacteria in industrially contaminated soils. *Plant Physiol. Biochem.* **2020**, *152*, 90–99. [CrossRef]
29. Zeng, F.; Mallhi, Z.I.; Khan, N.; Rizwan, M.; Ali, S.; Ahmad, A.; Hussain, A.; Alsahli, A.A.; Alyemeni, M.N. Combined Citric Acid and Glutathione Augments Lead (Pb) Stress Tolerance and Phytoremediation of Castorbean through Antioxidant Machinery and Pb Uptake. *Sustainability* **2021**, *13*, 4073. [CrossRef]
30. Ali, B.; Gill, R.A.; Yang, S.; Gill, M.B.; Farooq, M.A.; Liu, D.; Daud, M.K.; Ali, S.; Zhou, W. Regulation of cadmium-induced proteomic and metabolic changes by 5-aminolevulinic acid in leaves of *Brassica napus* L. *PLoS ONE* **2015**, *10*, e0123328. [CrossRef]
31. Bareen, F. Chelate assisted phytoextraction using oilseed brassicas. *Environ. Pollut.* **2012**, *21*, 289–311.
32. Hayat, K.; Menhas, S.; Bundschuh, J.; Zhou, P.; Niazi, N.K.; Amna; Hussain, A.; Hayat, S.; Ali, H.; Wang, J. Plant growth promotion and enhanced uptake of Cd by combinatorial application of *Bacillus pumilus* and EDTA on *Zea mays* L. *Int. J. Phytoremediat.* **2020**, *22*, 1372–1384. [CrossRef] [PubMed]
33. de Melo, É.E.C.; do Nascimento, C.W.A.; de Aguiar Accioly, A.M.; Santos, A.C.Q. Phytoextraction and fractionation of heavy metals in soil after multiple applications of natural chelants. *Sci. Agric.* **2008**, *65*, 61–68. [CrossRef]
34. Wuana, R.; Okieimen, F.; Imborvungu, J. Removal of heavy metals from a contaminated soil using organic chelating acids. *Int. J. Environ. Sci. Technol.* **2010**, *7*, 485–496. [CrossRef]
35. Freitas, E.V.; Nascimento, C.W.; Souza, A.; Silva, F.B. Citric acid-assisted phytoextraction of lead: A field experiment. *Chemosphere* **2013**, *92*, 213–217. [CrossRef]
36. Turgut, C.; Pepe, M.K.; Cutright, T.J. The effect of EDTA and citric acid on phytoremediation of Cd, Cr, and Ni from soil using *Helianthus annuus*. *Environ. Pollut.* **2004**, *131*, 147–154. [CrossRef]
37. Najeeb, U.; Jilani, G.; Ali, S.; Sarwar, M.; Xu, L.; Zhou, W. Insights into cadmium induced physiological and ultra-structural disorders in *Juncus effusus* L. and its remediation through exogenous citric acid. *J. Hazard. Mater.* **2011**, *186*, 565–574. [CrossRef]
38. Liu, J.; Zhou, Q.; Wang, X.; Zhang, Q.; Sun, T. Potential analysis of ornamental plant resources applied to contaminated soil remediation. In *Floriculture, Ornamental and Plant Biotechnology*; Global Science Books, Ltd.: Middlesec, UK, 2006; pp. 245–252.
39. Ma, Y. The role of domestic floriculture in prevention and treatment of pollution. *J. Changchun Univ.* **2003**, *13*, 21–29.
40. Liu, J.-N.; Zhou, Q.-X.; Sun, T.; Ma, L.Q.; Wang, S. Identification and chemical enhancement of two ornamental plants for phytoremediation. *Bull. Environ. Contam. Toxicol.* **2008**, *80*, 260–265. [CrossRef]

41. Zeng, P.; Guo, Z.; Cao, X.; Xiao, X.; Liu, Y.; Shi, L. Phytostabilization potential of ornamental plants grown in soil contaminated with cadmium. *Int. J. Phytoremediat.* **2018**, *20*, 311–320. [CrossRef]
42. Khan, A.A.; Wang, T.; Hussain, T.; Ali, F.; Shi, F.; Latef, A.A.H.A.; Ali, O.M.; Hayat, K.; Mehmood, S.; Zainab, N. Halotolerant-*Koccuria rhizophila* (14asp)-induced amendment of salt stress in pea plants by limiting Na^+ uptake and elevating production of antioxidants. *Agronomy* **2021**, *11*, 1907. [CrossRef]
43. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of Total Carotenoids and Chlorophylls a and b of Leaf Extracts in Different Solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. [CrossRef]
44. Balestri, M.; Bottega, S.; Spanò, C. Response of *Pteris vittata* to different cadmium treatments. *Acta Physiol. Plant.* **2014**, *36*, 767–775. [CrossRef]
45. Bates, L.S.; Waldren, R.P.; Teare, I. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
46. You, J.; Chan, Z. ROS regulation during abiotic stress responses in crop plants. *Front. Plant Sci.* **2015**, *6*, 1092. [CrossRef] [PubMed]
47. Nakano, Y.; Asada, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **1981**, *22*, 867–880.
48. Aebi, H. Formation or Removal of Oxygen Radicals. *Methods Enzymol.* **1984**, *105*, 121–126.
49. Zhang, H.; Jiang, Y.; He, Z.; Ma, M. Cadmium accumulation and oxidative burst in garlic (*Allium sativum*). *J. Plant Physiol.* **2005**, *162*, 977–984. [CrossRef]
50. Singh, H.P.; Batish, D.R.; Kohli, R.K.; Arora, K. Arsenic-induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting from enhanced lipid peroxidation. *Plant Growth Regul.* **2007**, *53*, 65–73. [CrossRef]
51. Cheraghi, M.; Lorestani, B.; Khorasani, N.; Yousefi, N.; Karami, M. Findings on the phytoextraction and phytostabilization of soils contaminated with heavy metals. *Biol. Trace Elem. Res.* **2011**, *144*, 1133–1141. [CrossRef]
52. Zubair, M.; Ramzani, P.M.A.; Rasool, B.; Khan, M.A.; Akhtar, I.; Turan, V.; Tauqeer, H.M.; Farhad, M.; Khan, S.A.; Iqbal, J. Efficacy of chitosan-coated textile waste biochar applied to Cd-polluted soil for reducing Cd mobility in soil and its distribution in moringa (*Moringa oleifera* L.). *J. Environ. Manag.* **2021**, *284*, 112047. [CrossRef]
53. Chen, L.; Long, C.; Wang, D.; Yang, J. Phytoremediation of cadmium (Cd) and uranium (U) contaminated soils by *Brassica juncea* L. enhanced with exogenous application of plant growth regulators. *Chemosphere* **2020**, *242*, 125112. [CrossRef] [PubMed]
54. Al Mahmud, J.; Hasanuzzaman, M.; Nahar, K.; Bhuyan, M.B.; Fujita, M. Insights into citric acid-induced cadmium tolerance and phytoremediation in *Brassica juncea* L.: Coordinated functions of metal chelation, antioxidant defense and glyoxalase systems. *Ecotoxicol. Environ. Saf.* **2018**, *147*, 990–1001. [CrossRef] [PubMed]
55. Redondo-Gómez, S.; Mateos-Naranjo, E.; Andrades-Moreno, L. Accumulation and tolerance characteristics of cadmium in a halophytic Cd-hyperaccumulator, *Arthrocnemum macrostachyum*. *J. Hazard. Mater.* **2010**, *184*, 299–307. [CrossRef] [PubMed]
56. Tusei, C. The effects of citric acid on pH and nutrient uptake in wheatgrass (*Triticum aestivum*). *IdeaFest Interdiscip. J. Creat. Work. Res. Humboldt State Univ.* **2019**, *3*, 7.
57. Yadav, S. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *S. Afr. J. Bot.* **2010**, *76*, 167–179. [CrossRef]
58. Ehlert, C.; Maurel, C.; Tardieu, F.; Simonneau, T. Aquaporin-mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. *Plant Physiol.* **2009**, *150*, 1093–1104. [CrossRef]
59. Riyazuddin, R.; Nisha, N.; Ejaz, B.; Khan, M.I.R.; Kumar, M.; Ramteke, P.W.; Gupta, R. A comprehensive review on the heavy metal toxicity and sequestration in plants. *Biomolecules* **2021**, *12*, 43. [CrossRef]
60. Ali, S.; Abbas, Z.; Seleiman, M.F.; Rizwan, M.; YavaŞ, İ.; Alhammad, B.A.; Shami, A.; Hasanuzzaman, M.; Kalderis, D. Glycine betaine accumulation, significance and interests for heavy metal tolerance in plants. *Plants* **2020**, *9*, 896. [CrossRef]
61. Gupta, D.; Nicoloso, F.; Schetinger, M.; Rossato, L.; Pereira, L.; Castro, G.; Srivastava, S.; Tripathi, R. Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. *J. Hazard. Mater.* **2009**, *172*, 479–484. [CrossRef]
62. Muhammad, D.; Chen, F.; Zhao, J.; Zhang, G.; Wu, F. Comparison of EDTA-and citric acid-enhanced phytoextraction of heavy metals in artificially metal contaminated soil by *Typha angustifolia*. *Int. J. Phytoremediat.* **2009**, *11*, 558–574. [CrossRef]
63. Gao, Y.; Miao, C.; Xia, J.; Luo, C.; Mao, L.; Zhou, P.; Shi, W. Effect of citric acid on phytoextraction and antioxidative defense in *Solanum nigrum* L. as a hyperaccumulator under Cd and Pb combined pollution. *Environ. Earth Sci.* **2012**, *65*, 1923–1932. [CrossRef]
64. Sabir, M.; Hanafi, M.M.; Zia-Ur-Rehman, M.; Saifullah; Ahmad, H.R.; Hakeem, K.R.; Aziz, T. Comparison of low-molecular-weight organic acids and ethylenediaminetetraacetic acid to enhance phytoextraction of heavy metals by maize. *Commun. Soil Sci. Plant Anal.* **2014**, *45*, 42–52. [CrossRef]
65. Najeeb, U.; Xu, L.; Ali, S.; Jilani, G.; Gong, H.; Shen, W.; Zhou, W. Citric acid enhances the phytoextraction of manganese and plant growth by alleviating the ultrastructural damages in *Juncus effusus* L. *J. Hazard. Mater.* **2009**, *170*, 1156–1163. [CrossRef]
66. Pena, L.B.; Pasquini, L.A.; Tomaro, M.L.; Gallego, S.M. Proteolytic system in sunflower (*Helianthus annuus* L.) leaves under cadmium stress. *Plant Sci.* **2006**, *171*, 531–537. [CrossRef]
67. Aghaz, M.; Bandehagh, A.; Aghazade, E.; Toorchi, M.; Ghassemi-Gholezani, K. Effects of cadmium stress on some growth and physiological characteristics in dill (*Anethum graveolens*) ecotypes. *Int. J. Agric.* **2013**, *3*, 409.

68. Lu, L.-L.; Tian, S.-K.; Yang, X.-E.; Peng, H.-Y.; Li, T.-Q. Improved cadmium uptake and accumulation in the hyperaccumulator *Sedum alfredii*: The impact of citric acid and tartaric acid. *J. Zhejiang Univ. Sci. B* **2013**, *14*, 106–114. [CrossRef]
69. Han, F.; Shan, X.-Q.; Zhang, J.; Xie, Y.-N.; Pei, Z.-G.; Zhang, S.-Z.; Zhu, Y.-G.; Wen, B. Organic acids promote the uptake of lanthanum by barley roots. *New Phytol.* **2005**, *165*, 481–492. [CrossRef] [PubMed]
70. Saleem, M.H.; Fahad, S.; Khan, S.U.; Din, M.; Ullah, A.; Sabagh, A.E.; Hossain, A.; Llanes, A.; Liu, L. Copper-induced oxidative stress, initiation of antioxidants and phytoremediation potential of flax (*Linum usitatissimum* L.) seedlings grown under the mixing of two different soils of China. *Environ. Sci. Pollut. Res.* **2020**, *27*, 5211–5221. [CrossRef] [PubMed]
71. Babushkina, E.A.; Belokopytova, L.V.; Grachev, A.M.; Meko, D.M.; Vaganov, E.A. Variation of the hydrological regime of Bele-Shira closed basin in Southern Siberia and its reflection in the radial growth of *Larix sibirica*. *Reg. Environ. Chang.* **2017**, *17*, 1725–1737. [CrossRef]
72. Parmar, P.; Kumari, N.; Sharma, V. Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. *Bot. Stud.* **2013**, *54*, 45. [CrossRef]
73. Kanwal, U.; Ali, S.; Shakoor, M.B.; Farid, M.; Hussain, S.; Yasmeen, T.; Adrees, M.; Bharwana, S.A.; Abbas, F. EDTA ameliorates phytoextraction of lead and plant growth by reducing morphological and biochemical injuries in *Brassica napus* L. under lead stress. *Environ. Sci. Pollut. Res.* **2014**, *21*, 9899–9910. [CrossRef] [PubMed]
74. Mahdavian, K. Effect of citric acid on antioxidant activity of red bean (*Phaseolus calcaratus* L.) under Cr+6 stress. *S. Afr. J. Bot.* **2021**, *139*, 83–91. [CrossRef]
75. Farid, M.; Ali, S.; Saeed, R.; Rizwan, M.; Bukhari, S.A.H.; Abbasi, G.H.; Hussain, A.; Ali, B.; Zamir, M.S.I.; Ahmad, I. Combined application of citric acid and 5-aminolevulinic acid improved biomass, photosynthesis and gas exchange attributes of sunflower (*Helianthus annuus* L.) grown on chromium contaminated soil. *Int. J. Phytoremediat.* **2019**, *21*, 760–767. [CrossRef] [PubMed]
76. Meng, H.; Hua, S.; Shamsi, I.H.; Jilani, G.; Li, Y.; Jiang, L. Cadmium-induced stress on the seed germination and seedling growth of *Brassica napus* L., and its alleviation through exogenous plant growth regulators. *Plant Growth Regul.* **2009**, *58*, 47–59. [CrossRef]
77. Haouari, C.C.; Nasraoui, A.H.; Bouthour, D.; Houda, M.D.; Daieb, C.B.; Mnai, J.; Gouia, H. Response of tomato (*Solanum lycopersicon*) to cadmium toxicity: Growth, element uptake, chlorophyll content and photosynthesis rate. *Afr. J. Plant Sci.* **2012**, *6*, 1–7.
78. Kurtyka, R.; Burdach, Z.; Siemieniuk, A.; Karcz, W. Single and combined effects of Cd and Pb on the growth, medium pH, membrane potential and metal contents in maize (*Zea mays* L.) coleoptile segments. *Ecotoxicol. Environ. Saf.* **2018**, *161*, 8–16. [CrossRef]
79. Zhang, F.; Zhang, H.; Wang, G.; Xu, L.; Shen, Z. Cadmium-induced accumulation of hydrogen peroxide in the leaf apoplast of *Phaseolus aureus* and *Vicia sativa* and the roles of different antioxidant enzymes. *J. Hazard. Mater.* **2009**, *168*, 76–84. [CrossRef]
80. Erdei, S. Heavy metal induced physiological changes in the antioxidative response system. *Acta Biol. Szeged.* **2002**, *46*, 89–90.
81. Mittler, R.; Vanderauwera, S.; Gollery, M.; Van Breusegem, F. Reactive oxygen gene network of plants. *Trends Plant Sci.* **2004**, *9*, 490–498. [CrossRef]
82. Santos, L.; Batista, B.; Lobato, A. Brassinosteroids mitigate cadmium toxicity in cowpea plants. *Photosynthetica* **2018**, *56*, 591–605. [CrossRef]
83. Agrawal, S.; Mishra, S. Effects of supplemental ultraviolet-B and cadmium on growth, antioxidants and yield of *Pisum sativum* L. *Ecotoxicol. Environ. Saf.* **2009**, *72*, 610–618. [CrossRef] [PubMed]
84. Chen, J.; Shafi, M.; Wang, Y.; Wu, J.; Ye, Z.; Liu, C.; Zhong, B.; Guo, H.; He, L.; Liu, D. Organic acid compounds in root exudation of Moso Bamboo (*Phyllostachys pubescens*) and its bioactivity as affected by heavy metals. *Environ. Sci. Pollut. Res.* **2016**, *23*, 20977–20984. [CrossRef] [PubMed]
85. Saffari, V.R.; Saffari, M. Effects of EDTA, citric acid, and tartaric acid application on growth, phytoremediation potential, and antioxidant response of *Calendula officinalis* L. in a cadmium-spiked calcareous soil. *Int. J. Phytoremediat.* **2020**, *22*, 1204–1214. [CrossRef] [PubMed]