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Mechanisms of Cadmium Tolerance and Detoxification in Two Ornamental Plants

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Abstract: Cadmium (Cd) is an important environmental heavy metal and one of the main soil pollutants in southwest China and even the Yangtze River Basin because of its toxicity to plants and humans. To clarify the potential of *Euryops pectinatus* L. and *Gardenia jasminoides* J. and the mechanism they use to remediate Cd-contaminated soil, a soil pot experiment with 0, 5, 10, 20, and 40 mg kg^{-1} of Cd was used to investigate the accumulation characteristics, subcellular distribution, chemical forms, and the antioxidative defense systems of the two ornamental plants. When the concentration of Cd was below 40 mg kg^{-1}, it promoted the growth of *E. pectinatus* shoots, and the tolerance index (TI) was >1. However, 20–40 mg kg^{-1} Cd significantly inhibited the growth of *G. jasminoides*, and the TI was <1. The shoots of both varieties accumulated more Cd than the roots, and the *E. pectinatus* shoots accumulated more Cd (1.45 mg $plant^{-1}$) than those of *G. jasminoides* (0.71 mg $plant^{-1}$). The Cd in *E. pectinatus* and *G. jasminoides* was primarily distributed in the soluble fraction (52.83–68.97%) and cell walls (44.62–54.98%), respectively. Higher proportions of Cd bound to NaCl and acetic acid (HAc) in *E. pectinatus* (55.32–73.44%) than in *G. jasminoides* (42.94–61.58%), while the inorganic and water-soluble proportions of Cd bound in the opposite manner. *E. pectinatus* maintained high activities of antioxidant enzymes under Cd treatments, and its levels of malondialdehyde (MDA) and relative electrical conductivity (REC) were comparable to those of the control. Nonetheless, *G. jasminoides* had low levels of activity of antioxidant enzymes, but its levels of MDA and REC were significantly higher than those of the control under the 20–40 mg kg^{-1} Cd treatment. Therefore, both types of plants have a strong ability to tolerate and accumulate Cd, which makes them suitable for the remediation of Cd-polluted soil. However, *E. pectinatus* is more effective at remediating Cd and tolerant to it than *G. jasminoides*. These plants utilize different mechanisms to detoxify Cd.

Keywords: Cadmium; *Euryops pectinatus* L.; *Gardenia jasminoides* J.; subcellular distribution; chemical form; antioxidative defense system

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1. Introduction

Rapid industrialization and urbanization in China have severely increased the pollution of soil with heavy metals [1,2]. According to the National Soil Pollution Survey Bulletin, 19.4% of the soil point exceeds the standard for cultivated land, and a large range of soil heavy metals exceed that standard in southwestern and south-central China. It is also clear that cadmium (Cd) is among the most important heavy metal pollutants with high toxicity and carcinogenicity, which exceeds the standard limit for China by up to 7.0% in soil samples and throughout the food chain [3]. Excessive Cd in the soil will lead to the

retardation of plant root growth, growth of short plants, leaf chlorosis, and a reduction in the yield of agricultural products and their quality [4]. In addition, Cd can affect human health through the food chain. For example, itai-itai disease is a well-known health hazard caused by the consumption of rice contaminated with Cd [5].

Phytoremediation, which uses plants that accumulate pollutants, such as hyperaccumulators, to remove heavy metals from the soil, has been proven to be a promising technique due to its cost-effectiveness and eco-friendliness [6,7]. As of July 2017, the Global Hyperaccumulator Database contained 721 species of hyperaccumulators (from 52 families and ~130 genera) that had been reported from around the world. Among these species, a few species accumulate Cd [8,9]. Although Cd-hyperaccumulative plants can absorb 50- to 500-fold more Cd than normal plants and can accumulate more than 100 mg kg^{-1} dry weight of Cd, most of these plants produce a small amount of biomass, are only weakly adaptive, and have no ornamental value or practical applications [10,11]. Screening new hyperaccumulators or accumulators with higher biomass and more efficient abilities to transport Cd is the key to phytoremediation [10,12].

Ornamental plants generally have features, such as a wide distribution, high adaptability, and large biomasses. If ornamental plants with hyperaccumulative properties can be screened for these features, they may be more economical and valuable for practical applications. Some herbaceous ornamental plants, such as *Calendula officinalis* L. [13] and *Cosmos bipinnatus* Cav. [14], are highly tolerant to heavy metal stress and can effectively accumulate these metals. Ornamental shrubs with developed roots and large biomass are more efficient at absorbing and accumulating heavy metals in the environment than herbaceous ornamental plants. Thus, they are more valuable and practical for phytoremediation. For example, *Lantana camara* L. is a hyperaccumulator of Cd [15]. However, there are currently few ornamental shrubs that are known to accumulate Cd. Therefore, there is an urgent need to identify new and native ornamental shrubs with a high biomass that can accumulate Cd.

Euryops pectinatus L. in the Composite family is a perennial ornamental shrub with bright flowers. It is a good plant that flowers in the shade and a ground cover that can conserve soil and water. A field study showed that the plant grew well in the central point of urban waste disposal, and the average concentration of Cd in rhizosphere soil reached 9.28 mg kg^{-1}. *Gardenia jasminoides* J. in the Rubiaceae family is a perennial ornamental shrub with evergreen leaves and fragrant flowers. It is a common garden plant and can also effectively accumulate heavy metals [16]. These two types of ornamental plants grow quickly and have a large biomass, long flowering period, beautiful plant shapes, and strong ecological adaptability, and they are common in flower belt materials in southwest China and even the Yangtze River Basin. However, the accumulation characteristics of Cd by these two plants and their tolerance mechanism to Cd have yet not been systematically reported.

Many plants have developed specific tolerance and detoxification mechanisms to adapt to heavy metals in the environment. These mechanisms include root retention, cell compartmentalization, changing the chemical forms of metals in plants, and enhancing their antioxidative defense system [17,18]. The compartmentalization of Cd in the cell wall or vacuole of plants can affect its mobility in the cell and reduce its concentration in sensitive organelles [19]. In *Sedum alfredii* Hance and *Kandelia obovate* (S., L), the cell wall had the highest concentrations of Cd, which reduced the transmembrane transportation of Cd^{2+} into cells [20,21]. In contrast, most of the Cd in *Phytolacca americana* L. and *Typha angustifolia* L. combines with amino acids, small molecular pigments, proteins, and polysaccharides in the cell soluble fraction to reduce its biological toxicity [22,23].

Proteins, pectins, oxalates, and other plant substances also combine with heavy metals to change the chemical forms and toxicity [24]. In *Athyrium wardii* (Hook.) [25] and soybean (*Glycine max* L.) [26], Cd primarily exists in less toxic forms, such as extracts of sodium chloride (NaCl) and acetic acid (HAc). However, in *Arenaria orbiculata* Royle [27] and *C. bipinnatus* [14], lead and zinc are primarily found in less toxic forms, such as extracts of

HAc and HCl. Moreover, some plants have antioxidative defense systems that can remove the reactive oxygen species (ROS) induced by heavy metals, thus, serving as a detoxification mechanism [28]. There are numerous reports that show that plants produce higher levels of antioxidant enzymes under stress conditions [29,30]. However, antioxidant enzymes are not always higher during abiotic stress [1,31]. Therefore, understanding how different plants tolerate Cd or detoxify it in their systems will help to develop phytoremediation systems for heavy metals in contaminated soil.

This study analyzed two perennial ornamental plants, including *E. pectinatus* and *G. jasminoides*. The objective was to study the changes in Cd accumulation characteristics, subcellular distribution, chemical forms, and the antioxidative defense system of two ornamental shrubs treated with Cd and analyze the roles of these characteristics in the tolerance and detoxification of this heavy metal by these two plants.

2. Materials and Methods

2.1. Plant Material

E. pectinatus and *G. jasminoides* were provided by Chengdu Huimei Flower Border Horticultural Engineering Co., Ltd. (Chengdu, China), and planted in the Wenjiang Campus of the Sichuan Agricultural University in Sichuan Province, China.

E. pectinatus and *G. jasminoides* were cultivated from cuttings. Selected branches that were approximately 10 cm long that had grown well and lacked pests and diseases were utilized as cuttings. After the cuttings had rooted, they were cultivated for an additional month.

2.2. Pot Experiment

The pot experiment was conducted in a greenhouse. Clean paddy soil was sampled from cultivated land in Wenjiang District, Chengdu City, Sichuan Province (30°43′ N, 103°52′ E). The soil properties were as follows: pH 6.57; soil organic matter (SOM) and total nitrogen (TN), 20.54 and 1.15 g kg^{-1}, respectively; available N (AN), Olsen P (AP), available K (AK), and total Cd were 69.64, 41.28, 151.32, and 0.13 mg kg^{-1}, respectively. The soil was air-dried, ground, and sieved through a 4-mm mesh before treatment with a solution of $CdCl_2$ to generate 0 (control) and concentrations of 5, 10, 20, and 40 mg kg^{-1} Cd^{2+}. Each plastic pot was 40 cm high and 30 cm in diameter and was filled with 8.0 kg of treated soil samples. The treated soils were stabilized for 2 months before transplanting. Each growing pot was fertilized with 0.1 g kg^{-1} of N, P_2O_5, and K_2O to meet the needs for plant growth.

Three seedlings with the same growth potential (plant height 12–15 cm, which contained 4–5 leaves) were transplanted to each pot that had been prepared. A tray was placed at the bottom of the pots to avoid the loss of heavy metals and environmental pollution. All the pots were watered with tap water (undetectable Cd) to ensure approximately 60% of soil field capacity. All treatments were replicated three times. After 4 months, the plants were harvested, separated into roots, stems, and leaves, and then washed with deionized water. The roots were immersed in 20 mM Na_2-EDTA for 15 min to remove Cd from the root surface [2]. The fresh plant samples (roots stems, and leaves) were divided into two portions. One portion was frozen in liquid nitrogen and stored at −80 °C for further physiological analysis, and the other was dried at 105 °C for 15 min and then at 80 °C to ensure a constant weight before the dry weights were measured.

2.3. Analysis of Cd in Plant Materials

The concentrations of Cd were determined as described by Zhang [1]. Briefly, plant samples were digested using HNO_3:$HClO_4$ (4:1) (v/v). The concentrations of Cd were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS).

2.4. Separation of Cd Subcellular Fractions

The subcellular fractions of Cd in plants were separated as described by Xu [2]. The frozen tissues of roots, stems, or leaves were homogenized in precooled extraction buffer (250 mM sucrose, 50 mM Tris-HCl pH 7.5, and 1.0 mM dithiothreitol) using a mortar and pestle. The homogenate was centrifuged at 3000 rpm for 15 min, and the solid residue was designated the cell wall fraction (F1). The supernatant solution was further centrifuged at 15,000 rpm for 30 min, and the precipitate (the organelle fraction [F2]) and supernatant (the soluble fraction [F3]) were separated. All the procedures were conducted at 4 °C. The three fractions were dried and digested as described in Section 2.3 before their contents of Cd were measured using ICP-MS.

2.5. Extraction of the Chemical Forms of Cd

The chemical forms of Cd were measured as described by Xu [2]. In this study, six chemical forms of Cd were sequentially extracted and differentiated in the following order: 80% ethanol for the inorganic Cd (FE), deionized water for the water-soluble Cd (FW), 1 M NaCl for the Cd bound to pectate and proteins (FNaCl), 2% HAc for the insoluble phosphate Cd complexes (FHAc), 0.6 M HCl for the oxalate Cd (FHCl), and the residual Cd remained as a precipitate (Fres). Each supernatant solution and its residue were evaporated on an electroplate at 70 °C to a constant weight and digested as described in Section 2.3. The concentrations of Cd were determined using ICP-MS.

2.6. Determination of the Antioxidative Enzyme Activity

The antioxidant enzymes were extracted at 4 °C as follows. Root and leaf samples were homogenized in an extraction buffer that contained 50 mM potassium phosphate buffer (pH 7.0) and 1 mM EDTA. The homogenate was centrifuged at 15,000× *g* for 20 min at 4 °C, and the supernatant was used to determine the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) as described by Liu [15].

2.7. Evaluation of Membrane Damage

The content of malondialdehyde (MDA) and the relative electrical conductivity (REC) were determined as described by Liu [15] and Ma [32], respectively. Briefly, the contents of MDA were estimated according to the absorbances of the supernatants that were produced using trichloroacetic acid at 450, 532, and 600 nm. The REC was calculated based on the ratio of electrolyte concentration of leaf discs that had been soaked in distilled water before and after boiling.

2.8. Calculation of Relevant Parameters

The tolerance of plants to Cd was evaluated using the tolerance index (TI). TI > 1 indicates that the plants are highly tolerant to Cd and can still grow under severe conditions of Cd pollution [33]. The effect of the accumulation of heavy metals and their transportation inside the plants was calculated using the bioaccumulation coefficient (BCF) and the translocation coefficient (TF), respectively. BCF > 1 and TF > 1 indicate that the plants have a strong ability to accumulate and transport heavy metals from the soil [7]. The phytoremediation potential was evaluated using the purification rate (PR). The PR represents the percentage of an element removed by the plant dry shoot biomass from the total content of elements in the soil [7].

TI, BCF, TF, and PR were determined using the following formulae [7]:

$$\text{TI} = \text{The root (shoot) biomass of the treatment} / \text{the root (shoot) biomass of the control} \quad (1)$$

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

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

$$PR = (\text{Cd concentration in the shoots} \times \text{dry weight of shoots} \times \text{the number of plants in one pot}/\text{Cd content in the soil} \times \text{soil weight in the pot}) \times 100\% \quad (4)$$

2.9. Statistical Analysis

All data are presented as the means ± SD of three independent replicates. The data were analyzed by a one-way ANOVA using SPSS 20.0 (IBM, Inc., Armonk, NY, USA). Tukey's multiple comparison tests detected the differences between treatments at $p < 0.05$. All the figures were plotted using Origin 9.0 (OriginLab, Northampton, MA, USA).

3. Results

3.1. Tolerance and Repair Potential of Two Ornamental Plants

3.1.1. Plant Growth and Tolerance Index

The two ornamental plants showed significant growth differences under Cd stress during the 120 days of experimentation (Table 1). All the treatments promoted shoot growth in *E. pectinatus*, particularly the 20–40 mg kg^{-1} Cd treatments, and the shoot TI > 1. However, the Cd treatments did not affect the root biomass of *E. pectinatus.*

Treatment with 5 mg kg^{-1} Cd significantly promoted the growth of *G. jasminoides* shoots with TI > 1. However, 20–40 mg kg^{-1} Cd significantly inhibited the growth and biomass of the roots and shoots by 21.94–39.38% and 14.36–33.54%, respectively. The root and shoot TIs were <1.

Table 1. Effects of Cd on the dry weight of *E. pectinatus* and *G. jasminoides*.

		Dry Weight				TI	
	Cd Concentrations (mg kg^{-1})	**Root (g $Plant^{-1}$)**	**Stem (g $Plant^{-1}$)**	**Leaf (g $Plant^{-1}$)**	**Shoot (g $Plant^{-1}$)**	**Root**	**Shoot**
E. pectinatus	0	5.14 ± 0.45 a	14.05 ± 1.00 b	12.31 ± 0.92 b	26.37 ± 1.26 c	-	-
	5	5.26 ± 0.39 a	15.21 ± 0.45 b	13.30 ± 0.43 ab	28.52 ± 0.48 b	1.02 ± 0.03 a	1.08 ± 0.04 a
	10	5.52 ± 0.54 a	15.73 ± 1.12 b	13.35 ± 0.75 ab	29.08 ± 0.51 b	1.09 ± 0.20 a	1.11 ± 0.07 a
	20	5.43 ± 0.35 a	16.91 ± 1.04 ab	14.22 ± 1.01 a	31.13 ± 0.38 a	1.06 ± 0.09 a	1.18 ± 0.07 a
	40	5.39 ± 0.46 a	18.44 ± 1.20 a	13.37 ± 0.64 ab	31.82 ± 1.46 a	1.05 ± 0.11 a	1.21 ± 0.08 a
G. jasminoides	0	5.77 ± 0.71 a	15.55 ± 1.34 a	18.86 ± 2.04 b	34.41 ± 3.31 b	-	-
	5	6.23 ± 0.58 a	15.65 ± 1.61 a	22.93 ± 1.66 a	38.58 ± 0.83 a	1.09 ± 0.16 a	1.13 ± 0.08 a
	10	5.49 ± 0.56 ab	14.35 ± 2.12 a	18.30 ± 1.3 bc	32.65 ± 1.03 b	0.97 ± 0.21 ab	0.95 ± 0.07 ab
	20	4.51 ± 0.69 b	13.71 ± 1.82 a	15.77 ± 1.18 c	29.47 ± 2.48 c	0.80 ± 0.23 ab	0.86 ± 0.13 bc
	40	3.50 ± 0.42 b	9.77 ± 1.33 b	13.09 ± 1.91 c	22.87 ± 2.17 d	0.61 ± 0.09 b	0.67 ± 0.09 c

Values are the means ± standard deviation ($n = 3$). Different letters in the same organs of each plant represent significant differences ($p < 0.05$) among different treatments according to ANOVA and Tukey's tests. Cd, cadmium; TI, and tolerance index.

3.1.2. Concentration and Accumulation of Cd

Increasing levels of Cd in the soil increased the concentrations of Cd in the different plant organs, and the concentrations of Cd in the plants reached their maximum levels following treatment with 40 mg kg^{-1} Cd (Figure 1a,b). The concentrations of Cd in the stems and leaves of *E. pectinatus* were higher than those of *G. jasminoides* at the same levels of Cd. However, the concentrations of Cd were higher in the roots of *G. jasminoides* than in those of *E. pectinatus*.

Similarly, increasing levels of Cd in the soil increased the accumulation of Cd in the different plant organs (Figure 2). *E. pectinatus* accumulated more Cd in the shoots (stem and leaf) than *G. jasminoides* in all the treatments. The roots of *E. pectinatus* accumulated less Cd than those of *G. jasminoides* except for the 40 mg kg^{-1} Cd treatment. The Cd that accumulated in the shoots of the two plants exceeded that in the roots and accounted for 80.90–86.44% and 70.21–75.17% of the total accumulation in the plants, respectively.

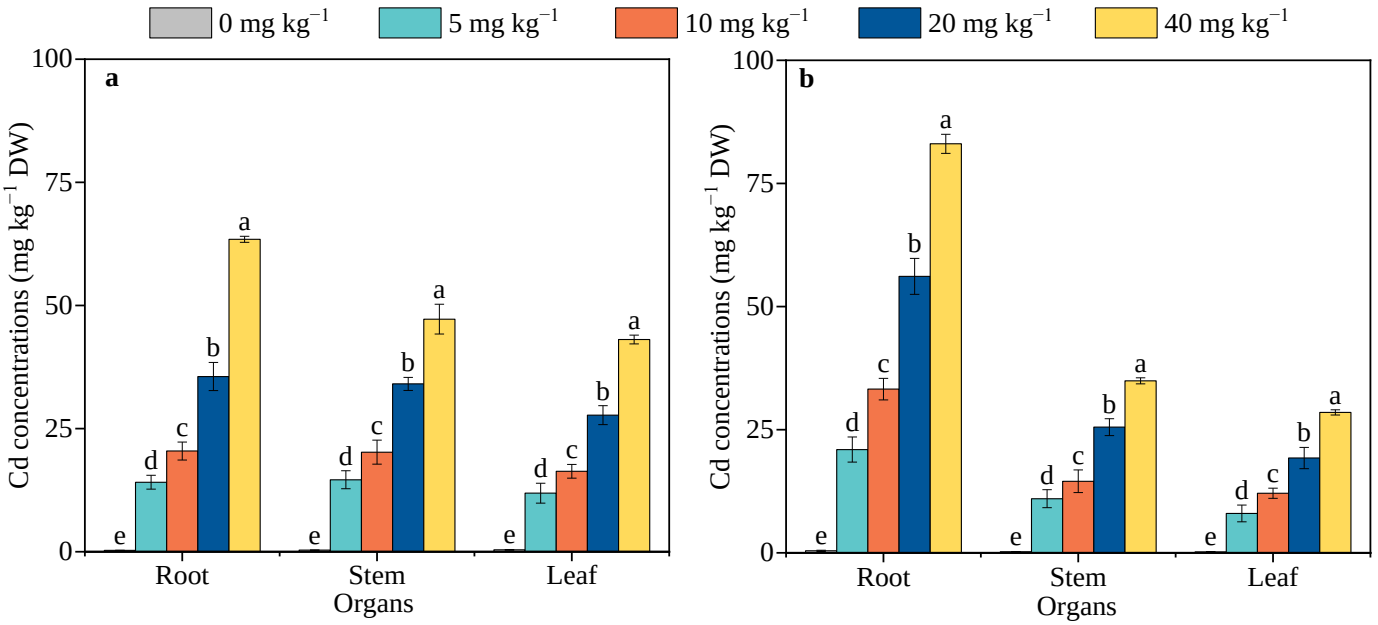

Figure 1. Cd concentration of *E. pectinatus* (**a**) and *G. jasminoides* (**b**) under different Cd treatments. Different letters in the same organs indicate the significant differences among treatments at $p < 0.05$. Cd, cadmium; DW, and dry weight.

With the increase in the concentration of Cd, the BCF, TF, and PR of the two plants tended to decrease (Table 2). The BCF of both plants were >1 except for the 40 mg kg^{-1} Cd treatment. The BCF of roots were higher than those in shoots at all treatments, with a TF < 1. However, the TF and PR of *E. pectinatus* were higher than those of *G. jasminoides*.

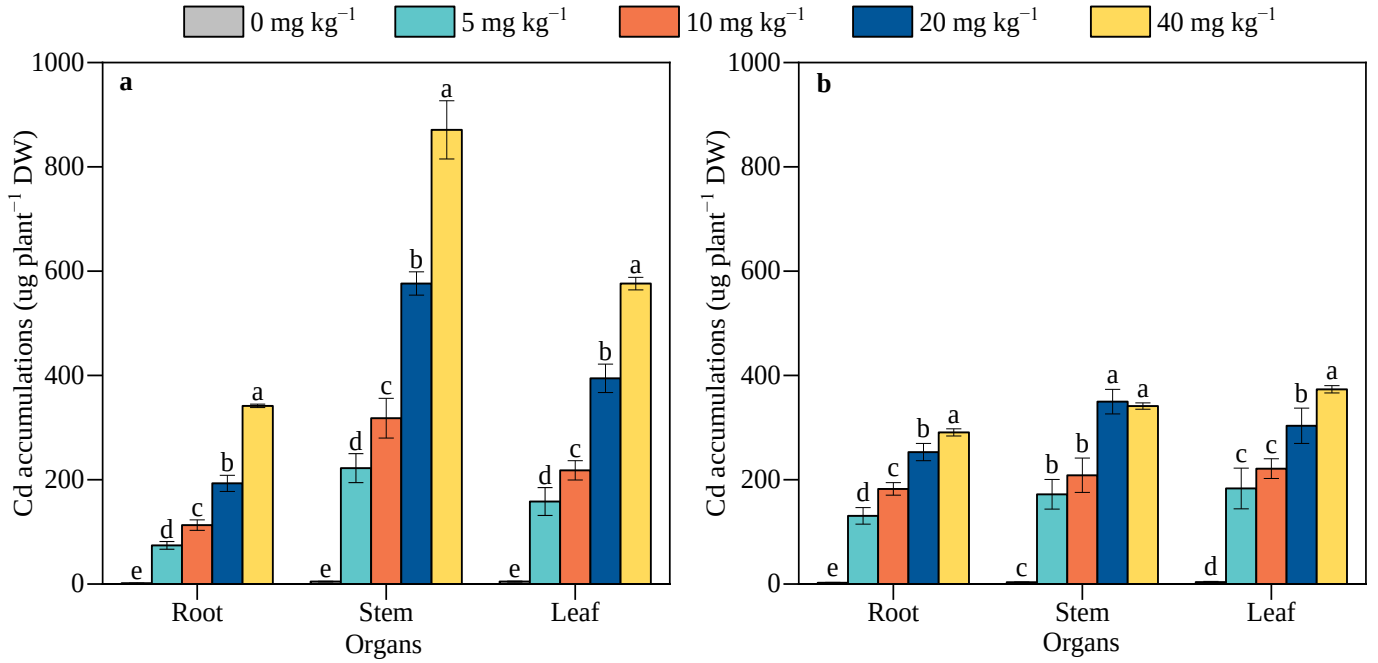

Figure 2. Cd accumulation of *E. pectinatus* (**a**) and *G. jasminoides* (**b**). Different letters in the same organs indicate the significant differences among treatments at $p < 0.05$. Cd, cadmium; DW and dry weight.

Table 2. Effects of Cd on the bioconcentration factor (BCF), translocation coefficient (TF), and Cd purification rate (PR).

	Cd Treatments (mg kg^{-1})	BCF		TF	PR (%)
		Root	Shoot		
E. pectinatus	0	--	--	1.26 ± 0.27 a	--
	5	2.82 ± 0.28 a	2.67 ± 0.38 a	0.96 ± 0.22 ab	2.85 ± 0.41 a
	10	2.05 ± 0.18 b	1.84 ± 0.07 b	0.91 ± 0.12 b	2.01 ± 0.08 b
	20	1.78 ± 0.14 bc	1.56 ± 0.04 b	0.88 ± 0.06 b	1.82 ± 0.05 b
	40	1.59 ± 0.02 c	1.13 ± 0.05 c	0.72 ± 0.03 b	1.36 ± 0.06 c
G. jasminoides	0	--	--	0.53 ± 0.12 a	--
	5	4.19 ± 0.51 a	1.84 ± 0.32 a	0.44 ± 0.07 ab	2.66 ± 0.46 a
	10	3.32 ± 0.22 b	1.32 ± 0.12 b	0.39 ± 0.02 b	1.61 ± 0.15 b
	20	2.81 ± 0.18 b	1.11 ± 0.06 bc	0.40 ± 0.03 b	1.22 ± 0.07 b
	40	2.08 ± 0.05 c	0.78 ± 0.01 c	0.38 ± 0.01 b	0.67 ± 0.01 c

Values are the means ± standard deviation (n = 3). Different letters in the same organs of each plant represent significant differences ($p < 0.05$) among different treatments according to ANOVA and Tukey's tests.

3.2. Subcellular Distribution of Cd in Two Ornamental Plants

Figure 3 shows the subcellular distribution of Cd in *E. pectinatus* and *G. jasminoides* where increasing the levels of Cd in the soil increased its concentrations in all the subcellular fractions. Most of the Cd in the roots (55.31–68.97%), stems (52.83–59.53%), and leaves (53.37–61.60%) of *E. pectinatus* (Figure 3a,c) accumulated in the soluble fraction, followed by the cell wall (22.76–35.53%, 29.82–36.46%, and 27.43–34.97%), and organelles (8.28–9.55%, 10.65–11.72%, and 10.75–11.66%). In *E. pectinatus*, increasing the concentration of Cd in the soil increased the proportion of Cd in the cell wall, decreased the proportion of Cd in the soluble fraction, and slightly changed the proportion in the organelle.

The reverse was observed in *G. jasminoides* (Figure 3b,d) where the cell wall accumulated the largest proportion of Cd (50.64–54.82%, 46.41–53.04%, and 44.62–54.98%), followed by the soluble fraction (31.61–35.87%, 30.68–38.93%, and 28.32–39.13%), and the organelle fractions (12.52–16.17%, 14.37–16.28%, and 16.06–16.86%). Increasing the concentration of Cd in the soil increased the proportion of the soluble fraction and decreased the proportion in the cell wall of *G. jasminoides*. Overall, the proportion of Cd in the organelle fraction of *G. jasminoides* exceeded that of *E. pectinatus*.

3.3. Chemical Forms of Cd in Two Ornamental Plants

The concentrations of Cd that bound to the different chemical forms in plants increased in a dose-responsive manner (Figure 4). For example, the concentrations of Cd extracted using 1 M NaCl and 2% HAc were predominant in the various organs of *E. pectinatus* and *G. jasminoides*. In contrast, the extractable concentrations in 80% ethanol and deionized water were relatively lower.

The extractable Cd in NaCl and HAc (55.32–61.48%, 67.15–73.44%, and 58.42–61.50%) of the total Cd were higher in the *E. pectinatus* roots, stems, and leaves (Figure 4c) than in those of *G. jasminoides* (48.38–57.59%, 42.94–55.91%, and 49.47–61.58%) (Figure 4d). However, the reverse was observed for the extractable proportion in 80% ethanol and deionized water. The proportions in *G. jasminoides* roots, stems, and leaves were 27.96–35.97%, 25.23–34.40%, and 26.80–35.78%, and in the *E. pectinatus* roots, stems, and leaves, the proportions were 23.99–28.58%, 11.06–15.87%, and 13.85–14.63%, respectively.

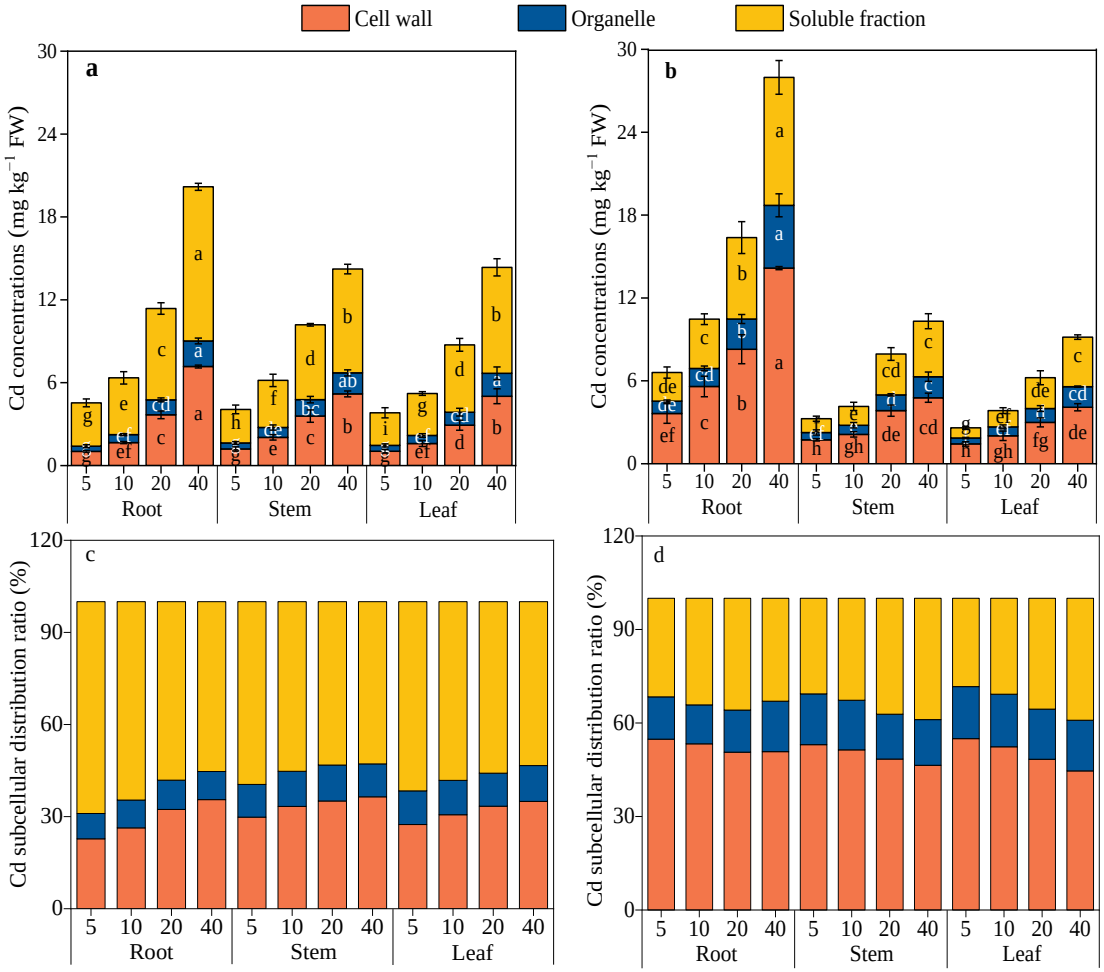

Figure 3. Subcellular distribution of Cd and its proportion in the root, stem, and leaf of *E. pectinatus* (**a**,**c**) and *G. jasminoides* (**b**,**d**) exposed to Cd. Different letters in the same subcellular fraction indicate significant differences among the treatments at $p < 0.05$. Cd, cadmium; FW, and fresh weight.

3.4. Antioxidant System in Both Ornamental Plants

3.4.1. Antioxidant Enzyme Activity

Treatment with Cd stimulated the activity of antioxidant enzymes in *E. pectinatus* (Figure 5). There were significantly higher levels of activities of SOD and POD in the roots and leaves of *E. pectinatus* than those of the control. In contrast, there was no difference in the activity of CAT under all the treatments. However, treatment with 5 mg kg^{-1} Cd increased the activities of SOD and POD in the roots and leaves of *G. jasminoides*, which were 1.19- and 1.30-fold and 1.15- and 1.26-fold of the control, respectively. At 20–40 mg kg^{-1} Cd treatment, the activities of SOD and POD were significantly lower by 24.78–50.87%, 22.04–38.67%, and 18.06–45.46%, 12.06–34.12%, than the control, respectively. However, 10–40 mg kg^{-1} Cd significantly decreased the activity of CAT in the roots and leaves of *G. jasminoides* to 56.33–74.32% and 64.66–77.92% lower than that of the control, respectively.

3.4.2. Lipid Peroxidation and Membrane Permeability

There was no difference in the contents of MDA and REC in the roots and leaves of *E. pectinatus* treated with Cd and those of the control plants (Figure 6). However, an increase in the levels of Cd in the soil increased the levels of MDA and REC in the roots and leaves of *G. jasminoides* with significantly higher values from the 20 mg kg^{-1} Cd treatment than that of the control. Following treatment with 40 mg kg^{-1} Cd, the levels of MDA and REC in the roots and leaves were 2.00- and 1.76-fold and 1.78- and 1.61-fold higher in *G. jasminoides* than in the control, respectively.

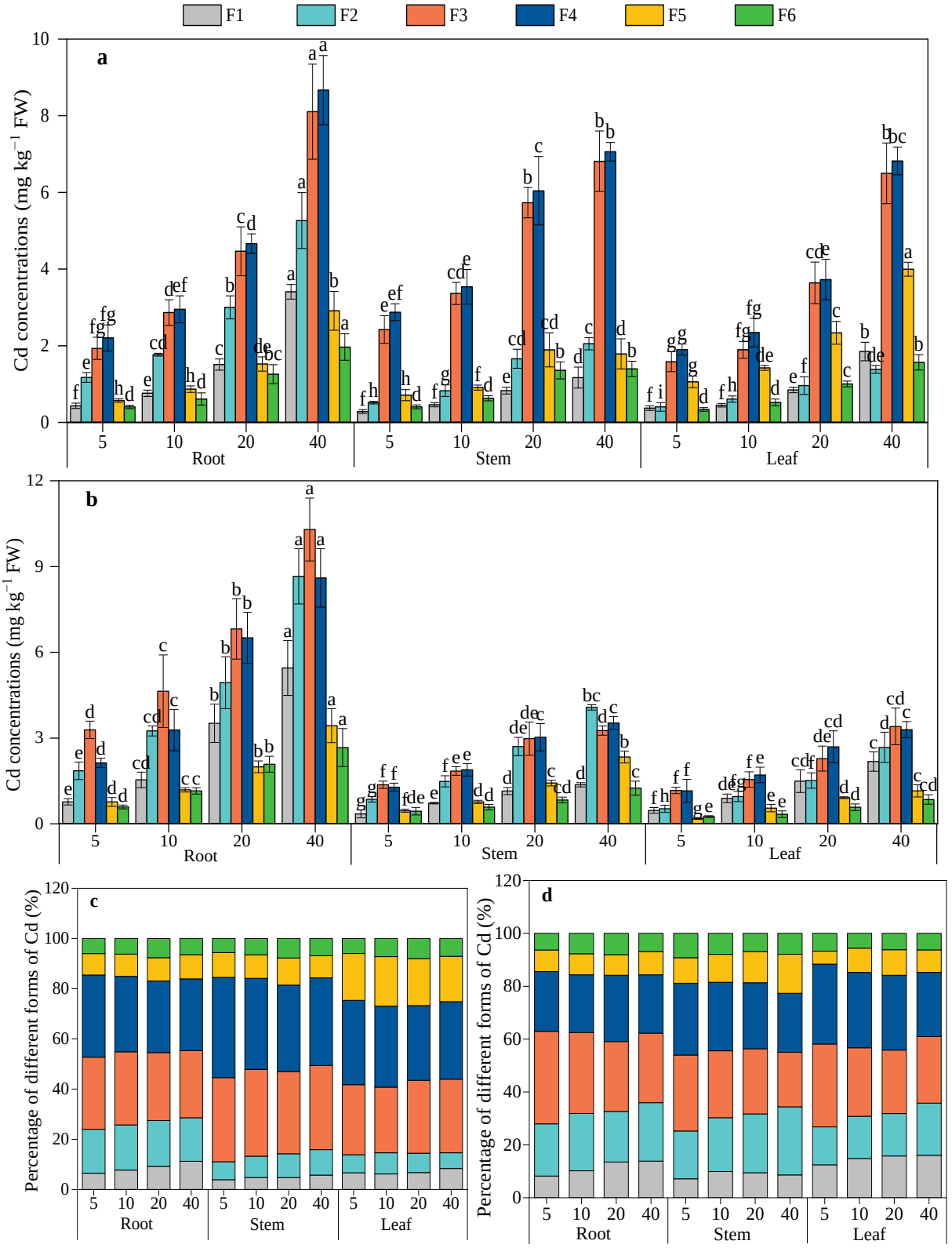

Figure 4. Different chemical forms of Cd and its proportion in root, stem, and leaf of *E. pectinatus* (**a**,**c**) and *G. jasminoides* (**b**,**d**) exposed to Cd. Different letters in the same chemical form indicate significant differences among the treatments at $p < 0.05$. Cd, cadmium; F1, inorganic Cd; F2, water-soluble Cd; F3, Cd bound to pectate and proteins; F4, insoluble phosphate Cd complex; F5, oxalate Cd; F6, Cd precipitate; FW and fresh weight.

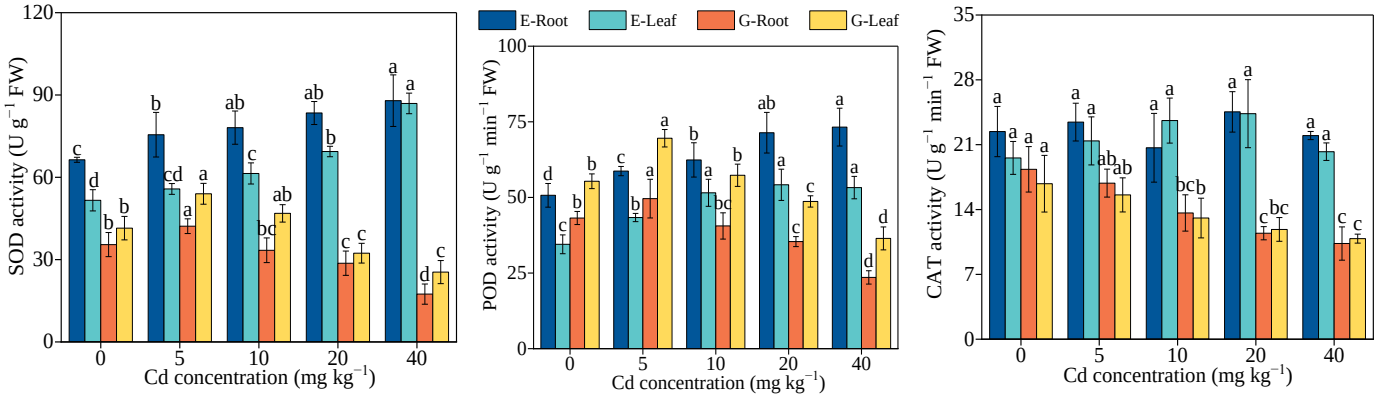

Figure 5. Antioxidant enzyme activities of *E. pectinatus* and *G. jasminoides* under a concentration gradient stress of Cd. Different letters indicate significant differences ($p < 0.05$) among different treatments. FW, fresh weight; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; Cd, cadmium; E, *E. pectinatus*; G, *G. jasminoides*.

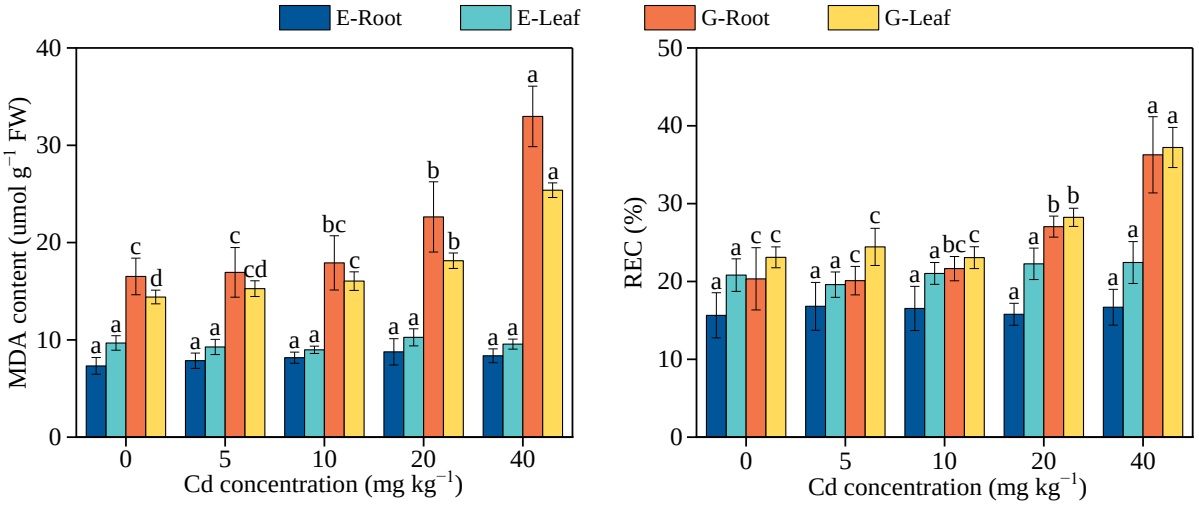

Figure 6. MDA and REC in roots and leaves of *E. pectinatus* and *G. jasminoides.* Different letters indicate significant differences ($p < 0.05$) among the different treatments. Cd, cadmium; FW, fresh weight; E, *E. pectinatus*; G, *G. jasminoides*; MDA, malondialdehyde; REC, relative electrical conductivity.

4. Discussion

4.1. Differences in Cd Tolerance and Remediation Potential of Two Ornamental Plants

The accumulation of Cd in plants, particularly in the shoots, is an important embodiment of their ability to accumulate this heavy metal and their potential to remediate it [2,34]. In this study, 5–40 mg kg^{-1} Cd treatments on *E. pectinatus* and *G. jasminoides* accounted for 80.90–86.44% and 70.21–75.17% of the levels of accumulation of Cd in their shoots compared with those in the whole plants, respectively. The highest accumulation reached 1.45 and 0.71 mg plant^{-1}, respectively (Figure 2). These results are higher than those of common Cd hyperaccumulator plants (*Bidens pilosa* L. [35] and *Solanum nigrum* L. [36]) and herbaceous flowers (*Tagetes patula* L. [37]), and the ornamental tree (*Robinia pseudoacacia* L.) that accumulates Cd [38]. The PR of Cd by *E. pectinatus* and *G. jasminoides* reached 1.36–2.85% and 0.67–2.66% (Table 2), respectively, which were similar to the Cd hyperaccumulator plants *Amaranthus mangostanus* L. [39]. Therefore, *E. pectinatus* and *G. jasminoides* have the characteristics of Cd hyperaccumulator plants and are usable for the phytoremediation of Cd-contaminated soil. Additionally, the accumulation of Cd and the tolerance of *E. pectinatus* to it exceeded that of *G. jasminoides*, suggesting that *E. pectinatus* has a greater potential to repair an environment contaminated with Cd than *G. jasminoides*.

The biomass and TI of plants stressed by Cd are important indicators of Cd tolerance and accumulation ability [34]. For example, a high concentration of Cd did not significantly affect the biomass of *Malva rotundifolia* L. and *L. camara* [15,34]. Low concentrations of Cd increased the biomass of *Celosia cristata* L. and *Sedum alfredii* Hance. However, high levels of Cd significantly decreased the biomass of *C. cristata* and *S. alfredii* [34,40]. In this study, all the Cd treatments significantly increased the shoot biomass of *E. pectinatus* compared with that of the control (Table 1). However, the TI was >1 (Table 2). In *G. jasminoides*, increasing the Cd concentration first increased the biomass of roots and shoots and then decreased them later. At a concentration of 20 mg kg^{-1} Cd, the biomass of *G. jasminoides* was significantly lower than that of the control (Table 1), and the TI was <1 (Table 2). However, treatment with 20–40 mg kg^{-1} Cd increased the TI of *E. pectinatus* compared with *G. jasminoides* (Table 2), indicating that *E. pectinatus* is more tolerant of Cd than *G. jasminoides*. Cadmium was more effective at promoting the growth of *E. pectinatus* shoots than its roots and was less inhibitory toward the *G. jasminoides* shoots than the roots (Table 1). Similar results were also noted in *Siegesbeckia orientalis* L. [2]. Roots have direct contact with the Cd^{2+} in the soil; thus, the higher content of Cd in the root affects the metabolic activity of root cells and inhibits cell division [41]. The different abilities and tolerances of the two ornamental plants to accumulate Cd may be related to their mechanisms of detoxification.

4.2. The Cd Tolerance and Detoxification Mechanisms of the Two Ornamental Plants

The Cd distribution in different plant organs is closely related to the tolerance and detoxification mechanism of plants and also reflects their abilities to accumulate Cd and repair potential damage [15]. Similar to most common plants [34], the content of Cd in the roots of the two ornamental plants in this study was greater than that in the shoots under all the treatments of Cd (Figure 1a,b). Although the BCF > 1; the TF was <1 (Table 2). These results indicate that the two ornamental plants had a strong ability to accumulate Cd but a weak ability to transfer it, thus, protecting the shoots from Cd poisoning. However, the content of Cd in the roots of *G. jasminoides* was higher than that in *E. pectinatus* (Figure 1), although the content of Cd in the shoots and TF were lower than those of *E. pectinatus* (Figure 1, Table 2). This difference may be due to the stronger ability of the nucleic acids, proteins, and polysaccharides in the roots of *G. jasminoides* to combine with heavy metals to form macromolecular substances or insoluble organic molecules [42]. The macromolecular substances or insoluble organic molecules are more conducive for forming complexes with Cd in the roots to improve their tolerance.

Plant cell walls contain pectic acid, polysaccharides, and proteins, which combine with heavy metal ions to limit transmembrane transport and maintain the normal physiological and metabolic process [2]. However, the cell walls cannot completely block heavy metals. Thus, most heavy metal ions that have entered the protoplast are transported to the vacuole where they combine with thiopeptides and organic acids and are then stored in the vacuole to reduce the damage to organelles [19]. In this study, much of the Cd in the roots, stems, and leaves of *E. pectinatus* was stored in the soluble fractions, including cell vacuoles, which compartmentalized Cd with organo-ligands, followed by the cell walls (Figure 3a,c). The results were similar to that of *P. americana* where 54–70% of the Cd was stored in the soluble fraction [22]. However, the Cd in the roots, stems, and leaves of *G. jasminoides* were primarily stored in the cell wall, followed by the soluble fraction (Figure 3b,d), which was similar to the findings observed with *K. obovata* [21]. These results indicate that vacuolar compartmentalization is an intracellular detoxification mechanism of *E. pectinatus*, and cell wall immobilization is an intracellular detoxification mechanism of *G. jasminoides*.

Nonetheless, the proportion of Cd in the soluble fraction of the *E. pectinatus* roots was much higher than that in the stems and leaves. In contrast, the proportion of Cd in the stem and leaf cell walls was higher than that in the root (Figure 3c). Cadmium-stressed *Impatiens walleriana* Hook. demonstrated similar results [43]. Thus, limiting the compartmentation of Cd in the *E. pectinatus* root cell walls promotes the transport of Cd to the shoot. This strategy could increase the concentration and accumulation of Cd in

the shoots of some hyperaccumulators [22]. However, *G. jasminoides* stores more Cd in the cell walls and protects the protoplast from Cd toxicity, while restricting Cd transport to the shoots (Figure 3d). This is an important mechanism to improve the tolerance of plants to Cd [44].

The toxicity and migratory ability of heavy metals in plants is closely related to their chemical forms. Inorganic and water-soluble Cd are the most toxic and likely to migrate, followed by pectate and protein-bound Cd, insoluble Cd phosphate, and Cd oxalate, which were extracted using 80% ethanol, H_2O, 1 M NaCl, 2% HAc, and 0.6 M HCl, respectively [2]. In this study, most of the Cd was in the chemical extractable forms integrated with NaCl and HAc among the different tissues of *E. pectinatus* and *G. jasminoides*. In contrast, ethanol and H_2O extracted a minority of the extractable Cd (Figure 4). These results are similar to those of other Cd hyperaccumulators, such as *S. nigrum* [45], indicating that Cd^{2+} primarily chelates with proteins, such as metallothionein, to form non-toxic complexes in the cells of *E. pectinatus* and *G. jasminoides* [46].

A higher percentage of Cd could be extracted with NaCl and HAc in *E. pectinatus* than in *G. jasminoides*, but the proportions of Cd that could be extracted with ethanol and H_2O were higher in *G. jasminoides* than *in E. pectinatus* (Figure 4c,d). These differences could possibly explain why *E. pectinatus* is more tolerant to Cd than *G. jasminoides*. Moreover, the proportions of Cd that could be extracted with ethanol and H_2O were higher in the roots of *E. pectinatus* than in its stems and leaves (Figure 4b). The Cd that could be extracted with ethanol and H_2O primarily combined with nitrate/nitrite, chloride, aminophenol, organic acid, and metaphosphate [46]. These forms of Cd in the roots of *E. pectinatus* are associated with the symplastic transportation of Cd, which indicates a higher ability to transfer Cd to the shoots [47]. This transportation probably contributes to the higher degree of accumulation of Cd by the shoots of *E. pectinatus*.

Heavy metal ions can damage cell membrane proteins and phospholipids, which cause an ion imbalance and cell metabolic disorders [15]. Normally, plants activate the SOD, POD, CAT, and other antioxidant enzyme protection systems to improve their ROS scavenging ability, thus, enhancing tolerance [48]. The levels of MDA and REC can reflect the degree of damage induced by Cd [49]. These results showed no significant difference in the levels of MDA and REC in *E. pectinatus* treated with Cd and the control (Figure 6). However, increasing the concentration of Cd gradually increased the contents of MDA and REC in *G. jasminoides* (Figure 6), which was similar to previous research on *L. camara* [15], indicating that membrane lipid peroxidation damaged *G. jasminoides*.

Additionally, treatments with Cd changed the activities of antioxidant enzymes in both ornamental plants. For example, treatment with Cd significantly increased the activities of SOD and POD in *E. pectinatus* compared with the control (Figure 5). Therefore, *E. pectinatus* plants treated with Cd had a strong antioxidant capacity, which could possibly explain its strong tolerance. The activities of SOD and POD were significantly higher in the control *G. jasminoides* than in the plants treated with 20–40 mg kg^{-1} Cd. Moreover, the activity of CAT was significantly higher in the control *G. jasminoides* than in the plants treated with 10–40 mg kg^{-1} Cd (Figure 5), which induced a small degree of antioxidant activity. However, the levels of MDA and REC of the plants treated with 20–40 mg kg^{-1} Cd were significantly higher than those of the control (Figure 6) and thus, caused greater oxidative damage. These results are similar to those of previous studies on *S. orientalis* [47]. The reason may be that the accumulation of Cd in *G. jasminoides* exceeds its limits of tolerance and thus, inhibits its growth.

5. Conclusions

Both *E. pectinatus* and *G. jasminoides* can accumulate a large amount of Cd, and the former plant accumulated more of this heavy metal than the latter. At a concentration of 20–40 mg kg^{-1} Cd, *E. pectinatus* can grow normally with a TI > 1, while the growth of *G. jasminoides* is inhibited with a TI < 1. This indicated that these plants are highly tolerant to Cd and effective at accumulating it, which makes them suitable for the remediation

of Cd-contaminated soil. In addition, *E. pectinatus* is more effective at remediating Cd and tolerant to it than *G. jasminoides*. There are differences between *E. pectinatus* and *G. jasminoides* in the detoxification mechanism of Cd. The former primarily includes vacuolar compartmentalization, presence in a less toxic form, and a higher antioxidant system. The latter primarily includes cell wall retention and presence in a less toxic form. Further research is needed on the molecular mechanisms that underlie the detoxification of Cd.

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