Impacts of root pruning intensity and direction on the phytoremediation of moderately Cd-polluted soil by *Celosia argentea*

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Abstract

Root pruning can impact the nutrient uptake capacity, photosynthetic efficiency, transpiration rate, root pressure, and biomass yield of various plants, which influence phytoremediation. A series of root pruning treatments with different combinations of direction (two-side pruning and four-side pruning) and intensity (10%, 25%, and 33% pruning) were performed on *Celosia argentea*. All two-side pruning treatments, regardless of intensity, decreased the dry biomass of the *C. argentea* roots at the end of the experiment relative to that of the control, and the lowest value occurred in the two-side-33% pruning treatment. However, the two-side-10% and two-side-25% pruning treatments stimulated the growth rate of the plant leaves significantly by 58.6% and 41.4%, respectively, relative to that of the control, and even offset the weight loss of the plant roots. Contrastingly, the two-side-33% pruning treatment reduced the biomass yield of leaves by 24.1%. For the four-side pruning treatments, low intensity increased the dry weight of both the plant roots and leaves, while both decreased under high intensity root pruning. The dry weight, Cd content, pigment level, and photosynthetic efficiency in the four-side-10% treatment were higher than those in the other treatments during the experiment, thereby corresponding to the best phytoremediation effect. Except for the two-side-33% pruning treatment, all the treatments increased the phytoremediation efficiency of *C. argentea*, thereby indicating that root pruning with a suitable combination of direction and intensity can positively influence the Cd removal ability of *C. argentea*.

1. Introduction

With the development of society and the expanding population, a large amount of contaminants have been discharged into various environmental media (Miao et al., 2017; Zeng et al., 2019). Among various contaminants, the emission of heavy metals has gained extensive attention in China because the last national soil quality survey that covered more than 90% of China's territory showed that nearly 20% of the survey fields were polluted by different metals (Wang et al., 2018). Moreover, approximately 7% of the survey soils are polluted by Cd to different degrees. Cd has been ranked 7th among the top 20 hazardous materials in the list of the US Agency for Toxic Substances and Disease Registry (Chaturvedi et al., 2019) owing to its detrimental impacts on the physiological functions of cells. Except for those in some microalgae (Park et al., 2007), no biological functions of Cd have been reported. Severe environmental issues can be induced by the accumulation of Cd in soil and water, and the metal can endanger human health via the food web owing to its high bioavailability and biological toxicity. Although the emission of Cd is strictly controlled worldwide, its contamination in different environments has been worsening, especially in developing nations (Snousy et al., 2019; Sydow et al., 2018). Cd-polluted soil, especially in agricultural land, should be remediated to ensure global food safety and security.

Some physical and chemical remediation techniques such as covering, chemical washing, in situ stabilization, biochar application, and electrokinetic remediation (Ottosen et al., 2007; Park et al., 2016; Shen et al., 2018) have been developed to treat metal-polluted fields. These methods can remediate heavily polluted sites with a small area in a short time period, but are not qualified for low to moderately contaminated fields with a large area owing to their high cost. Besides the cost, the complex topography and need for heavy equipment also limit the widespread use of these physical and chemical methods. In addition, conventional soil remediation techniques generally cause negative impacts on the soil structure, fertility, and ecological system, and can even result in irreversible damage to the soil, thereby decreasing the area of farmland. Considering the growing demand for food, which is expected to increase by two to five fold in 2030 (St. Clair and Lynch, 2010), traditional methods are unsuitable for agricultural land with large areas and low but ecologically significant contamination.

Phytoremediation, which involves the use of green plants and associated microorganisms to decrease the bioavailability and toxicity of pollutants in different environmental media or to remove them from various substrates, is regarded as a potential candidate to remediate metal-contaminated soils (Luo et al., 2016). This plant-based soil remediation method generally contains four subcategories, namely phytoextraction, phytostabilization, phytofiltration, and phytoevaporation (Ma et al., 2011). Among these subcategories, phytoextraction, which uses plants to translocate and accumulate pollutants in aerial tissues, and phytostabilization, which uses plants to reduce the chemical activity of pollutants in the rhizosphere, are used more widely.

Qu et al., (2013) suggested that the expenditure of phytoremediation using a hyperaccumulator is approximately \$400–800 $\cdot ha^{-1} \cdot y^{-1}$ based on the labor cost and the price in China. Although this cost is lower than that of conventional physical and chemical methods by several orders of magnitude, it is still too expensive considering the contaminated soil area. In addition, compared with traditional methods, which only require a one-time investment, phytoremediation requires consistent investment to maintain the replanting and harvesting procedures. The longer the duration of the phytoremediation process, the higher the cost. Unfortunately, the efficiency of phytoremediation is significantly lower than that of traditional methods, and it will take centuries for plants, even hyperaccumulators, to decontaminate excessive pollutants in soil (Luo et al., 2019b). According to the remediation mechanism of phytoextraction, the biomass yield and metal extraction ability of plants are the decisive factors determining the success and the duration of the decontamination process. Therefore, a series of supplementary means, i.e., chelator application (Wang et al., 2018), plant growth promoting rhizobacteria inoculation (Rehman et al., 2018), transgene introduction (Das et al., 2016), electric field addition (Cameselle and Gouveia, 2019), and magnetic field pretreatment (Luo et al., 2019a), have been exploited to increase the dry weight and metal uptake capacity of various plants. However, these means generally result in some secondary problems, such as pollutant leaching, high energy consumption, and species invasion. Therefore, economical and feasible supplementary means that can be conducted in a real-scale field should be developed.

Root pruning is a common agronomic management practice. The application of this practice can remove the apical dominance that impedes the development of lateral roots, thereby increasing the biomass yield, water use efficiency, and nutrient uptake capacity of plants (Valdés-Rodríguez and Pérez-Vázquez, 2019). However, there are conflicting results from previous studies regarding the impacts of root cutting of different plants. Generalized statements are not accurate because multiple factors can influence the responses of plants to root pruning. Physiological responses depend on the type of species, pruning deposition, pruning time, pruning size, and pruning intensity. For example, low pruning intensity results in high root vigor in *Ricinus communis* L.; contrastingly, the severity of root pruning increases the rooting ability of *Pyrus communis* L. Moreover, suitable pruning practices can disrupt the physiological equilibrium of plants and change the hormone levels in their tissues. Feng et al., (2018) reported that the content of indole-3-acetic acid, which is a type of plant hormone that stimulates the growth rate of plants, increased in *Platycladus orientalis* roots when the taproots were removed. In addition, the loss of roots is inevitable during the transplanting process, which is an important procedure of phytoremediation because the integrity of the roots could be damaged to varying degrees when excavating the cultivated plants from the soil.

Celosia argentea is a native Chinese plant belonging to the Amaranthaceae family. The ornamental plant is grown throughout China, and can even survive in extreme environments, i.e., mining areas (Shen et al., 2017). Liu et al. (2018) reported that more than 80% of Cd extracted by *C. argentea* was accumulated in its above-ground parts. This indicates that the species can provide great potential for phytoextraction of metal-polluted soils without species invasion.

The physiological responses of *C. argentea* have rarely been reported. Therefore, it is necessary to determine the impacts of agronomic practices on the phytoremediation effect of the ornamental plant because agronomic practices that do not induce ecological risks are enforceable and economical in the real world. The major objectives of the current study were to evaluate the impacts of root pruning treatments with different directions and intensities on (1) the biomass yield of plant tissues, (2) the photosynthesis efficiency and transpiration rate of *C. argentea*, and (3) the Cd uptake capacity and phytoremediation effect of the species.

2. Materials And Methods

2.1 Collection and preparation of soil

Soils treated in the current experiment were sampled from Guiyu, which has been involved in electronic waste dismantling and recycling businesses for approximately 40 y. According to reports from the local statistical bureau, there are more than 6000 disassembly and recycling workshops in the town, which receive approximately 1.7 million tons of e-waste from local areas and abroad (Zhang et al., 2014). Most recycling activities are operated in village-owned or private family-sized enterprises without sufficient occupational hygienic and pollution control measures. Primitive dismantling and recycling methods including acid washing, burning, and hydrometallurgy melting, which discharge a large amount of solid and liquid waste that contain multiple metals, induce severe environmental pollution (Jiang et al., 2019). The results of the current geochemical survey show that soil pollution level significantly varies among various regions because different regions specialize in different dismantling and recycling stages (Li et al., 2011). The highest pollution level was found in the open burning fields, followed by dismantling sites, waste stack regions, residential quarters, agricultural soil, and wastelands. Previous studies verified that the dismantling and recycling business has already caused detrimental effects on local residents, especially pregnant women and children. Kim et al. (2019) reported that the Cd content in cord blood in pregnant women from Guiyu was 0.78 times higher than that in a control area. However, there was little difference in the blood Cd content between women involved in e-waste dismantling and those without involvement within Guiyu, thereby indicating widespread pollution of metals. In addition, Zeng et al. (2019) suggested that development parameters including birth weight and length, body mass index, and chest circumference in preschool children from Guiyu were significantly lower than those of their peers from a control area, and they attributed the lower development parameters to the high metal exposure. Therefore, it is necessary to remediate metal-polluted soil in the town, particularly in residential quarters. Because weeds can grow spontaneously in residential quarters, phytoremediation can be applied to decontaminate soil in these areas.

Considering the different geological backgrounds, soil types, and terrain in the study area (Luo et al., 2017), 200 soils (0–20 cm) were collected using a 200 m × 200 m plot to produce synthetical data representing the overall features of the region. After being air dried, the gathered soils were sieved using 2 mm meshes to remove foreign materials. The sieved soils were blended to generate a composite substrate. To ensure the homogeneity of the substrate in the experiment, the composite soil underwent several mixing, moistening, and drying procedures. Twenty soils were sampled randomly in the blended soil after each mixing cycle to analyze the content of Cd. Homogenization was considered to be achieved after the third blending cycle, which manifested as a low variable coefficient (< 15%) of Cd. The homogeneous soil was divided into 35 aliquots of 6 kg, and each was filled to a height of 16 cm in prepared cylindrical containers with a height of 20 cm and a bottom diameter of 20 cm for further treatment.

Soil pH, organic matter, and exchangeable cations were analyzed at the beginning of the experiment. For pH analysis, the prepared soil was mixed with deionized water at a ratio of 1:2.5 (w/v), and a pH meter (HI98160, Hanna Instruments, USA) was used to read the pH in the dispersion. For the organic matter measurement, a mixture of 20 mL of concentrated sulfuric acid and 10 mL of potassium dichromate (0.8 $mol \cdot L^{-1}$) was applied to dissolve 100 mg of soil. After cooling, the suspension was shaken at 100 rpm for 30 min and then heated for 10 min. A mixed solution of 200 mL of deionized water and 10 mL of phosphoric acid was used to dilute the suspension, and ammonium ferric sulfate was added to titrate dichromate in the suspension (Zhang et al., 2016). For the exchangeable cation determination, 4 g of prepared soil was mixed with 33 mL of sodium acetate (1 $mol \cdot L^{-1}$). After ion extraction, ammonium acetate (1 $mol \cdot L^{-1}$) was applied to replace the sodium ions.

2.2 Experimental design

Healthy *C. argentea* seeds with no visible defects were provided by the Guyue landscaping company. After being sterilized using hydrogen peroxide (10%), the seeds were grown in sand at room temperature according to the methods suggested by (Pan et al., 2019). Germinated seedlings were cultivated in clean sand until two pairs of true leaves were developed, and plants with a similar morphology were chosen for the root pruning treatments. The experiment had a completely randomized block design with seven treatments with five replications each, as follows: (1) the control (without root pruning); (2) two-side-10% treatment (cutting 10% of the roots from both sides of each plant; approximately 5% from each side); (3) two-side-25% treatment (cutting 25.0% of the roots from both sides of each plant; approximately 12.5% from each side); (4) two-side-33% treatment (cutting 33.0% of the roots from both sides of each plant; approximately 16.5% from each side); (5) four-side-10% treatment (cutting 10.0% of the roots from four sides of each plant; approximately 2.5% from each side); (6) four-side-25% treatment (cutting 25% of the roots from four sides of each plant; approximately 6.5% from each side); and (7) four-side-33% treatment (cutting 33.0% of the roots from four sides of each plant; approximately 8.2% from each side). Distilled water was applied in the tray under each container to keep the soil moist. The transpiration rate and photosynthetic efficiency of *C. argentea* were determined with a photosynthesis instrument (LI-6400 XT, LI-COR Biosciences, Lincoln, NE, USA) on day 50 after transplanting. The measurements lasted 1 h from 9:00 to 10:00.

After the transpiration rate and photosynthetic efficiency measurements, all the plants were harvested and divided into roots and leaves. The plant roots were cleaned with running water, then with deionized water under sonication (10 min) to completely eliminate attached materials, and finally with calcium chloride (5 mmol·L^{-1}; 10 min) to clean the adsorbed metals. The leaves were cleaned with tap water to remove the surface dust and then immersed in Na_2EDTA (10 mM; 10 min) to eliminate adsorbed metals. Deionized water was used to remove the residual calcium chloride and Na_2EDTA from the plant tissues. The cleaned plants were lyophilized at -80°C.

2.3 Cd measurement

The prepared samples were ground onto 74 μm meshes, and the powder was dissolved in aqua regia according to the method suggested by Chamba et al. (2017). After being diluted using deionized water, the digestion was filtered through an OE67 Whatman membrane. The concentration of Cd in the supernatant was analyzed using an inductively coupled plasma mass spectrometer (Agilent 7700, Agilent Technologies, Santa Clara, USA). Soil (GBW07410) and plant (GBW10012) standard references were analyzed to ensure the quality of the analysis. A drift monitoring solution was also utilized to control the signal drift of the apparatus.

2.4 Pigment measurement

The concentrations of chlorophyll and carotenoid in the plant leaves were analyzed according to the method suggested by Lichtenthaler (1987). In brief, the cut fresh leaves were dissolved using 80% acetone in the dark until the color faded from the leaf surface. The extracts were measured with a UV-Vis spectrophotometer (UV mini 1240, Shimadzu, Japan) to determine the chlorophyll and carotenoid concentrations, which were calculated from the visible absorbances of 470, 645, and 663 nm (Lichtenthaler, 1987).

2.5 Lipid peroxidation and electrolyte leakage assay

For evaluation of lipid peroxidation according to the malondialdehyde (MDA) concentration, the plant leaves were homogenized in trichloroacetic acid according to Iqbal et al. (2019). The absorbance of the solution was determined at 450, 532, and 600 nm. The concentration of MDA was calculated using the equation described by Iqbal et al. (2019).

For the electrolyte leakage (EL) analysis, the plant leaves were cut into approximately 4 mm^2 pieces and immersed in 8 mL of deionized water for 120 min of heating (32°C) in a water bath. The initial electrical conductivity (EC_1) of the supernatant was measured using an electrical conductivity meter. The supernatant was then heated for 20 min (120°C) for electrolyte release. The electrical conductivity (EC_2) of the supernatant was determined again using the same instrument, and the EL was calculated according to the equation suggested by Wang et al. (2017), as follows:

$EL = EC_1/EC_2$

2.6 Statistical analysis

A one-way analysis of variance was performed to assess the differences in the dry weight, transpiration rate, photosynthetic efficiency, pigment content, and stress injury of *C. argentea* under different root pruning treatments. The mean values were compared using the protected Fisher's least significant difference test at 0.05 confidence using SPSS 15.0.

Bioaccumulation factors (BCFs), transfer factors (TFs), and accumulation factors (AFs) were calculated according to Beiyuan et al. (2017) to evaluate the ability of *C. argentea* to extract, translocate, and decontaminate contaminants, as follows:

BCF = Cd content in plant tissues/Cd content in substrate

TF = Cd content in aerial parts/Cd content in below-ground parts

AF = Cd content in plant tissue × corresponding dry weight of tissue

3. Results And Discussion

3.1 Soil characteristics

The soil pH at the beginning of the experiment ranged from 5.5 to 6.5 with a mean value of 6.1. After the experiment, the pH values decreased by 3.3%, 3.3%, 8.2%, 1.6%, 9.8%, 8.2%, and 3.3% in the control, two-side-10%, two-side-25%, two-side-33%, four-side-10%, four-side-25%, and four-side-33% treatments, respectively. Although the pH values among all the root pruning treatments were not statistically significant, the four-side treatments tended to generate lower pH values than the control and two-side treatments. At the end of the experiment, the organic matter content in the soil increased by 5.3%, 6.2%, 5.7%, 2.3%, 7.7%, 6.3%, and 4.9% in the control, two-side-10%, two-side-25%, two-side-33%, four-side-10%, four-side-25%, and four-side-33% treatments, respectively, compared with the initial content. The organic matter content did not vary significantly among all treatments, and was higher in the four-side treatments than in the two-side treatments. At the beginning of the experiment, the content of exchangeable cations in the soil was 13.6 ± 3.9 cmolc·kg^{-1}, and the value decreased by 2.9% in the control at the termination of the treatment. In addition, the values reduced by 4.1%, 3.3%, 1.7%, 6.6%, 5.2%, and 3.5%, respectively, in the corresponding root pruning treatments. Perveen et al. (2015) reported that *C. argentea* roots secrete organic acids, such as protocatechuic acid, caffeic acid, genetic acid, and gallic acid, and acidize its rhizosphere soil. Wang et al. (2018) suggested that root cutting enhances the root activity of *Zea mays* L., and thus stimulated the species to secrete more organic matter, thereby supporting the results of this study. Although there were no statistically significant differences in pH, organic matter, and exchangeable cations under any of the treatments, the four-side pruning treatments, regardless of intensity, generated greater impacts on the soil characteristics relative to those of the two-side treatments.

The initial soil Cd content ranged from 2.9 mg·kg^{-1} to 4.2 mg·kg^{-1} with an average of 3.5 mg·kg^{-1}. The variable coefficient of Cd was 14.3%, thereby indicating the homogeneous distribution of Cd in the composite soil. The concentrations of Cd severely exceeded the statutory limit of Cd (0.3 mg·kg^{-1}) established by the Ministry of Environmental Protection of China for farmland. The concentrations of Cd were only analyzed at the beginning of the experiment because the duration of phytoremediation was not sufficient for *C. argentea* to alter the pseudo-total Cd content in the soil. A well-known Cd hyperaccumulator, *Noccaea caerulescens*, failed to change the soil pseudo-total Cd content in 132 d treatments (Martínez-Alcalá et al., 2016).

3.2 Biomass production

As shown in Fig. 1, the lowest dry weight of *C. argentea* roots was observed in the two-side-33% treatment, which decreased significantly by 47.9% compared with that in the control, and was successively higher in the four-side-33%, two-side-25%, four-side-25%, two-side-10%, control, and four-side-10% treatments. Although only slightly, the four-side-10% treatment increased the dry weight of the plant roots relative to that of the control during the experiment. Contrastingly, all the root pruning treatments, except for the two-side-33% treatment, increased the biomass yield of the leaves and even offset the weight loss of the roots induced by pruning. The highest dry weight of plant leaves was obtained in the four-side-10% treatment, which increased the value significantly by 113.8% compared with that of the control (Fig. 1). The results exhibited that both pruning intensity and direction can impact the biomass yield of the species. The four-side treatments alleviated the detrimental effects of the severity of root pruning on plant growth, as manifested in the higher dry weight of the plant roots and leaves in the four-side treatments than in the corresponding two-side treatments.

The effects of pruning size, pruning location, pruning intensity, and pruning time on the growth status of various plants have been fully discussed in previous works, but conflicting phenomena dominate the previous works in terms of the impacts of this agronomic practice. Generalized rules are difficult to establish because multiple factors may influence physiological responses. For instance, two *Acer* species, namely *Acer palmatum* L. and *Acer negundo* L., showed significantly different growth responses to different pruning intensities. Specifically, root pruning resulted in negative impacts on the above-ground growth of *A. palmatum*, but did not influence the growth of *A. negundo* (Benson et al., 2019). For the same species, the dry weight and root plasticity of *Glycine max* L. were increased by root pruning, with the increase being proportional to the extent of root pruning (Fanello et al., 2020). These studies indicate the complexity of the impacts of root pruning on plant growth.

Although the physiological responses of various plants to root pruning were different, almost all previous studies verified that root pruning, except for excessive pruning, which induced irrecoverable damage to the plant roots, can significantly stimulate the emergence of new roots at the incision site, thereby increasing the vitality and absorption area of the root system and extracting and translocating more nutrients from the soil to its aerial parts (Feng et al., 2018; Gao et al., 2018; Miller and Graves, 2019). A greater number of lateral roots emerged in the four-side treatments compared with that in the two-side treatments because there were more incisions in the former, which explained why the species in the four-side pruning treatments, regardless of intensity, could produce greater root and leaf biomass than those in the two-side pruning treatments.

In addition, the severity of root pruning can result in irreversible damage to the physiological functions of the root system (Feng et al., 2018), thereby impeding the compensatory growth of plant tissues, which explained why the two-side-33% root pruning treatment inhibited the growth status of the plant roots and leaves.

3.3 Photosynthesis efficiency and pigment level

The transpiration rate of *C. argentea* did not vary significantly among the treatments, except for the two-side-33% treatment, during phytoremediation. The highest transpiration rate of 3.9 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$ was recorded in the four-side-10% treatment, and was successively lower in the four-side-25%, two-side-10%, two-side-25%, control, four-side-33%, and two-side-33% treatments (Fig. 2). The results exhibited a general trend that low to moderate pruning stimulated the transpiration rate of the species, and severe pruning inhibited it. Although the suitable pruning direction did not further enhance the beneficial effect induced by low to moderate root pruning, it alleviated the detrimental impact caused by severe pruning to some extent. Regarding the photosynthesis efficiency, a similar variation trend with transpiration rate was found in all the root pruning treatments. The quotient of photosynthesis efficiency and transpiration rate is the water use efficiency of the species (Larchevêque et al., 2011). In contrast to the variation tendency of the photosynthesis efficiency and transpiration rate, the highest water use efficiency was observed in the two-side-33% treatment, and was successively lower in the two-side-10%, four-side-33%, control, two-side-25%, four-side-25%, and four-side-10% treatments (Fig. 2). In the two-side-25%, four-side-10%, and four-side-25% treatments, the reduction in the transpiration rate was greater than the photosynthetic efficiency, thereby causing the increase in water use efficiency.

Chlorophyll and carotenoid can be used as biological indicators to reflect the level of external stresses (Shiri et al., 2015). In the present study, the highest chlorophyll content in the plant leaves was observed in the four-side-10% treatment. Except for that in the four-side-10% treatment, no statistically significant differences in chlorophyll concentrations were recorded among the treatments (Fig. 3). Compared with the control, only the two-side-33% treatment showed a slightly lower chlorophyll level. The results indicate that the root pruning treatments conducted in the present study generally tend to increase the content of chlorophyll, although not significantly. The severity of root pruning, regardless of direction, induced detrimental effects on the synthesis of carotenoid, as manifested in the significantly lower carotenoid content in the plant leaves in the two-side-33% and four-side-33% treatments compared with that in the control. Contrastingly, low to moderate pruning treatments increased the carotenoid content (Fig. 3). The results show that both suitable pruning intensity and direction can enhance the carotenoid synthesis efficiency.

Root pruning can increase the level of plant hormones, such as indole-3-acetic acid, in plant roots and shoots. For instance, Wang et al. (2018) found that root cutting increased the content of cytokinin in lateral roots, xylem saps, and shoots of *Z. mays*. They established a relationship between the leaf cytokinin content and morphology of the plant roots, and revealed that the new emerged roots from incisions with a large amount of meristem served as a cytokinin-generating area, and the loss of deep roots impeded the production of cytokinin. The enhanced level of plant hormones can indirectly increase the photosynthesis efficiency and transpiration rate of the species by stimulating the synthesis of chlorophyll and carotenoid in the plant leaves. The results explain why moderate root cutting can improve the photosynthesis efficiency and pigment level of *C. argentea* at a molecular level.

In addition, Feng et al. (2018) evaluated the influences of root pruning on the root morphology, physiology, and anatomy of *P. orientalis* seedlings, and found that the total root length, root surface area, average root diameter, and number of root tips of the plants subjected to root pruning were higher than those of the non-root pruned seedlings. They reported that well-structured roots are conducive to increasing the nutrient absorption and photosynthetic rate of the species. This explains why 10% and 25% root cutting, regardless of direction, in the current study improved the transpiration rate and pigment concentration of *C. argentea*. Moreover, Benson et al. (2019) reported that the severity of root pruning caused *A. palmatum* to lose 70% of its aerial tissues because the species redistributed more photoassimilates to the roots to restore the damage resulting from root cutting. Moreover, Jing et al. (2018) suggested that the below-ground and aerial parts of *Populus*×*euramericana* can communicate through signaling molecules, which results in a reduction in the transpiration rate in leaves when the roots are partially pruned. This explains why intense root pruning inhibited the transpiration rate of *C. argentea* in this experiment.

In contrast to the transpiration rate and photosynthesis efficiency, it was observed that the severity of the root pruning treatments, regardless of direction, increased the water use efficiency of *C. argentea*. This might have been because the root pruning treatment produced a stronger impact on transpiration than on photosynthesis. The transpiration rate is more dependent on the stomatal conductance compared with photosynthesis (Kumari et al., 2013); thus, partial stomatal closure caused by intense root pruning can improve the water use efficiency of the species.

3.4 Cd concentration and oxidative damage

The concentrations of Cd were significantly lower in the plant roots than in the leaves (Fig. 4). Compared with that of the control, the four-side-10% and four-side-25% treatments had slightly higher Cd contents in the *C. argentea* roots, and other treatments had lower root Cd concentrations. Significantly lower root Cd concentrations were observed in the case of intense root pruning treatments regardless of direction relative to the corresponding low and moderate pruning strategies. In addition, the root Cd content in the four-side-33% treatment was significantly higher than that in the two-side-33% treatment. The results indicate that root pruning generally tends to reduce the Cd content in the below-ground parts of the species, and a suitable cutting direction can alleviate the negative effect, especially under severe root damage. For the plant leaves, the highest content of Cd was observed in the four-side-10% treatment, followed by that in the four-side-25%, two-side-10%, two-side-25%, four-side-33%, control, and two-side-33% treatments. Compared with that in the control, the Cd concentrations in the leaves were significantly higher in the four-side-10% and four-side-25% treatments. The four-side treatments had higher leaf Cd contents than the corresponding two-side treatments; moreover, the four-side pruning increased the leaf Cd content back to a normal level under severe root damage. The results indicate that except for the unsuitable pruning treatment, which might cause irreversible damage to the root system, root pruning can increase the content of Cd in the plant leaves. Furthermore, the combination of intensity and direction can further promote the individual positive impacts, and a suitable cutting direction can fully offset the detrimental impact on the Cd uptake and accumulation ability of the species induced by severe root damage.

The highest BCF values of Cd in the plant roots were observed in the four-side-10% treatment, and were successively lower in the four-side-25%, control, two-side-25%, two-side-10%, four-side-33%, and two-side-33% treatments (Table 1), thereby indicating that root pruning generally tends to decrease the root BCF values of Cd. In contrast, all the pruning treatments, except for the two-side-33% treatment, had higher leaf BCF values than the control. In addition, the BCFs of Cd in the leaves were significantly higher than those in the roots.

Table 1
BCF, TF, and AF of Cd in *C. argentea* grown in different treatment

	Roots BCF	**Shoots BCF**	**TF**	**AF (μg per plant)**
control	4.4	18.9	4.3	21.4
2−10%	3.8	25.2	6.7	43.8
2−25%	4.2	23.6	5.6	36.5
2−33%	2.5	15.5	6.2	12.9
4−10%	4.8	31.7	6.5	74.1
4−25%	4.5	29.2	6.5	56.2
4−33%	3.3	22.7	6.9	31.6

The TF values of Cd in *C. argentea* were 4.3, 6.7, 5.6, 6.2, 6.5, 6.5, and 6.9, respectively. All the values were higher than 1, thereby indicating that the species tends to transfer more extracted Cd from its below-ground parts to its aerial tissues. As shown in Table 1, the TF values increased with the increase in cutting sides and intensity, and the combination of these two factors further improved the positive impact resulting from the individual factors, as manifested by the highest TF in the four-side-33% treatment.

The levels of MDA and EL were calculated to evaluate the membrane lipid peroxidation and integrity in the current study. None of the root pruning treatments exhibited regular impacts on both factors (Fig. 5). No statistically significant MDA and EL contents among all the treatments were observed, thereby indicating that root pruning may not result in oxidative damage to *C. argentea*.

Overall, the root pruning treatments generally decreased the Cd content in the plant roots, but increased the content in the leaves. According to previous studies, the agronomic practice can increase the concentrations of specific nutrient elements in plant tissues via three pathways, which can be used to explain the variation in Cd content observed in this experiment. First, the application of moderate root pruning resulted in the significantly higher contents of organic acids and amino acids in the rhizosphere soil of *Populus*×*euramericana* compared with those of the control, slight, and intense cutting groups, thereby lowering the pH in the soil and consequently mobilizing the soil Cd (Jing et al., 2017). Second, root cutting promoted the development of lateral roots from the incisions and increased the productivity of the plant roots, thereby resulting in the high absorption area of the root system and enhancing the metal uptake capacity of the species (Lordan et al., 2019). Finally, the current study proved that slight and moderate root pruning can enhance the transpiration rate and photosynthetic efficiency of *C. argentea*, and verified that the transpiration rate of plants is one of the most important determining factors for phytoextraction (Bagheri et al., 2019; Luo et al., 2019c). In addition, severe root pruning can result in irrecoverable damage to the plant roots, thereby decreasing the metal uptake ability of the species, which explained the low Cd contents in the roots and leaves of *C. argentea* observed in the present study. Based on the variation trends of BCFs and TFs in the plant tissues, root pruning was conducive to accelerating the extraction rate and translocation rate of Cd in *C. argentea*; the translocation rate increased faster than the extraction rate, so the TFs increased.

Whether root pruning can cause stress injuries such as lipid peroxidation and EL has rarely been discussed in previous studies. In this phytoremediation period, none of the root pruning treatments varied the levels of MDA and EL in the plant leaves. The highest MDA and EL values were in the two-side-25% treatment, which had a higher content of Cd in the leaves compared with that of the control. Moderate root pruning associated with two cutting directions (two-side-25%) can drive more Cd into the aerial parts of *C. argentea*, which may induce oxidative damage to the plant tissues. However, the four-side-25% treatment had a higher content of Cd and lower levels of MDA and EL in the plant leaves compared with those of the two-side-25% treatment. We hypothesize that a suitable pruning direction can alleviate the oxidative damage caused by the increased content of Cd. More precise experiments should be designed in the future to reveal the relationships among root pruning, stress injury, and antioxidant enzyme activity.

3.5 Decontamination effect

AFs were calculated to estimate the time required for *C. argentea* to eliminate excessive Cd in the soil. The difference between the determined Cd content in the substrate (3.7 mg·kg^{-1}) and its corresponding statutory limit (0.3 mg·kg^{-1}) multiplied by the weight of the soil in each container (6 kg) was regarded as the excess that needed to be eliminated. The excess amount of Cd divided by the AF value was the necessary harvesting cycling in each treatment.

The AF values of Cd in the different root pruning treatments were 21.4, 43.8, 36.5, 12.9, 74.1, 56.2, and 31.6 μg per plant in the control, two-side-10%, two-side-25%, two-side-33%, four-side-10%, four-side-25%, and four-side-33% treatments, respectively. Relative to that of the control, the AF values of the two-side-10%, two-side-25%, four-side-10%, four-side-25%, and four-side-33% treatments increased by 104.5%, 70.8%, 246.5%, 162.8%, and 47.8%, respectively, and that of the two-side-33% treatment decreased by 39.5%. This result indicates that severe root cutting with two pruning directions reduced the phytoremediation effect of *C. argentea* by simultaneously decreasing its biomass production and Cd uptake capacity. In addition, a suitable pruning direction can alleviate the detrimental effect, as manifested in the higher AF value in the four-side-33% treatment than that in the control.

It would take 159, 78, 93, 262, 46, 61, and 108 planting cycles using *C. argentea* to reduce the initial Cd content of 3.7 mg·kg^{-1} to an acceptable level in the control, two-side-10%, two-side-25%, two-side-33%, four-side-10%, four-side-25%, and four-side-33% treatments, respectively. According to the results, the four-side-10% treatment was the optimal method in the current study.

In order to ensure food security, the Chinese government strictly bans the growing of ornamental plants in arable land, although a significantly higher economic benefit can be gained from ornamental plants than from traditional agricultural products. However, ornamental plants can be cultivated in lightly to moderately metal-polluted soils, which cannot be used to grow crops. Common ornamental plants generally have some fundamental features, i.e., drought resistance, metal tolerance, and a short growing cycle, to acclimate to inhospitable environments, such as highways and green belts (Guo et al., 2012). In this study, the concentration of Cd in the aerial parts of *C. argentea* without root pruning did not meet the criteria for a Cd hyperaccumulator (100 mg·kg^{-1}). However, in the four-side-10% and four-side-25% treatments, the leaf Cd content achieved the threshold, thereby indicating that *C. argentea* can be used as a hyperaccumulator to remediate Cd-polluted soil when associated with suitable root pruning treatments.

A complete process of phytoremediation in a real-scale field at least includes soil preparation, plant cultivation, transplantation, field management, and harvesting. The lower the repetitions, the lower the remediation cost. The optimal treatment requires 113 less planting cycles than the control to clean the soil. Agronomic practices conducted in real fields are easier and more economical than other supplementary methods, i.e., chelator addition, endophyte and fungi inoculation, genetic engineering, and magnetic field pretreatment. Moreover, the performance of agronomic practices may not induce associated secondary environmental risks such as pollutant leaching and biological invasion. The optimal root pruning strategy can shorten the duration of phytoremediation by 71.1% without decreasing the phytoremediation efficiency.

4. Conclusions

The impacts of different root pruning strategies with different intensities and directions on the phytoremediation effect of a common ornamental plant, *C. argentea*, were assessed in the current study. Except for the two-side-33% treatment, all the root pruning treatments increased the phytoremediation effect of *C. argentea* through enhancing both the dry weight and Cd content of the species. A pruning treatment with a suitable cutting direction and intensity can increase the transpiration rate of *C. argentea* and consequently translocate more Cd to the aerial parts of the species. Severe root pruning reduced the phytoremediation effect of *C. argentea* because the treatment induced irreversible damage to the root system of the species. However, a suitable cutting direction can alleviate the negative influences caused by the severe pruning to some extent. The results of this study show that root pruning treatment with a suitable strategy can improve the phytoremediation efficiency of *C. argentea*, and this supplementary method can be easily and economically used in real-scale fields. However, the variation in the root morphological characteristics, such as the root length, absorption area, average diameter, and amount of root tips, which can influence the metabolic activity of plants, was not measured in the present study. In addition, the relationships among metal accumulation, oxidative stress, and root pruning were not fully revealed. More precise experiments should be designed to solve these limitations and to validate our results in a real field.

Declarations

Ethical Approval: Not applicable. This work did not describe experiments with animals, human subjects, or human tissue samples.

Consent to Participate: Not applicable. This work did not describe experiments with animals, human subjects, or human tissue samples.

Consent to Publish: The manuscript entitled, "Impacts of root pruning intensity and direction on the phytoremediation of moderately Cd-polluted soil by *Celosia argentea*" is prepared in accordance with the Guide for authors available on the journal's website and it has not been published elsewhere in part or in its entirety. All authors attest to the validity of its contents, and agree to its submission in Environmental Science and Pollution Research.

Authors Contributions: Conceptualization, Youjun Tang, Jie Luo; Data curation, Tian Gan, Jie Luo; Methodology, Jinnuo Song, Dan Chen; Funding acquisition, Jie Luo; Writing – original draft, Youjun Tang, Tian Gan, Jie Luo; Writing – review & editing, Min Cao, Dan Chen. All authors read and approved the final manuscript.

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Figures

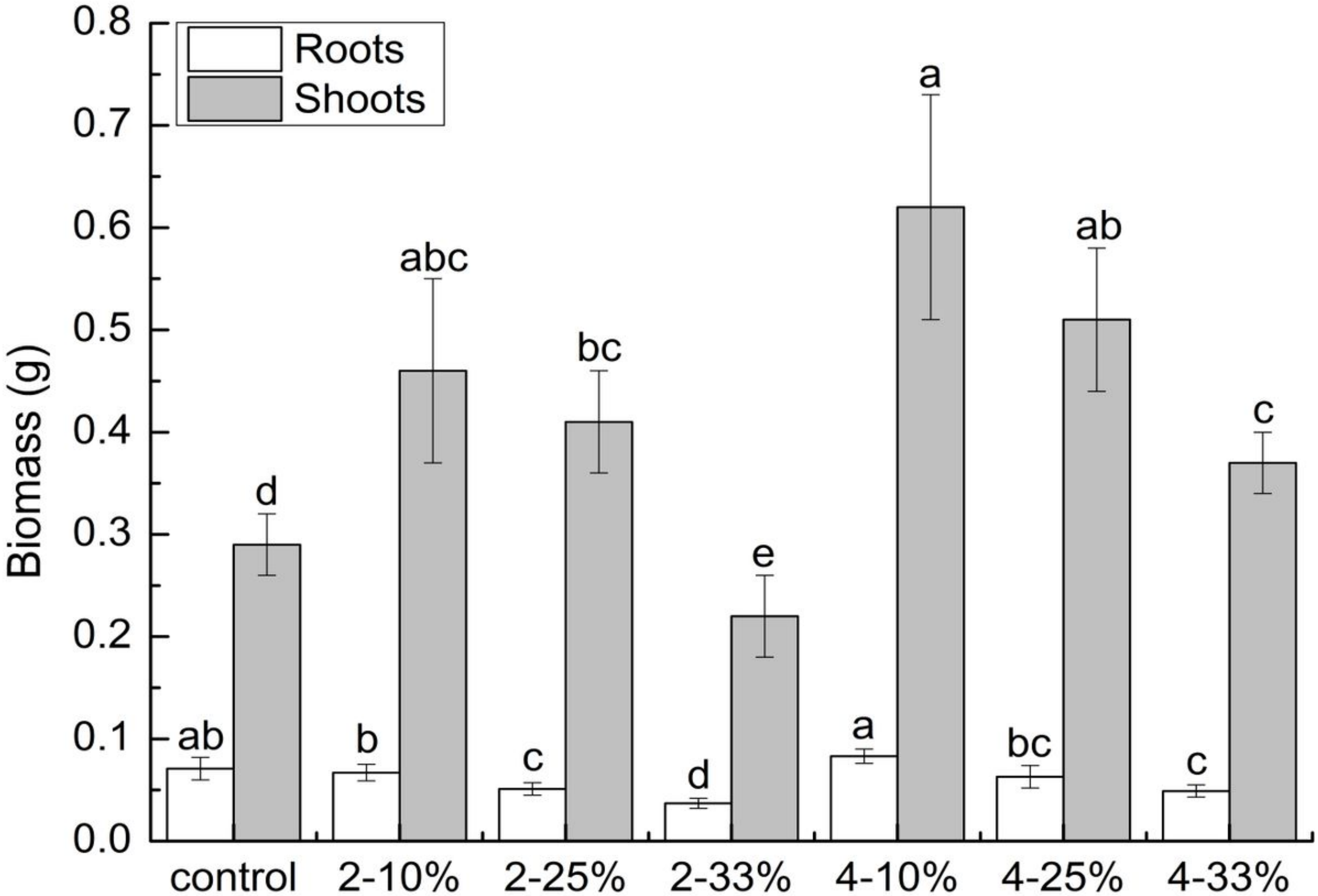

Figure 1

Biomass yield of C. argentea roots and leaves in different treatments Different letters represent significant differences in biomass yield ($p < 0.05$) determined by Fisher's LSD post-hoc tests in different pruning treatments.

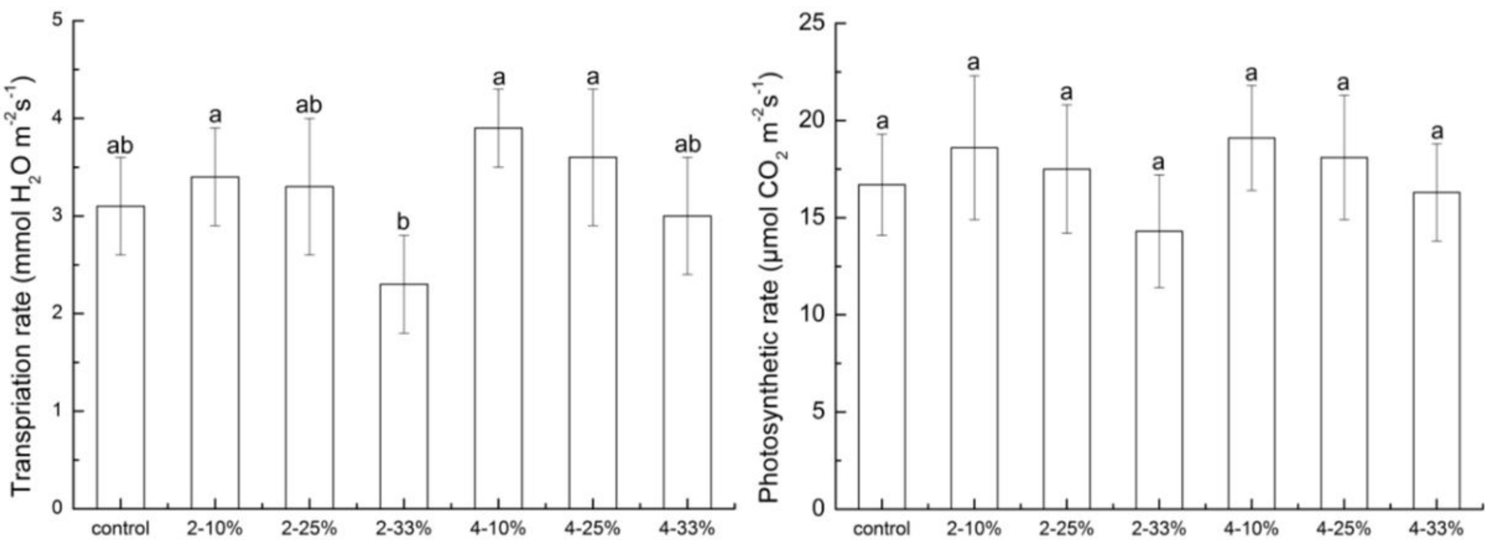

Figure 2

Transpiration rate and photosynthesis efficiency of C. argentea leaves in different root pruning treatments. Different letters represent significant differences in transpiration rate and photosynthesis efficiency ($p < 0.05$) determined by Fisher's LSD post-hoc tests in different pruning treatments.

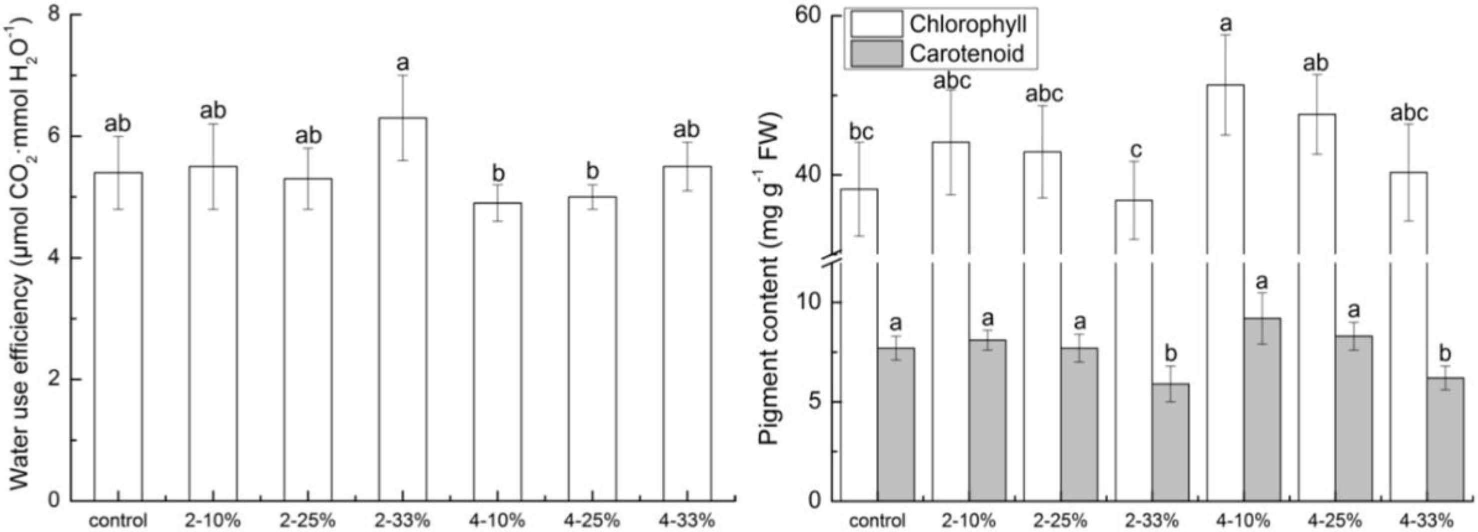

Figure 3

Water use efficiency and pigment content of C. argentea leaves in different root pruning treatments. Different letters represent significant differences in water use efficiency and pigment content ($p < 0.05$) determined by Fisher's LSD post-hoc tests in different pruning treatments.

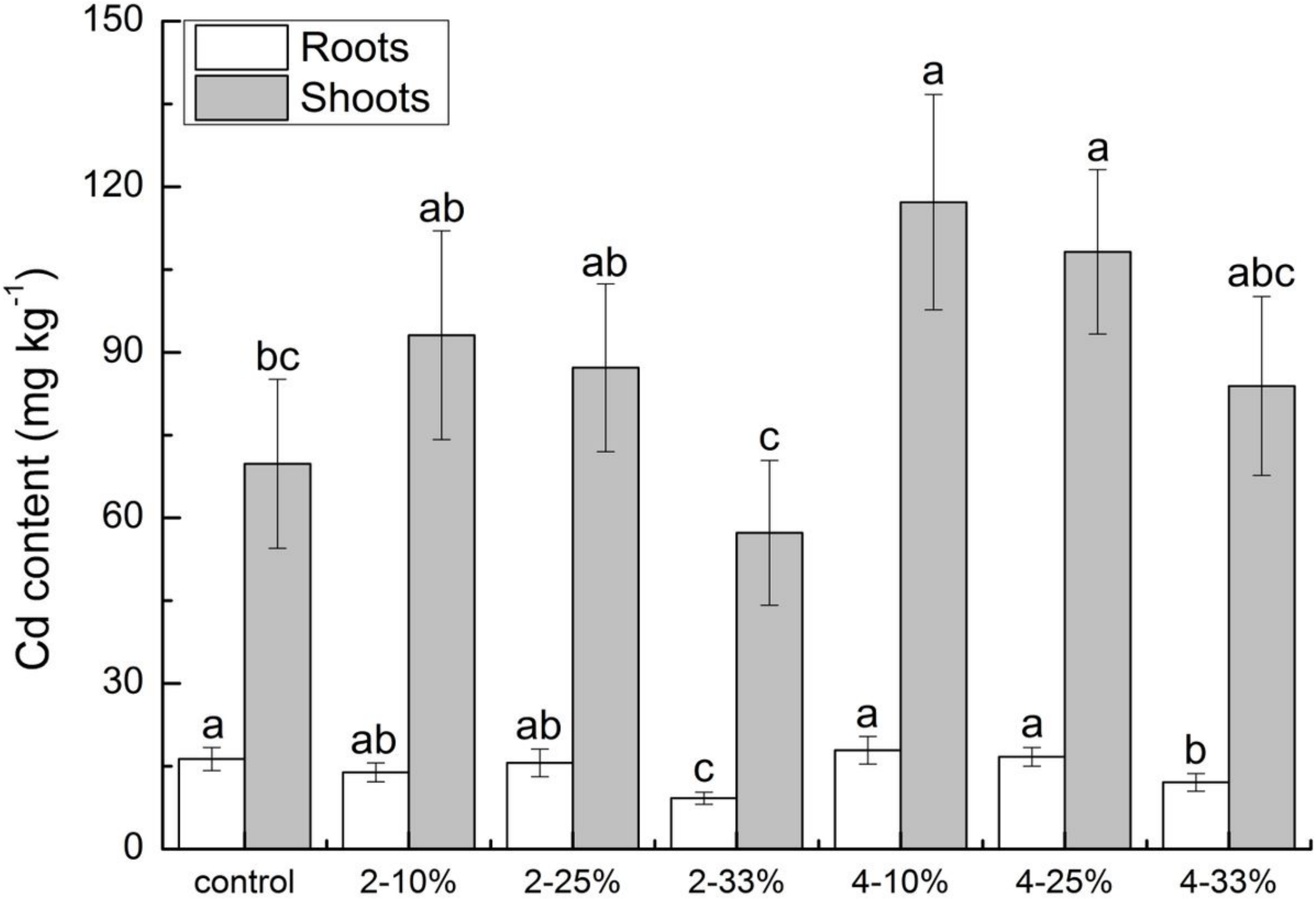

Figure 4

Cd content in C. argentea leaves in different root pruning treatments Different letters represent significant differences in Cd content in plant tissues ($p < 0.05$) determined by Fisher's LSD post-hoc tests in different pruning treatments.

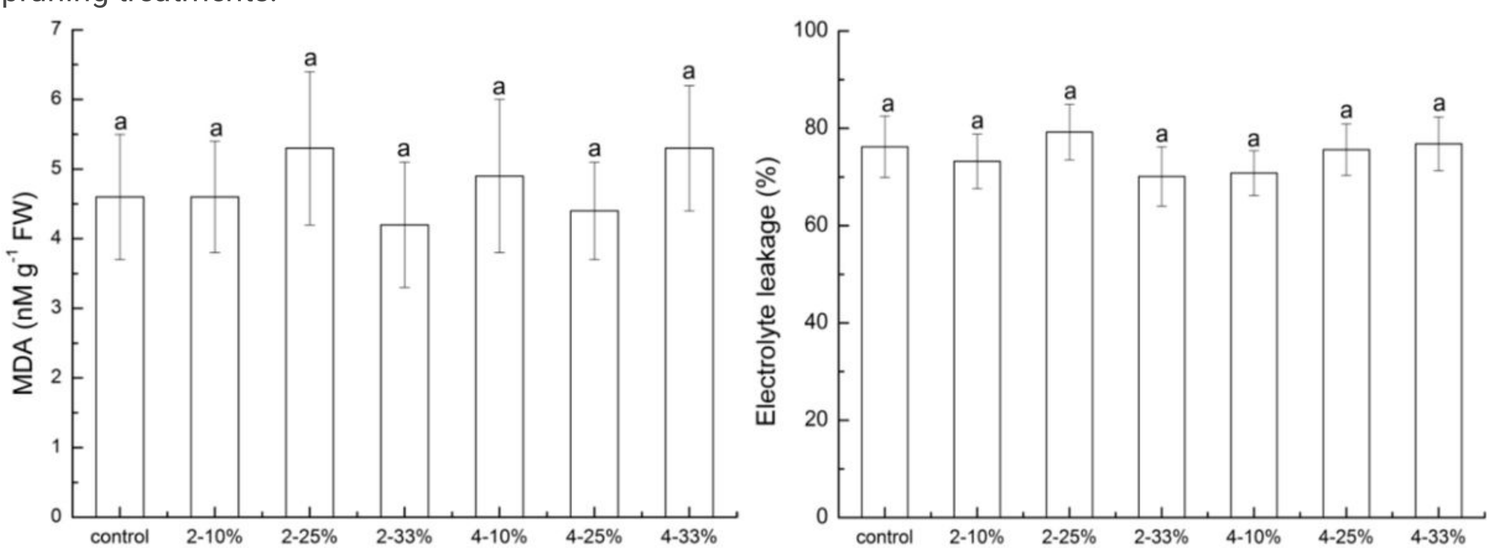

Figure 5

MDA content and electrolyte leakage level in C. argentea leaves in different root pruning treatments Different letters represent significant differences in levels of MDA and electrolyte leakage in plant tissues ($p < 0.05$) determined by Fisher's LSD post-hoc tests in different pruning treatments.