agronomy

MDPI

Article

Heavy Metals Can Affect Plant Morphology and Limit Plant Growth and Photosynthesis Processes

Zhewei Hu, Caiyi Zhao, Qi Li, Yanhong Feng, Xiaoyu Zhang, Yuanyuan Lu, Rongrong Ying *, Aijing Yin and Wenbing Ji

Nanjing Institute of Environmental Science, Ministry of Ecology and Environment, Nanjing 210042, China; fengyanhong@nies.org (Y.F.); zhangxiaoyu@nies.org (X.Z.); jiwenbing@nies.org (W.J.)
* Correspondence: rongrongying1@163.com

Abstract: Soil heavy metal pollution caused by human activities has become one of the most critical environmental issues with a global concern. Phytoremediation is widely used due to its low cost and environmental friendliness. However, the impact of heavy metals on plant growth remains unclear. This study investigated the effects on the growth and photosynthetic activity of *Picris divaricata Vant.* under different cadmium concentrations using a hydroponics cultivation system. The results showed that the growth and photosynthetic processes of *P. divaricata* exhibited a phenomenon of promotion in low Cd concentrations and inhibition in high Cd concentrations. Under a low to medium Cd concentration (≤25 μM), there was no Cd toxicity in terms of plant growth, but high concentrations of Cd inhibited plant growth. The Fe content of leaves gradually increased as the Cd concentration increased; it reached 201.8 mg kg^{-1} in 75 μM Cd. However, there was no significant difference in Mn between the 75 μM Cd treatment and the control ($p > 0.05$). The contents of carotenoid ranged between 3.06 and 3.26 mg/g across the different Cd treatments, showing no significant differences. The treatment with 5–75 μM Cd did not directly affect the photosynthesis of *P. divaricata*. Higher Cd concentrations reduced the stomatal density on the of *P. divaricata* leaves, resulting in stomatal and mesophyll conductance limitations, indirectly affecting *P. divaricata* photosynthesis. These research results provide a reference for evaluating and selecting heavy metal tolerant plants and provide environmentally friendly approaches to remediate heavy metal pollution.

Keywords: heavy metal pollution; *Picris divaricata Vant.*; cadmium; photosynthesis; biomass

Citation: Hu, Z.; Zhao, C.; Li, Q.; Feng, Y.; Zhang, X.; Lu, Y.; Ying, R.; Yin, A.; Ji, W. Heavy Metals Can Affect Plant Morphology and Limit Plant Growth and Photosynthesis Processes. *Agronomy* **2023**, *13*, 2601. https://doi.org/10.3390/agronomy13102601

Academic Editor: Changfeng Ding

Received: 28 July 2023
Revised: 21 September 2023
Accepted: 25 September 2023
Published: 12 October 2023

1. Introduction

With industrial development, human activities have led to an enormous increase in the content of heavy metals in soil [1]. In the early 1990s, the average amount of cadmium (Cd) entering the soil through various sources reached 22,000 tons per year [2]. However, Cd is a non-essential and toxic trace element in plants, and it is easily absorbed by plant roots and transported to the leaves and the fruits [3]. After Cd enters the plant, it may impact plant growth and photosynthesis. The photosynthetic reaction sites of plants are most susceptible to heavy metal ions, and Cd is considered the most effective inhibitor of photosynthesis [4]. Cd has been shown to affect the net photosynthetic rate of many plants, such as tomatoes, rice, corn, and Indian mustard. Although only a small proportion of Cd^{2+} can enter the chloroplast, it can strongly inhibit pigment functions, chloroplast structure, and photosynthesis's light and dark reactions. In addition, Cd disrupts the integrity of the chloroplast membrane system [5], leading to a significant increase in osmolytes. It was reported that low-concentration Cd treatment resulted in a sparse structure of the thylakoids in maize chloroplasts [6]. As Cd concentration increased, the thylakoids and grana disappeared, and the chloroplasts became spherical and shrunk. However, studies have also shown that increasing the concentration of Mn ions in the culture medium under Cd stress can partially restore the damaged structure of chloroplasts [7]. The inhibition

of chlorophyll synthesis by Cd is considered the initial mechanism by which Cd affects photosynthesis [8]. The total chlorophyll content of *P. australis* decreased by 30% and 60% compared to the control, under 50 and 100 μM Cd, respectively [9]. In addition to reducing the total chlorophyll content, Cd directly affects the photon capture by chlorophyll molecules, leading to the reduction and eventual shutdown of photosynthesis. Certain studies have demonstrated that Cd can replace the carotenoid binding in PSII [10], thus affecting carotenoids that play an important role in plant absorption of light energy and chlorophyll protection.

Presently, research on plants that have metal hyperaccumulator properties mainly focuses on heavy metal absorption, transportation, storage, and detoxification mechanisms by different super-enriched plants at the physiological and molecular levels [11,12]. Moreover, the inhibitory effect of heavy metal stress on photosynthesis has been extensively demonstrated [13]. However, Cd can also promote growth and increase photosynthetic rates in certain species. *Picris divaricata Vant.* is a Zn/Cd polymetallic-tolerant and hyperaccumulator plant that is able to grow in Lanping County, Yunnan Province, where China's largest lead and zinc mine is located. It has high heavy metal tolerance, enrichment ability, and relatively large biomass [14]. In this study, we compared and analyzed the growth and photosynthetic response of *P. divaricata* under Cd stress using a hydroponics-based cultivation system and investigated the mechanisms underlying photosynthesis maintenance under these conditions. The research results provide a basis for heavy metal phytoextraction approaches using hyperaccumulator plants and insights into the physiological adaptation mechanisms of plants in extreme environments.

2. Materials and Methods

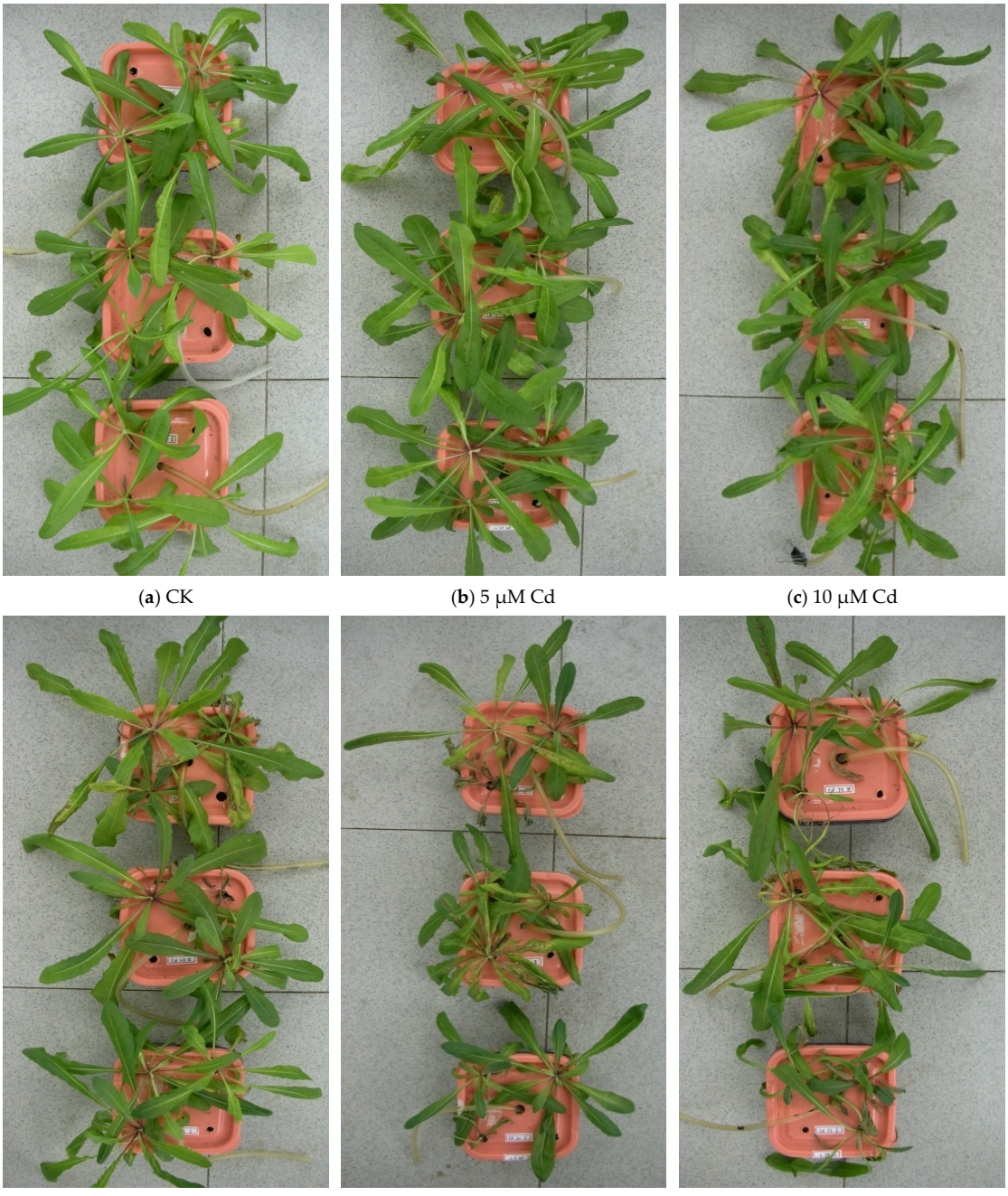
2.1. Experimental Design

Seedlings of *P. divaricata* were collected from the Jinding lead–zinc mining area in Lanping County, Nujiang Prefecture, Yunnan Province. Seedlings with similar growth were transferred to plastic pots containing 500 mL of 20% Hoagland nutrient solution for pre-cultivation. After 14 days of plant pre-cultivation, five Cd concentrations were applied in the above nutrient solution, namely 0 (CK), 5, 10, 25, 50, and 75 μM (added as $CdCl_2 \cdot 2.5\ H_2O$). Each treatment has 3 replicates, with three plants per replicate (Figure 1). The nutrient solution was changed every 3 days, and continuous aeration was maintained. The greenhouse growth conditions were day/night temperature of 25/18 °C, relative humidity of 70–75%, and light intensity of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with 14 h of light and 10 h of darkness per day.

2.2. Biomass Measurements and Root Growth Analyses of P. divaricata under Cd Stress

After 14 days of plant pre-cultivation, the entire *P. divaricata* seedlings were removed from the medium, and the plant roots were soaked in 5 mM $CaCl_2$ for 5 min to remove heavy metal ions adsorbed on the surface of the roots. The samples were collected from 3 plants in each pot and pooled for the analyses. Then, the samples were washed with deionized water, and, after separating the above-ground parts and the roots, their fresh weight was measured separately. The plants were then dried at 105 °C for 30 min and at 75 °C for 48 h to a constant weight, and then the dry weight was measured.

The *P. divaricata* root morphological characteristics were measured using the WinRHIZO root analysis system manufactured by Regent Instruments in Canada. The root sample was placed in a 30 cm × 40 cm transparent glass tray, and water was added, forming a 3–4 mm layer to disperse the root system fully. The root system was scanned with a double-sided light source and analyzed by dedicated digital software (WinRHIZO 2008a). Morphological indicators such as root length, root surface area, root volume, and average root diameter were obtained.



(**a**) CK (**b**) 5 μM Cd (**c**) 10 μM Cd

(**d**) 25 μM Cd (**e**) 50 μM Cd (**f**) 75 μM Cd

Figure 1. Growth of *P. divaricata* in different levels of Cd. The (**a**, **b**, **c**, **d**, **e**, and **f**) correspond to the 0, 1, 5, 10, 25, 50, and 75 μM Cd treatments, respectively.

2.3. Determination of Photosynthetic Parameters

A total of 1~2 fully expanded leaves were selected from the plants in each pot for the determination of photosynthetic parameters with a photosynthesis meter (LI-6400XT, LI-COR, Lincoln, NE, USA). Using a combined red and blue light source leaf chamber, the net photosynthetic rate (AN), stomatal conductance (gs), intercellular CO_2 concentration (Ci), and other gas exchange parameters were measured under different light density flux (PPFD) settings (2000, 1800, 1500, 1200, 1000, 800, 700, 600, 500, 400, 200, 100, 50, 20, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The measurement conditions were leaf temperature of 25 °C, CO_2 concentration 380 ± 5 μmol/mol, and airflow rate of 500 $\mu mol \cdot s^{-1}$.

2.4. Determination of Chlorophyll Fluorescence Parameters

The chlorophyll of leaves was determined by 80% acetone extraction spectrophotometry. Remove the weighed leaves from the refrigerator at −80 °C and place them in a mortar. Then add a small amount of calcium carbonate and 2–3 mL of 80% acetone to grind into a homogenate. Filter the extract into a 25 mL colorimetric tube wrapped in tin foil, rinse the mortar, grinding rod, and residue several times with a small amount of 80% acetone, pour into a funnel, and filter together. Dilute to 25 ml with 80% acetone and shake well. Measure the extinction of the extraction solution at wavelengths 663, 646, and 470 nm using 80% acetone as the blank.

The chlorophyll fluorescence parameters of the plants were measured with the LI-6400XT photosynthesis meter. The measured leaf position was the same as those selected for photosynthetic gas exchange parameter measurements. The specific steps followed were:

(1) After dark adaptation of the selected leaf for 30 min, a light with a wavelength of 630 nm was flashed on the leaf to measure the initial fluorescence (F_o). Then, light with a wavelength of 630 nm and an intensity of approximately 6000 μmol·m^{-2}·s^{-1} was used to saturate the reaction centers of photosystem II (Duration 0.8 s, Intensity 7, Blue 10%, Modulation 20 KHz, Filter 50 KHz) to determine the maximum fluorescence (F_m).

(2) The leaves sampled for the measurements were exposed to continuous photochemical active light (constant light in a greenhouse with a light intensity of 80 μmol·m^{-2}·s^{-1} for 30 min at steady-state fluorescence (F_s)). Then, the light intensity was increased to 6000 μmol·m^{-2}·s^{-1} to measure the maximum fluorescence (F_m') after adaptation under saturated light irradiation. Then, the photochemically active light was stopped, and far-red light with a wavelength of 740 nm was applied (Duration 6 s, Intensity 8, Modulation 0.25 KHz, Filter 1 KHz) to measure the minimal fluorescence, F_o'.

(3) Other chlorophyll fluorescence parameters of PSII, such as the maximum photochemical efficiency (F_v/F_m), actual photochemical efficiency of PSII (Φ_{PSII}), the photochemical quenching (q_P), non-photochemical quenching (NPQ), and electron transfer rate (ETR) of PSII were calculated according to the following formulas:

$$F_v/F_m = (F_m - F_o)/F_m \quad (1)$$

$$F_v'/F_m' = (F_m' - F_o')/F_m' \quad (2)$$

$$\Phi_{PSII} = (F_m' - F_s')/F_m' \quad (3)$$

$$q_P = (F_m - F_s)/(F_m' - F_o') \quad (4)$$

$$NPQ = (F_m - F_m')/F_m' = F_m/F_m' - 1 \quad (5)$$

$$ETR = \Phi_{PSII} \times PPFD \times 0.5 \times 0.84 \quad (6)$$

2.5. Stomatal Pore Observation by Scanning Electron Microscopy

Samples with a width of 2–3 mm were excised from the middle transverse section of a plant leaf. The samples were washed with deionized water and immersed in a 1% Na_2S aqueous solution. They were then subjected to vacuum infiltration for 5 min to appropriately soak with the solution. After soaking at room temperature for another 25 min, they were rinsed twice with 0.1 mmol L^{-1} phosphate buffer (pH 7.2). A single-sided blade pre-cooled with liquid nitrogen was used to cut the sample and obtain an undisturbed transverse section, which was then quickly transferred to a freeze-drying machine (JFD-320, JEOL, Tokyo, Japan) for drying. The dried samples were mounted on the plastic platform, sprayed with carbon, and observed under the scanning electron

microscope (SEM, Model Quanta 400, FEI, Eindhoven, The Netherlands) under thermal field emission.

2.6. Fluorescence CO_2 Response Curve

The chlorophyll fluorescence CO_2 response curves of plants were measured by the LI-6400XT photosynthesis analyzer and a fluorescence leaf chamber (LI-COR, Lincoln, NE, USA). Please refer to annex 1 for specific measurement methods (File S1).

2.7. Statistical Analysis

Statistical analyses were performed using SPSS 16.0 (IBM, New York, NY, USA). Descriptive statistics were used to calculate the means and standard deviations for each set of replicates. One-way ANOVA with LSD-t test was performed to test the differences in the biomass, root growth, and metals of different Cd treatments. A one-way ANOVA was also used to analyze the effect of Cd treatments on PSII, chlorophyll a, chlorophyll b, and carotenoid contents. All data were tested for normal distribution and homogeneity of variance.

3. Results

3.1. Effects of Cd Stress on the Biomass, Root Growth, and Metals Content of P. divaricata

Low to medium Cd concentrations had a beneficial effect on the total biomass and root growth of *P. divaricata*, which were gradually reduced as the Cd concentration increased to high levels (Figure 2). The FW of *P. divaricata* was the highest in the 5 μM Cd treatment, 1.27-fold higher than that of the CK, but gradually decreased with the increase in Cd concentration. There was no significant difference between the 10 and 25 μM Cd treatments ($p > 0.05$). The FWs of *P. divaricata* under 5, 10, and 25 μM Cd were 2-, 1.33-, and 1.24-fold higher than the CK, respectively. The FW of *P. divaricata* under the 50 and 75 μM Cd treatments was significantly lower compared to 25 μM Cd. The DW of *P. divaricate* followed the same patterns as the FW under the different Cd treatments. However, the DW of *P. divaricata* exhibited a more pronounced Cd concentration response. In the 5–25 μM Cd treatment, the DW was 1.85–2.19-fold higher than that of the CK. The length, average diameter, total surface area, and total volume of the roots increased with the increase in Cd concentration (Table 1). In the 5–25 μM Cd treatments, they were significantly higher than those of CK ($p < 0.05$). However, they decreased significantly in the 50 and 75 μM Cd treatments ($p < 0.05$).

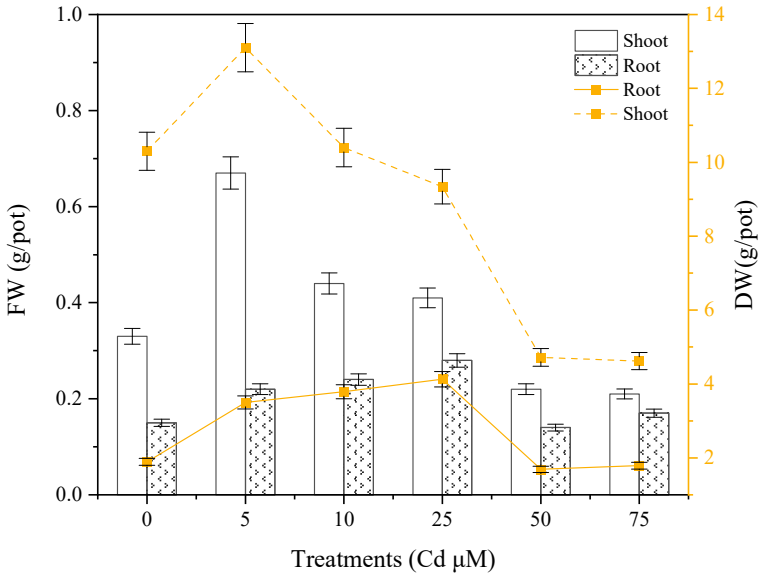


Figure 2. Biomass of *P. divaricata* under different Cd concentrations.

Table 1. Root system growth of *P. divaricata* in different Cd treatments. Values represent the mean and the variance of three independent replicates. Different letters in the same column indicate values which significantly differ according to one-way ANOVA at $p < 0.05$.

Treatment (Cd μM)	Root Length (cm)	Average Root Diameter (mm)	Total Surface Area of Root System (cm^2)	Total Volume of Root System (cm^3)
0	1681.8 ± 154.3 ^{b}	0.30 ± 0.03 ^{a}	156.46 ± 5.80 ^{b}	1.17 ± 0.17 ^{c}
5	2555.4 ± 356.8 ^{a}	0.31 ± 0.02 ^{a}	252.92 ± 52.91 ^{a}	2.00 ± 0.56 ^{ab}
10	2429.2 ± 139.2 ^{a}	0.35 ± 0.02 ^{a}	262.90 ± 23.20 ^{a}	2.27 ± 0.33 ^{a}
25	2741.0 ± 196.8 ^{a}	0.34 ± 0.01 ^{a}	288.28 ± 10.03 ^{a}	2.43 ± 0.07 ^{a}
50	1501.3 ± 385.8 ^{b}	0.33 ± 0.00 ^{a}	155.79 ± 38.02 ^{b}	1.27 ± 0.30 ^{bc}
75	1678.0 ± 473.5 ^{b}	0.31 ± 0.03 ^{a}	165.18 ± 57.93 ^{b}	1.30 ± 0.55 ^{bc}

There were no significant changes in the Mg, Ca, and Cu in leaves of *P. divaricata* under Cd treatment (Table 2). The Fe content of leaves were 144.1 and 153.9 mg kg^{-1} in the 5–10 μM Cd treatment, showing a significant difference from the control ($p < 0.05$). However, the Fe content of leaves gradually increased as the Cd concentration increased, and it reached 201.8 mg kg^{-1} in 75 μM Cd. Similarly, the content of Mn in the 5–25 μM Cd treatment was lower than the control; they were 60.4%, 78.2%, and 52.3% of the control, respectively. There was no significant difference in Mn between 75 μM Cd treatment and control ($p > 0.05$).

Table 2. Metals contents in *P. divaricata*. Values represent the mean and the variance of three independent replicates. Different letters in the same column indicate values which significantly differ according to one-way ANOVA at $p < 0.05$.

Treatment (Cd μM)	Fe (mg kg^{-1})	Mg (g kg^{-1})	Mn (mg kg^{-1})	Ca (g kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
0	227.4 ± 52.8 ^{a}	6.58 ± 0.47 ^{a}	234.5 ± 123.1 ^{a}	22.9 ± 2.59 ^{a}	3.11 ± 0.50 ^{a}	37.2 ± 10.2 ^{a}
5	144.1 ± 13.0 ^{b}	6.57 ± 0.62 ^{a}	307.7 ± 164.9 ^{a}	22.0 ± 1.83 ^{a}	2.09 ± 1.62 ^{a}	30.8 ± 2.57 ^{a}
10	153.9 ± 45.8 ^{b}	5.77 ± 0.44 ^{a}	222.1 ± 45.6 ^{a}	20.1 ± 0.33 ^{a}	3.27 ± 0.15 ^{a}	35.0 ± 7.04 ^{a}
25	164.7 ± 14.6 ^{ab}	5.28 ± 1.12 ^{a}	259.1 ± 76.1 ^{a}	17.8 ± 0.73 ^{a}	2.95 ± 1.14 ^{a}	22.9 ± 8.50 ^{a}
50	173.8 ± 40.6 ^{ab}	5.06 ± 0.26 ^{a}	449.3 ± 174.1 ^{a}	20.2 ± 4.62 ^{a}	4.62 ± 0.85 ^{a}	22.1 ± 14.3 ^{a}
75	201.8 ± 21.8 ^{ab}	5.81 ± 1.47 ^{a}	281.7 ± 132.1 ^{a}	20.4 ± 5.66 ^{a}	4.40 ± 2.57 ^{a}	26.7 ± 11.4 ^{a}

3.2. Effects of Cadmium on Leaf Pigment Content of P. divaricata

The chlorophyll a, chlorophyll b, and carotenoid contents in the leaves of *P. divaricata* were increased as the Cd treatment concentration increased, and then they decreased under higher Cd concentrations (Figure 3). They were significantly higher in the 25 μM Cd concentration compared to the CK ($p < 0.05$). Whereas, under 50 and 75 μM of Cd, they were significantly lower compared to CK ($p > 0.05$). There was no significant difference in chlorophyll a/b between the 5 and 10 μM Cd treatments ($p > 0.05$). Similarly, there were also no differences in chlorophyll a/b between the 50 and 75 μM Cd treatments ($p > 0.05$). The contents of carotenoid ranged between 3.06 and 3.26 mg/g across the different Cd treatments, showing no significant differences ($p > 0.05$).

3.3. Distribution Pattern of Blade Cross-Section

Compared to the control, the 5 and 10 μM Cd treatments resulted in a thickening of the *P. divaricata* leaf cross-section, with thicknesses of 147.3 and 142.0 μM, respectively (Figures 4 and 5). As the concentration of Cd applied increased, the thickness of cross-section of the leaves became compact, and the intercellular spaces became smaller. The lowest value was observed at 75 μM Cd at 103.3 μm, which was 22.3% lower than that of CK, at 133.0 μM.

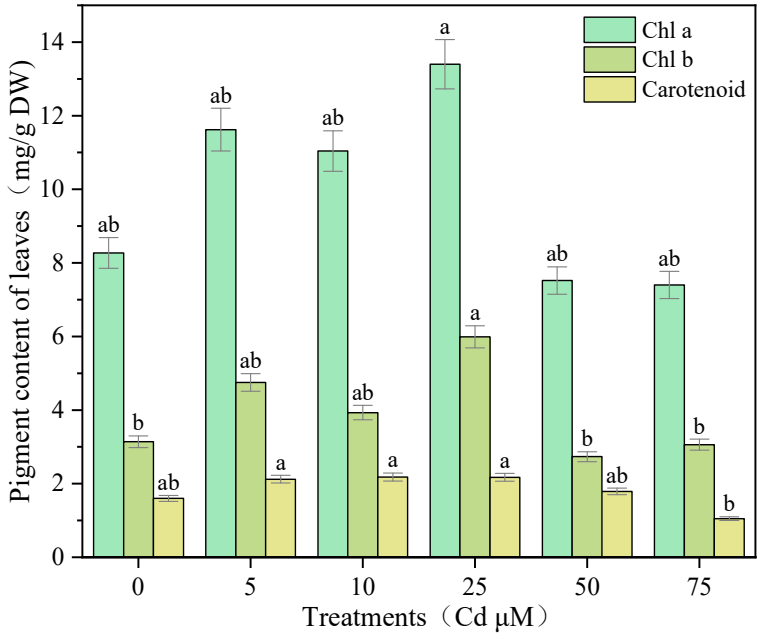
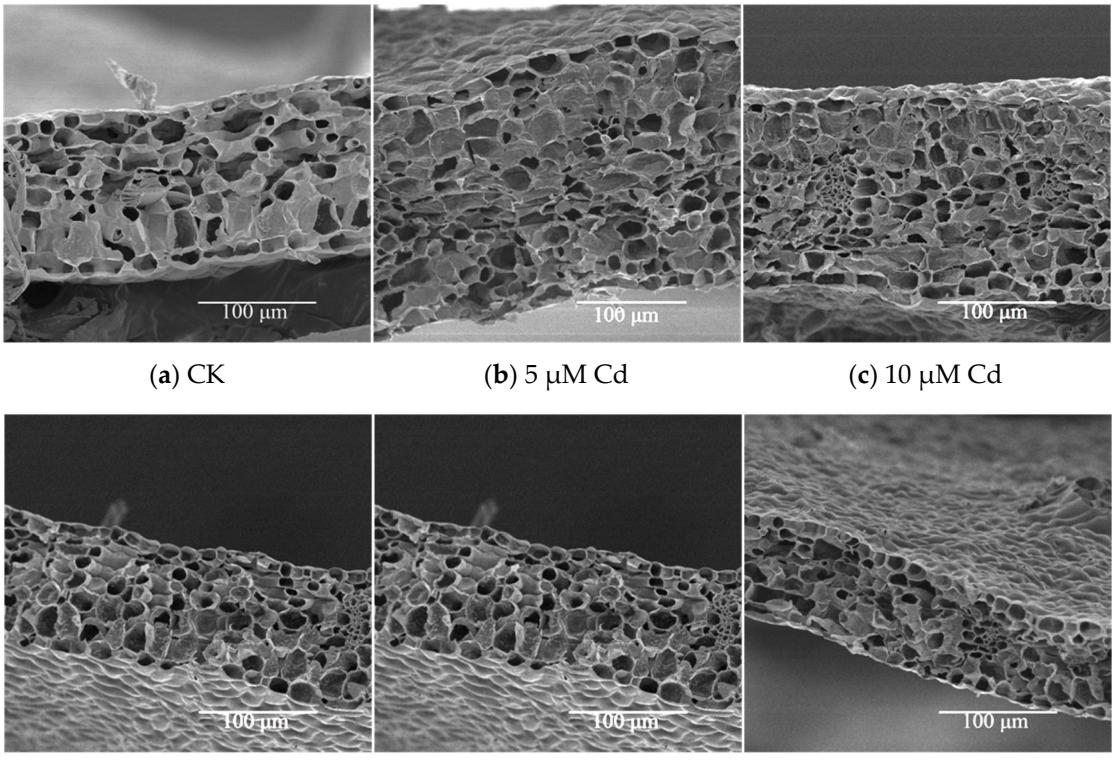


Figure 3. The effect of different cadmium concentrations on the leaf pigment content of *P. divaricata*. Significant differences ($p < 0.05$) among different Cd treatments are indicated with different lower-case letters.



(**a**) CK (**b**) 5 μM Cd (**c**) 10 μM Cd

(**d**) 25 μM Cd (**e**) 50 μM Cd (**f**) 75 μM Cd

Figure 4. Scanning electron microscopy micrographs of the upper and lower epidermis and stomata of control and 75 μM Cd treated *P. divaricata* (magnification ×250 and 3000). (**a**) The upper epidermis of control (magnification ×250); (**b**) the lower epidermis of control (magnification ×250); (**c**) the stomata in the lower epidermis of control (magnification ×250); (**d**) the upper epidermis of 75 μM Cd (magnification ×250); (**e**) the lower epidermis of 75 μM Cd (magnification ×3000); (**f**) stomata in lower epidermis of 75 μM Cd (magnification ×3000). White arrows, stoma. Bars (**a**,**b**,**d**,**e**) = 100 μm.

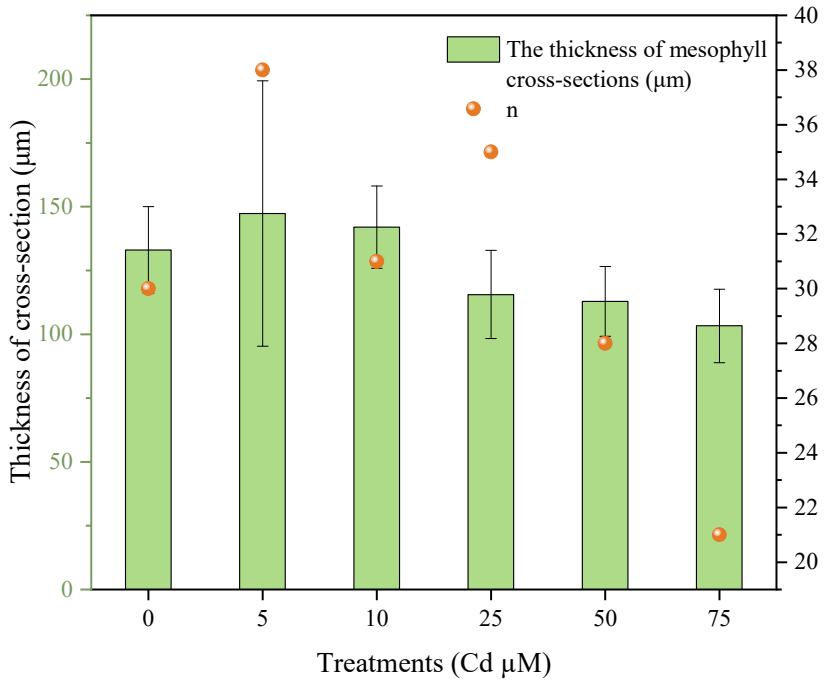


Figure 5. The thickness of mesophyll cross-sections of leaves in control and Cd-treated *P. divaricata plants.* The n is the number of samples.

3.4. Effects of Cadmium on the Photochemical Reactions of P. divaricata

3.4.1. Effects of Cadmium on the Chlorophyll Fluorescence Parameters of *P. divaricata*

The F_v/F_m increased with the increase in Cd treatment concentrations (Figure 6). The maximum photochemical quantum yield (F_v/F_m) remained around 0.8 across all treatments. The F_m', and F_v' increased with increasing Cd concentration up to 10 μM Cd, while they decreased under 25–75 μM Cd, showing an inverted "U"-shaped trend under Cd treatment. Compared with the control, there were no significant differences in F_v'/F_m' between each Cd treatment and the CK.

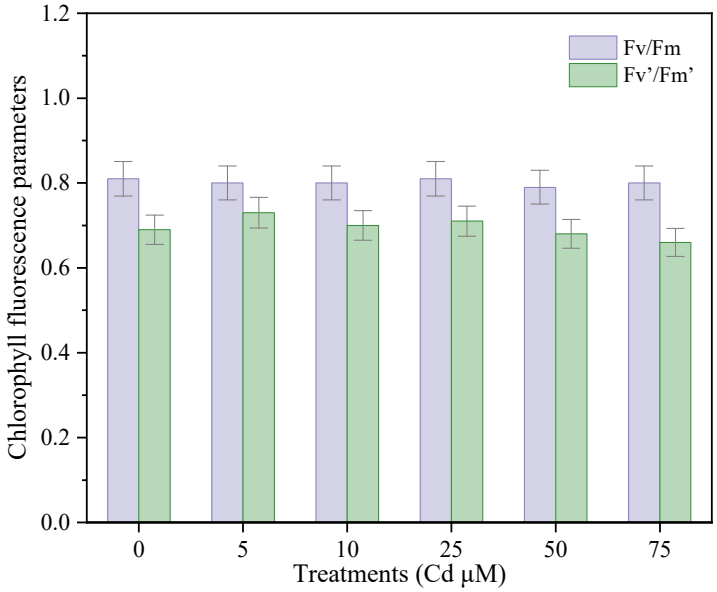


Figure 6. Chlorophyll fluorescence parameters (F_v/F_m, F_v'/F_m', Φ_{PSII}) in *P. divaricata* under different Cd concentrations.

The Φ_{PSII} of the plants in the Cd treatments ranged between 0.5 and 0.6, and there were no significant differences between each Cd treatment and the CK (Figure 7). No significant differences could be identified in the ETR between the Cd treatments and the CK. Similarly, no significant differences were observed in q_P between the Cd treatments and CK, except for the 10 µM Cd concentration. The NPQ was also unaffected by Cd, showing no significant differences among the Cd treatments.

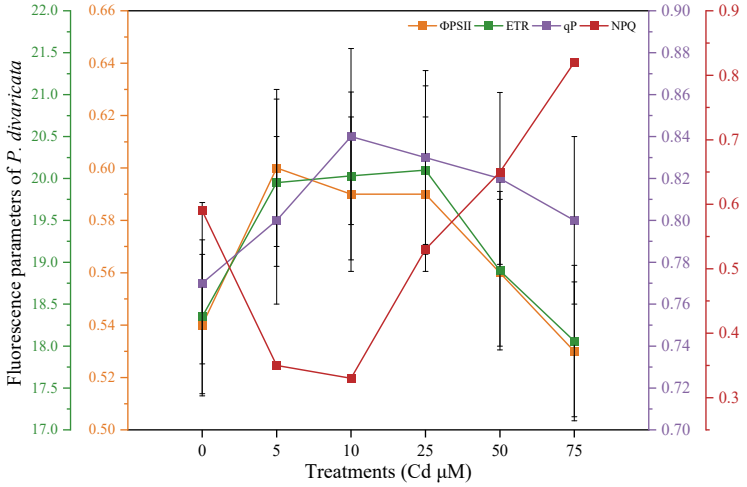


Figure 7. Effects of cadmium on fluorescence parameters (Φ_{PSII}, ETR, q_P, NPQ) of *P. divaricata* under different Cd concentrations.

3.4.2. Effects of Cadmium on the Excited-State Energy Distribution and the Photoreaction Quantum Yield in *P. divaricata*

The P used for the photochemical reactions did not exhibit significant changes under different Cd treatments, and the p value remained between 0.53 and 0.59 (Figure 8). The heat dissipation D of the antenna pigment–protein complex showed no significant difference between Cd treatments and CK. However, the D values in the leaves under 75 and 5 µM Cd were 0.34 and 0.27, showing significant differences ($p < 0.05$). The excess excitation energy Ex absorbed by PSII was the lowest at 10 and 50 µM Cd, significantly different compared to CK. On the other hand, there was no difference in Ex between the 5 and 75 µM Cd treatments.

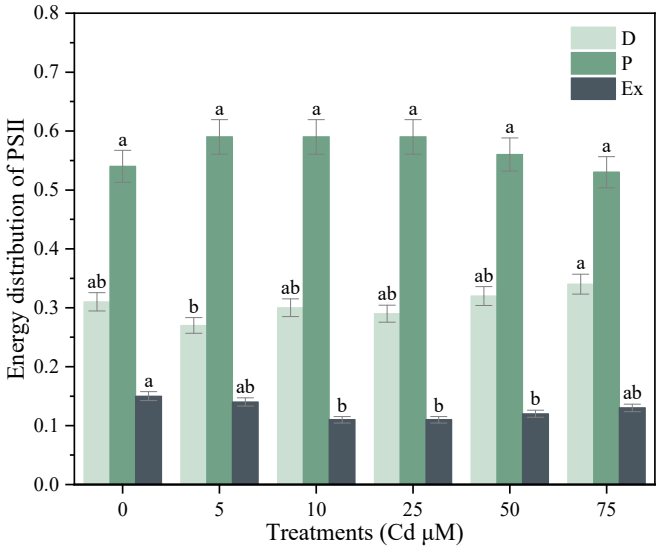


Figure 8. Energy distribution of PSII in *P. divaricata* under different Cd concentrations. Significant differences ($p < 0.05$) among different Cd treatments are indicated with different lower-case letters.

3.5. Effects of Cadmium on the Photosynthetic Parameters of P. divaricata

3.5.1. Effects of Cadmium on Photosynthetic Parameters and the Light Response Curve of *P. divaricata*

The net photosynthetic rate (Pn) of *P. divaricata* under different Cd treatments showed a consistent trend. It initially increased with the increase in photon flux density (PPFD) and gradually decreased after being suppressed by light (Figure 9). The maximum Pn value decreased with increased Cd concentration. The maximum Pn value was reached under 5 μM Cd at 7.00 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and was significantly higher compared to that of CK at 6.07 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The maximum Pn values under the 10, 25, 50, and 75 μM Cd treatments decreased significantly with the increase in Cd concentration, reaching 5.99, 5.13, 4.93, and 3.86 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. This indicates that the photosynthesis of *P. divaricata* was inhibited as the Cd concentration increased.

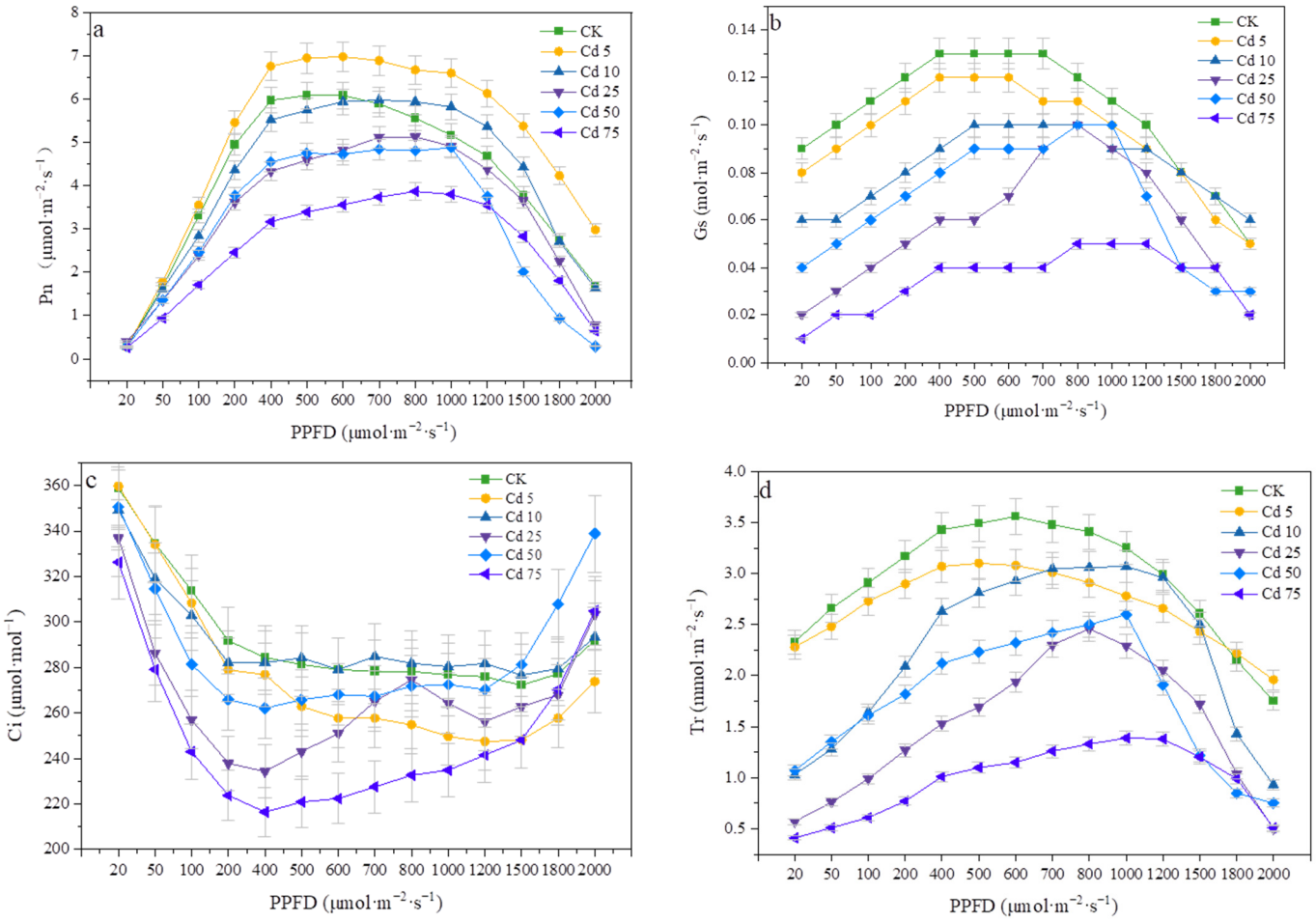


Figure 9. Gas exchange parameters of *P. divaricata* leaves under different Cd treatments and different photosynthetic photon flux densities (PPFD). (**a**, **b**, **c**, and **d**) correspond to the net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (T_r), respectively.

Similar to Pn, the stomatal conductance (g_s) and transpiration rate (T_r) showed a trend of initially increasing and then decreasing with the increase in PPFD. There was an inhibition of gs and T_r at 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The trend in the intercellular CO_2 concentration (Ci) with increasing PPFD was opposite to that of AN, g_s, and T_r, initially decreasing and then gradually increasing.

3.5.2. Analysis of the Factors Affected by Cadmium That Influence the Photosynthesis of *P. divaricata*

Using the fluorescence CO_2 response curve, the corresponding limiting values of the photosynthetic limiting factors of *P. divaricata* were calculated under different Cd concentrations (Table 3). The 5 μM Cd concentration was set as the reference, and, as a result, its various limit values were all 0. Because diffusion limitation ($D_L = S_L + MC_L$) accounted for the majority of the total limitation value (T_L) (73–96%), both D_L and T_L increased with the Cd treatment concentration increase. Their values and trends were similar to that of Pn. There were no significant differences between 10 μM Cd and the CK. The biochemical limitation (B_L) accounted for a small percentage of the total photosynthesis limitation, at only 4–27%. The mesophyll conductance limitation (MC_L) accounted for most of the non-stomatal limitation factors (N_L; $N_L = MC_L + B_L$).

Table 3. Photosynthesis limitation parameters (%) of *P. divaricata* under different Cd concentrations.

Treatment (Cd μM)	S_L	MC_L	B_L	N_L	D_L	T_L
0	9.0 ± 1.3 ^{c}	4.2 ± 3.3 ^{c}	5.1 ± 1.2 ^{a}	9.7 ± 2.7 ^{c}	13.6 ± 2.3 ^{c}	18.7 ± 2.4 ^{c}
10	12.1 ± 0.3 ^{c}	2.6 ± 0.4 ^{c}	0.6 ± 0.0 ^{c}	3.1 ± 0.4 ^{c}	14.7 ± 0.1 ^{c}	15.3 ± 0.2 ^{c}
25	17.0 ± 1.9 ^{b}	11.7 ± 2.1 ^{b}	0.5 ± 0.2 ^{c}	12.2 ± 2.0 ^{c}	28.7 ± 1.8 ^{b}	39.2 ± 2.0 ^{b}
50	23.0 ± 1.5 ^{b}	18.5 ± 3.0 ^{b}	2.9 ± 0.8 ^{b}	21.4 ± 4.0 ^{b}	41.5 ± 3.3 ^{b}	44.4 ± 3.7 ^{b}
75	28.8 ± 2.0 ^{a}	36.8 ± 7.2 ^{a}	3.1 ± 0.4 ^{b}	40.0 ± 7.2 ^{a}	65.7 ± 6.2 ^{a}	68.8 ± 6.3 ^{a}

The 5 μM Cd treatment was used as a reference, for which all limitations were set to 0. SL, stomatal limitations; MCL, mesophyll conductance limitations; B_L, biochemical limitations; N_L, non-stomatal limitations; D_L, diffusional limitations; T_L, total limitations. Data are the mean ± SE of three replicates. Different letters in the same column indicate values that are significantly different according to one-way ANOVA at $p < 0.05$.

4. Discussion

4.1. Cadmium Resulted in Growth Promotion in Low Concentrations and Growth Inhibition in High Concentrations in P. divaricata

The root system is an important plant organ that absorbs mineral nutrients and water. Under abiotic stress conditions, the plant root system is the first organ to be under stress. Plant roots have certain developmental plasticity [15], and plants can alter the morphology and distribution of their roots to adapt to environmental stress [16]. Cadmium exerts a strong toxicity to plants, and its toxicity symptoms are first expressed in plant roots [17]. Root length is an indicator of the potential for plants to absorb nutrients or water [18], while root area is an indicator of root absorption efficiency [19]. The heavy metal absorption efficiency by plant roots directly affects their metal enrichment ability. The metal hyperaccumulator model plant *T. caerulescens*, when compared to non-metal hyperaccumulator plants such as *T. arvense*, has more lateral roots, denser and longer root hairs (>2.1 mm), and longer effective root length [20], which enhance the ability of *T. caerulescens* to absorb and utilize metals faster and more efficiently for normal growth. Similarly, compared with the CK, the root length and the root surface area of the metal hyperaccumulator *Sedum alfredii* significantly increased under Cd treatment (≥50 μM). However, Cd did not affect root diameter and volume [21]. The study showed that low to medium concentrations of Cd (5, 10, 25 μM) promoted root growth of *P. divaricata*. However, at higher concentrations (50 and 75 μM), the root system growth was inhibited to a certain extent, indicating that *P. divaricata*, as a metal hyperaccumulator plant, has strong adaptability to Cd.

The apparent growth status and biomass of *P. divaricata* also showed that Cd had "growth promotion in low concentrations and growth inhibition in high concentrations" effects in *P. divaricata*, consistent with previous experimental results [22]. In toxicology, the phenomenon where chemicals exhibit beneficial effects on organisms at low doses (such as stimulating growth and development) but exhibit negative effects at high doses is known as hormesis [23]. However, in general, the stimulus–response amplitude and the stimulus–dose range for a hormesis response are very small [24]. The maximum stimulus–response amplitude generally does not exceed twice that of the control and is usually only 30% to 60% higher

than the control; the dose range of the stimulus is generally around one percent to one-fifth of the no-observed-adverse-effect level [25]. In this study, *P. divaricata* exhibited toxicity symptoms under the 50 μM Cd treatment; however, 5–25 μM Cd stimulated growth, which is higher than the normal stimulus dose range to induce hormesis. Moreover, the fresh and dry weights of the below-ground parts of *P. divaricata* treated with Cd were 2.2- and 2.6-fold higher than the control, respectively, and the dry weight of the above-ground part was 1.5-fold higher than the control. Moreover, the root length and root surface area under Cd treatment were up to 63% and 84% higher than the control, respectively. The maximum stimulus response amplitude in *P. divaricata* was also greater than what is commonly observed in hormesis responses. The effect of Cd resulting in growth promotion in low concentrations and growth inhibition in high concentration in *P. divaricata* is likely not a hormesis effect as described in toxicology, and an overcompensation mechanism cannot explain the growth-promoting effects of Cd in *P. divaricata*. This phenomenon observed in metal hyperaccumulator plants may be related to their long-term biological evolution in environments with extremely heavy metal concentrations. A certain concentration of heavy metals may have become a necessary condition for the normal growth of metal hyperaccumulator plants, and this hypothesis also needs to be further investigated in the future.

4.2. Effects of Cadmium on P. divaricata Photosynthesis

The photosynthetic organs of plants are very sensitive to heavy metal stress [26]. Cd can affect various processes of photosynthesis. Zhu et al. concluded that Cd affects photosynthesis in two different ways: (1) directly, by affecting the chloroplast structure, photosynthetic pigments, fluorescence parameters, electron transfer, and the Calvin cycle enzyme activities [27,28]; and (2) indirectly, through factors such as water status, stomatal opening and closing, and other factors that can affect intercellular and intracellular CO_2 contents [29]. In this study, Pn was inhibited at high Cd levels, indicating that Cd did not directly affect the photosynthesis of *P. divaricata*. At the same time, deficiencies in different nutrient elements can affect chlorophyll and fluorescence parameters. In this study, the element content was similar to the leaf pigment content; therefore, Cd did not significantly reduce it. This also indicates that Cd did not affect photosynthesis indirectly through nutrient element deficiencies.

Stomatal density was positively correlated with stomatal conductance g_s, net photosynthetic rate Pn, and water use efficiency [30]. Stomatal closure is an important factor controlling biological carbon fixation [31]. In this study, the plant water content was altered under 50–75 μM Cd. This significant decrease is directly related to stomatal conductance, mesophyll conductance, stomatal density, stomatal opening and closure, and leaf density. Tang et al. suggested that when SL increases, Ci decreases [32]. We can conclude that stomatal closure is the main reason for the photosynthetic rate decrease. In this study, we can conclude that stomatal limitations were one of the reasons for the reduction of the photosynthetic rate of *P. divaricata*. Our research results are consistent with those of many studies under different stress conditions [33]. However, certain studies have demonstrated that Cd could reduce AN, GS, and E in rice [34] and peanut [35] but could increase stomatal density and Ci. These differences may be related to the different plants studied. The mesophyll conductance (g_m) is considered to play a more important role than stomatal conductance in leaf photosynthesis [36]. Many studies have shown that mesophyll conductance g_m is relatively very low in high Cd concentration, which results in the chloroplast matrix CO_2 concentration (Cc) being relatively lower compared to the intercellular CO_2 (Ci) concentration, further confirming its limiting effect on photosynthesis [37]. In this study, g_m decreased due to the reduction of mesophyll and chloroplast surfaces and changes in leaf morphology caused by Cd, consistent with the research results under water, salt, and Zn stress [38,39].

5. Conclusions

This study demonstrated that *P. divaricata* has strong Cd tolerance and Cd accumulation capacity. *P. divaricata* showed a phenomenon of "growth promotion in low concentrations and growth inhibition in high concentrations" under Cd treatment. At Cd concentrations below 25 μM, there was no Cd toxicity in plant growth and photosynthesis, but high concentrations of Cd inhibited plant growth and photosynthetic physiological processes. The above-ground and below-ground parts of *P. divaricata* differed in their response to Cd stress. In the above-ground tissues, membrane lipid peroxidation were mainly eliminated through the action of various antioxidants. At the same time, in the roots, mainly Cd stress tolerance was exerted by reduced enzyme production. Diffusion limitations (stomatal limitation and mesophyll conductance limitation) mainly contributed to the total photosynthetic limitations under Cd treatment. To conclude, 5–75 μM Cd did not directly affect the photosynthesis of *P. divaricata*. The inhibition of the photosynthetic efficiency of *P. divaricata* under high Cd concentrations was indirectly caused by the impact of Cd on leaf morphology.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13102601/s1. Refs. [40–43] are listed in File S1.

Author Contributions: C.Z. and Z.H. wrote the paper; Q.L. and Y.F. conceived and designed the experiments; X.Z. and Y.L. analyzed the data; R.Y., A.Y., Z.H. and W.J. performed the experiments and collected the data. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Baishanzu National Park scientific research project, grant number 2021ZDLY01; and the National Natural Science Foundation of China, grant number 42007182.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the authors upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pouresmaieli, M.; Ataei, M.; Forouzandeh, P.; Azizollahi, P.; Mahmoudifard, M. Recent progress on sustainable phytoremediation of heavy metals from soil. *J. Environ. Chem. Eng.* **2022**, *10*, 108482. [CrossRef]
2. Wang, L.; Yang, D.; Ma, F. How a functional soil animal-earthworm affect arbuscular mycorrhizae-assisted phytoremediation in metals contaminated soil? *J. Hazard. Mater.* **2022**, *435*, 128991. [CrossRef] [PubMed]
3. Tavarez, M.; Macri, A.; Sankaran, R.P. Cadmium and zinc partitioning and accumulation during grain filling in two near isogenic lines of durum wheat. *Plant Physiol. Biochem.* **2015**, *97*, 461–469. [CrossRef] [PubMed]
4. Sigfridsson, K.G.V.; Bernat, G.; Mamedov, F.; Styring, S. Molecular interference of Cd^{2+} with photosystem ii. *Biochim. Biophys. Acta-Bioenerg.* **2004**, *1659*, 19–31. [CrossRef]
5. Bora, M.S.; Sarma, K.P. Anatomical and ultrastructural alterations in *Ceratopteris pteridoides* under cadmium stress: A mechanism of cadmium tolerance. *Ecotoxicol. Environ. Saf.* **2021**, *218*, 112285. [CrossRef]
6. Waheed, A.; Haxim, Y.; Islam, W.; Ahmad, M.; Ali, S.; Wen, X. Impact of cadmium stress on growth and physio-biochemical attributes of *Eruca sativa* mill. *Plants* **2022**, *11*, 2981. [CrossRef]
7. Sebastian, A.; Prasad, M.N.V. Iron and manganese-assisted cadmium tolerance in *Oryza sativa* L.: Lowering of rhizotoxicity next to functional photosynthesis. *Planta* **2015**, *241*, 1519–1528. [CrossRef]
8. Chen, X.J.; Tao, H.F.; Wu, Y.Z.; Xu, X.M. Effects of cadmium on metabolism of photosynthetic pigment and photosynthetic system in *Lactuca sativa* L. revealed by physiological and proteomics analysis. *Sci. Hortic.* **2022**, *305*, 111371. [CrossRef]
9. Abidli, A.; Huang, Y.; Rejeb, Z.B. Sustainable and efficient technologies for removal and recovery of toxic and valuable metals from wastewater: Recent progress, challenges, and future perspectives. *Chemosphere* **2022**, *292*, 133102.
10. Raheem, A.; Prinsen, P.; Vuppaladadiyam, A.K. A review on sustainable microalgae based biofuel and bioenergy production: Recent developments. *J. Clean. Prod.* **2018**, *181*, 42–59.
11. Seth, C.S. A review on mechanisms of plant tolerance and role of transgenic plants in environmental clean-up. *Bot. Rev.* **2012**, *78*, 32–62.

12. Sharma, P.; Ngo, H.H.; Khanal, S.; Larroche, C.; Kim, S.H.; Pandey, A. Efficiency of transporter genes and proteins in hyperaccumulator plants for metals tolerance in wastewater treatment: Sustainable technique for metal detoxification. *Environ. Technol. Innov.* **2021**, *23*, 101725. [CrossRef]
13. Sharma, A.; Kapoor, D.; Gautam, S.; Landi, M.; Kandhol, N.; Araniti, F. Heavy metal induced regulation of plant biology: Recent insights. *Physiol. Plant.* **2022**, *174*, e13688. [CrossRef] [PubMed]
14. Broadhurst, C.L.; Bauchan, G.R.; Murphy, C.A.; Tang, Y.T.; Pooley, C.; Davis, A.P. Accumulation of zinc and cadmium and localization of zinc in *Picris divaricata* Vant. *Environ. Exp. Bot.* **2013**, *87*, 1–9. [CrossRef]
15. Mohd-Radzman, N.A.; Drapek, C. Compartmentalisation: A strategy for optimising symbiosis and tradeoff management. *Plant Cell Environ.* **2023**, *46*, 2998–3011. [CrossRef]
16. Ru, C.; Hu, X.; Chen, D.; Wang, W.; Song, T. Heat and drought priming induce tolerance to subsequent heat and drought stress by regulating leaf photosynthesis, root morphology, and antioxidant defense in maize seedlings. *Environ. Exp. Bot.* **2022**, *202*, 105010. [CrossRef]
17. Li, Y.; Rahman, S.U.; Qiu, Z.; Shahzad, S.M.; Nawaz, M.F.; Huang, J. Toxic effects of cadmium on the physiological and biochemical attributes of plants, and phytoremediation strategies: A review. *Environ. Pollut.* **2023**, *325*, 121433.
18. Liu, Y.; Wiren, N. Integration of nutrient and water availabilities via auxin into the root developmental program. *Curr. Opin. Plant Biol.* **2022**, *65*, 102117. [CrossRef]
19. Zhu, S.; Luo, L.; Zhang, X.; Zhao, M.; Wang, X.; Zhang, J. Study on the relationship of root morphology and phosphorus absorption efficiency with phosphorus uptake capacity in 235 peanut (*Arachis hypogaea* L.) germplasms. *Front. Environ. Sci.* **2022**, *10*, 855815. [CrossRef]
20. Abdullah, A.; Abdullah, A.Z.; Ahmed, M. A review on recent developments and progress in sustainable acrolein production through catalytic dehydration of bio-renewable glycerol. *J. Clean. Prod.* **2022**, *341*, 130876.
21. Li, T.; Yang, X.; Lu, L.; Islam, E.; He, Z. Effects of zinc and cadmium interactions on root morphology and metal translocation in a hyperaccumulating species under hydroponic conditions. *J. Hazard. Mater.* **2009**, *169*, 734–741. [CrossRef]
22. Liu, S.; Tang, Y.; Qiu, R.; Ying, R.; Ge, R.; Ji, X. Responses of carbonic anhydrase to cadmium in the zinc/cadmium hyperaccumulator *Picris divaricata* vant. *Pedosphere* **2016**, *26*, 709–716. [CrossRef]
23. Xu, N.; Sun, Y.; Wang, Y.; Cui, Y.; Jiang, Y.; Zhang, C. Hormesis effects in tomato plant growth and photosynthesis due to acephate exposure based on physiology and transcriptomic analysis. *Pest Manag. Sci.* **2023**, *79*, 2029–2039. [CrossRef]
24. Calabrese, E.J. Hormesis mediates acquired resilience: Using plant-derived chemicals to enhance health. *Annu. Rev. Food Sci. Technol.* **2021**, *12*, 355–381. [CrossRef] [PubMed]
25. Ray, A.; Nayak, M.; Ghosh, A. A review on co-culturing of microalgae: A greener strategy towards sustainable biofuels production. *Sci. Total Environ.* **2022**, *802*, 149765. [PubMed]
26. Zhou, W.; Xin, J.; Tian, R. Photosynthetic response, antioxidase activity, and cadmium uptake and translocation in monochoria korsakowii with cadmium exposure. *Water Sci. Technol.* **2022**, *86*, 2974–2986. [CrossRef] [PubMed]
27. Zhu, T.T.; Li, L.Y.; Duan, Q.X.; Liu, X.L.; Chen, M. Progress in our understanding of plant responses to the stress of heavy metal cadmium. *Plant Signal. Behav.* **2021**, *16*, 1836884. [CrossRef]
28. Parmar, P.; Kumari, N.; Sharma, V. Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. *Bot. Stud.* **2013**, *54*, 45. [CrossRef]
29. She, W.; Cui, G.X.; Li, X.L.; Su, X.H.; Jie, Y.C.; Yang, R.F. Characterization of cadmium concentration and translocation among ramie cultivars as affected by zinc and iron deficiency. *Acta Physiol. Plant.* **2018**, *40*, 104. [CrossRef]
30. Xu, Z.; Zhou, G. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J. Exp. Bot.* **2008**, *59*, 3317–3325. [CrossRef]
31. Hoshika, Y.; Fares, S.; Pellegrini, E.; Conte, A.; Paoletti, E. Water use strategy affects avoidance of ozone stress by stomatal closure in mediterranean trees—A modelling analysis. *Plant Cell Environ.* **2020**, *43*, 611–623. [CrossRef] [PubMed]
32. Ha, D.V.D.; Bundervoet, B.; Verstraete, W. A sustainable, carbon neutral methane oxidation by a partnership of methane oxidizing communities and microalgae. *Water Res.* **2011**, *45*, 2845–2854. [PubMed]
33. Tang, L.; Ying, R.R.; Jiang, D.; Zeng, X.W.; Morel, J.L.; Tang, Y.T. Impaired leaf CO_2 diffusion mediates cd-induced inhibition of photosynthesis in the Zn/Cd hyperaccumulator *Picris divaricata*. *Plant Physiol. Biochem.* **2013**, *73*, 70–76. [CrossRef]
34. Lee, H.J.; Abdula, S.E.; Jang, D.W.; Park, S.H.; Yoon, U.H.; Jung, Y.J. Over expression of the glutamine synthetase gene modulates oxidative stress response in rice after exposure to cadmium stress. *Plant Cell Rep.* **2013**, *32*, 1521–1529. [CrossRef] [PubMed]
35. Shi, G.R.; Cai, Q.S. Photosynthetic and anatomic responses of peanut leaves to cadmium stress. *Photosynthetica* **2008**, *46*, 627–630. [CrossRef]
36. Niinemets, U.; Diaz-Espejo, A.; Flexas, J.; Galmes, J.; Warren, C.R. Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. *J. Exp. Bot.* **2009**, *60*, 2249–2270.
37. Okcu, G.D.; Eustance, E.; Lai, Y.J.S. Evaluation of co-culturing a diatom and a coccolithophore using different silicate concentrations. *Sci. Total Environ.* **2021**, *769*, 145217. [CrossRef]
38. Bunce, J.A. Use of the response of photosynthesis to oxygen to estimate mesophyll conductance to carbon dioxide in water-stressed soybean leaves. *Plant Cell Environ.* **2009**, *32*, 875–881. [CrossRef]

39. Sagardoy, R.; Vazquez, S.; Florez-Sarasa, I.D.; Albacete, A.; Ribas-Carbo, M.; Flexas, J. Stomatal and mesophyll conductances to CO_2 are the main limitations to photosynthesis in sugar beet (*Beta vulgaris* L.) plants grown with excess zinc. *New Phytol.* **2010**, *187*, 145–158. [CrossRef]
40. Harley, P.C.; Loreto, F.; Di Marco, G.; Sharkey, T.D. Theoretical Considerations when Estimating the Mesophyll Conductance to CO_2 Flux by Analysis of the Response of Photosynthesis to CO_2. *Plant Physiol.* **1992**, *98*, 1429–1436. [CrossRef]
41. Bernacchi, C.J.; Portis, A.R.; Nakano, H.; Von Caemmerer, S.; Long, S.P. Temperature Response of Mesophyll Conductance. Implications for the Determination of Rubisco Enzyme Kinetics and for Limitations to Photosynthesis in Vivo. *Plant Physiol.* **2002**, *130*, 1992–1998. [CrossRef] [PubMed]
42. Grassi, G.; Magnani, F. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant Cell Environ.* **2005**, *28*, 834–849. [CrossRef]
43. Farquhar, G.D.; Firth, P.M.; Wetselaar, R.; Weir, B. On the Gaseous Exchange of Ammonia between Leaves and the Environment: Determination of the Ammonia Compensation Point. *Plant Physiol.* **1980**, *66*, 710–714. [CrossRef] [PubMed]