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The Tolerance, Absorption, and Transport Characteristics of *Macleaya cordata* in Relation to Lead, Zinc, Cadmium, and Copper under Hydroponic Conditions

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Featured Application: The different absorption and transport characteristics of *Macleaya cordata* relative to lead, zinc, cadmium, and copper can be important for the plant to be applied to the remediation of com-pound-polluted soil or industrial sewage.

Abstract: Heavy metal pollution has potential hazards to plant, animal, and human health, and phytoremediation is recognized as a safe and efficient technique for the revegetation of heavy-metal-polluted soil. *Macleaya cordata* was found in heavily tailing areas with fast growth rates, large biomass, and huge taproots. In our study, the seedlings of *M. cordata* were exposed to cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) in a Hoagland solution. After 20 days, the tolerance index as well as the content and distribution of Cd, Pb, Cu, and Zn in roots, stems, and leaves were determined. The results showed *M. cordata* had higher tolerance to Pb and Zn than to Cd and Cu under hydroponic culture conditions. Pb and Cu mainly accumulated in the roots, and the translocation efficiency to the shoots was very low, while about three-quarters of Zn concentrations in the plants were accumulated in the shoots; even the Cd content per shoot of *M. cordata* exceeded some Cd hyperaccumulators. In the present study, the metal ions in the roots or leaves of *M. cordata* were firstly determined in situ using dithizone staining, and the degree of root-tip staining was consistent with the amountof the total metal content in the roots. The addition of Zn or Cu in the Pb treatment solution increased the Pb content in the stems and leaves of *M. cordata*, while the addition of Zn or Cu in the Cd treatment solution had the opposite effect. Pb or Cd in the compound treatment decreased the Zn content in all parts of *M. cordata*. Our results suggest that Pb can be transported above ground via some special pathways in *M. cordata*. The different absorption and transport mechanisms of *M. cordata* in relation to Cd, Zn, Cu, and Pb can be important for the plant to be applied for the remediation of compound-polluted soil or water.

Keywords: *Macleaya cordata*; lead; zinc; copper; cadmium; phytoremediation

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Citation: Zhang, H.; Zhou, W.; Chen, Y.; Xu, H.; Hou, D.; Lv, S.; Sun, X.; Wang, F.; Yang, L. The Tolerance, Absorption, and Transport Characteristics of *Macleaya cordata* in Relation to Lead, Zinc, Cadmium, and Copper under Hydroponic Conditions. *Appl. Sci.* **2022**, *12*, 9598. https://doi.org/10.3390/app12199598

Academic Editor: Zhujian Huang

Received: 19 August 2022
Accepted: 21 September 2022
Published: 24 September 2022

1. Introduction

With the development of industry and agriculture, ever-increasing volumes of soil have been polluted by the discharge of mining and the massive use of pesticides and chemical fertilizers. Heavy metal pollution has become a global problem, posing environmental and health risks. Lead (Pb) and cadmium (Cd) are the two most universal inorganic pollutants in mining sites [1,2], and they are often found to be naturally associated with zinc (Zn) and copper (Cu) due to mining activities [3–8].

Phytoremediation has been recognized as a type of environmentally safe and efficient technique to rehabilitate heavy-metal-contaminated soils [2,9]. Phytoremediation is mainly divided into two categories: phytoextraction and phytostabilization; the former utilizes hyperaccumulator plants to remove heavy metals from the soil, while the latter uses some tolerance plants with special abilities to transform and immobilize heavy metals in the soil or plants to prevent their migration [10,11]. Thus, the selection and utilization of appropriate plants are crucial steps for the successful applications of both phytoextraction and phytostabilization programs.

Macleaya cordata (Willd.) is a perennial herb in the family Papaveraceae, which contains various bioactive alkaloids [12], and this plant has been approved by the European Food Safety Authority (EFSA) as a safe plant for the manufacture of feed additives [13,14]. Meanwhile, this plant was found in tailing areas, with fast growth rates, large biomass, and huge taproots, making it a good candidate species for studying phytoremediation. *M. cordata* had been studied for the phytoextraction of uranium- and molybdenum-contaminated soil [15,16]. *M. cordata* has also been reported to show a good ability to accumulate mercury, cadmium, zinc, and manganese [16–19], but little is known about the absorption and transport mechanisms of this plant relative to these heavy metals. Moreover, previous studies on *M. cordata* usually adopted soil substrates with complex components [14,16–18,20], which makes it quite difficult to explore the underlying mechanisms [20]. Hydroponic experiments that can eliminate the disturbance of soil substrates are usually conducted to unravel the response mechanisms of plants to toxic materials [21–23].

The objectives of this study were (1) to investigate the differences in the tolerance of *M. cordata* under varying concentrations of Zn, Cu, Cd, and Pb in solutions; (2) to compare the effects of metal absorption and transport in *M. cordata* exposure to Zn, Cu, Cd, and Pb treatment; and (3) to analyze the changes in metal accumulation in the *M. cordata* exposed to two or more heavy metals under hydroponic conditions.

2. Materials and Methods

2.1. Hydroponic Experiments

The seeds of *M. cordata* were collected from the tailings of Huaguoshan Town of Luoyang City in China (Lat. 39°19′ N, Long. 111°53′ E). After the seeds were germinated in vermiculite, the seedlings were cultured under the same conditions as those presented in Wang et al. (2018) [16]. The seedlings with uniformity were treated with various concentrations of Pb, Zn, Cd, and Cu for 20 days when they had four leaves. The control (CK) was cultivated only in a complete Hoagland solution (1 mM KH_2PO_4; 1 mM KNO_3; 1 mM $Ca(NO_3)_2$; 1 mM $MgSO_4$; 20 μM Fe-EDTA; 46 μM H_3BO_3; 9 μM $MnCl_2$; 0.77 μM $ZnSO_4$; 0.32 μM $CuSO_4$; 0.11 μM H_2MoO_4). The elements were applied as $ZnSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$, $CdCl_2$, and $Pb(NO_3)_2$, respectively, for the Zn, Cu, Cd, and Pb treatment, and every element had varying treatment concentrations of 100, 500, and 1000 $\mu mol{\cdot}L^{-1}$. The Pb treatment using these three concentrations was denoted as Pb100, Pb500, and Pb1000; similarly, the Zn, Cd, and Cu treatments were separately designated as Zn100, Zn500, and Zn1000; Cd100, Cd500, and Cd1000; and Cu100, Cu500, and Cu1000. The compound treatments were designed with 500Pb, 500Zn, 100Cd, and 100Cu, either as a combination in pairs, for instance, 500Pb + 500Zn; 500Pb + 100Cu; 500Zn + Cd100; 100Cd + 100Cu, or as a mixed treatment of 500Pb, 500Zn, 100Cd, and 100Cu (PZCC). Each treatment had three replicate vessels. The root elongation was calculated with the length difference in the longest root measured at the beginning and at the end of the metal exposure period. After 20 days of exposure, the plants were removed and separated for the analysis of chlorophyll content, dry weight, and metal content, and the detection of metal in situ.

2.2. Estimation of Chlorophyll Content

Chlorophyll (a and b) were extracted from 1.0 g fresh leaves (the second youngest) of *M. cordata* in 10 mL 80% acetone under dark conditions, according to the method of Arnon [24]. The chlorophyll content was calculated on a fresh weight basis ($mg{\cdot}g^{-1}$ FW).

2.3. Dry Weight and Metal Element Analysis

The leaves, stems, and roots of *M. cordata* were collected after being washed with distilled water, and the roots were immersed in a 25 mmol·L^{-1} EDTA–Na_2 solution for 10 min and then washed with distilled water again. The plant samples were then dried in an air circulation oven at 70 °C, then weighed to obtain the shoot dry weight (the sum dry weight of the stems and leaves) and the root dry weight. About 0.2 g of the dried samples were digested using HNO_3:$HClO_4$ (a *v*:*v* of 87:13) mixed acid, following the procedure described by Wang et al. [16]. The ICP-OES (Optima 8000, PerkinElmer, Waltham, MA, USA) was used to analyze the contents of Zn, Cu, Cd, and Pb in *M. cordata*. The translocation factor (TF) was calculated as the metal concentration of the shoot (the aboveground part of the plant) divided by that of the root (the underground part of the plant) [19].

2.4. Detection of Metals In Situ

For the histochemical detection of Pb, Zn, Cd, and Cu in the roots and leaves of *M. cordata*, the dithizone (DTZ) staining method developed by He et al. [25] was used, with some modifications. The roots were stained and microscopically photographed, following the procedure described by Zhang et al. [26]. Specifically, the roots were immersed in a 25 mmol·L^{-1} EDTA–Na_2 solution for 10 min and then washed with distilled water before the detection of metals in situ. Subsequently, the cut roots were immersed in a staining solution (30 mg of dithizone dissolved in 60 mL of acetone, 5–7 drops of glacial acetic acid, and finally fixed to 80 mL) for 15 min. After being washed with distilled water, the well-stained root tips were immediately observed under an Olympus microscope fitted with a Nikon D7100 digital camera. The leaves were stained, discolored, and photographed following the procedure described by Zhang et al. [27]. In practical terms, the second youngest leaves were cut and immersed in the same staining solution, vacuum-infiltrated for 10 min, and then incubated at room temperature for 4 h in the dark. Subsequently, the leaves were bleached in boiling ethanol, and the images were captured with a Nikon D7100 digital camera.

2.5. Statistical Analysis

The data were analyzed using SPSS 16.0 and drawn with GraphPad Prism 8. All the data are expressed as the mean values ± standard errors (SEs) of three independent replicates; the means denoted by different letters refer to the significant differences ($p < 0.05$, Duncan's test). The staining experiments were repeated at least five times, with similar results.

3. Results

3.1. The Tolerance of M. cordata under the Treatment of Pb, Zn, Cd, and Cu in the Solution

Compared with the control, 100 and 500 µmol·L^{-1} Pb and 100 µmol·L^{-1} Zn did not significantly inhibit root elongation. The other treatments inhibited root elongation to a significant level. Among them, all Cu treatments, 500 and 1000 µmol·L^{-1} Cd, and 1000 µmol·L^{-1} Pb completely inhibited root elongation (Figure 1).

After 20 days of exposure, only 100 µmol·L^{-1} Zn caused a significant increase in the root biomass of *M. cordata* when compared with the control, while the other treatments resulted in a significant decrease. Among them, 100 µmol·L^{-1} Pb showed less inhibition to the biomass of the roots than the other treatments (Figure 2a). All Pb treatments and 100 µmol·L^{-1} Zn had no significant influence on the aboveground biomass of *M. cordata*. All Cd and Cu treatments, as well as 500 and 1000 µmol·L^{-1} Zn, significantly decreased the aboveground biomass of *M. cordata* (Figure 2b).

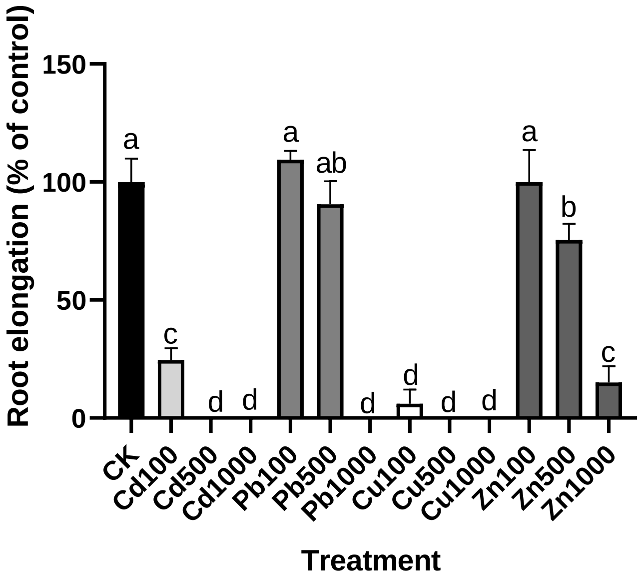

Figure 1. Root elongation (% of control) in roots of *M. cordata* under control (CK) and different concentrations of Cd, Pb, Cu, and Zn after 20 days of exposure. Values are means ± SEs (n = 3) of three different experiments. Means denoted by different letters refer to the significant differences ($p < 0.05$, Duncan's test).

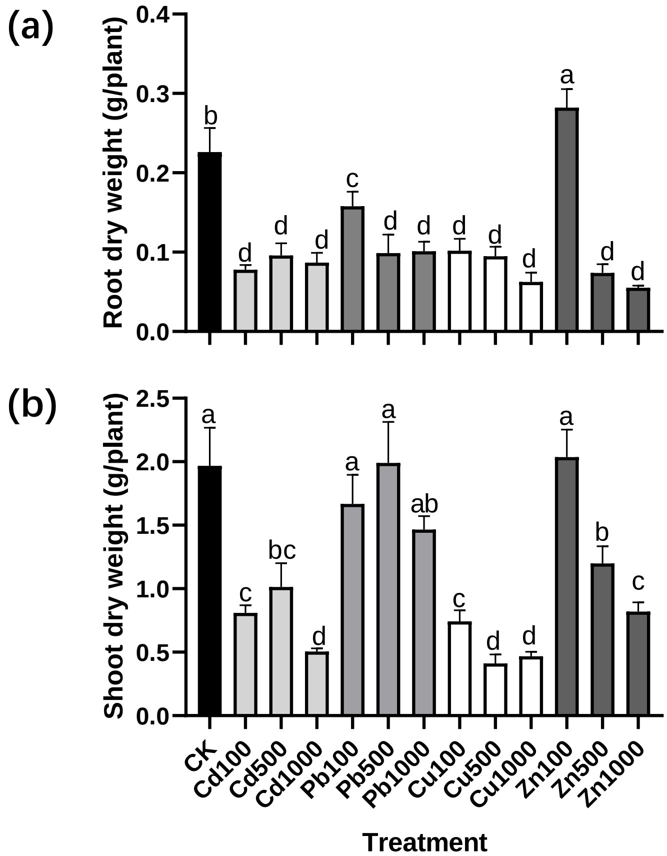

Figure 2. Dry weight of the roots (**a**) and shoots (**b**) of *M. cordata* under control (CK) and different concentrations of Cd, Pb, Cu, and Zn after 20 days of exposure. Values are means ± SEs (n = 3) of three different experiments. Means denoted by different letters refer to the significant differences ($p < 0.05$, Duncan's test).

All the treatments except 100 μmol·L^{-1} Pb resulted in a significant decrease in the chlorophyll content of *M. cordata*. However, the plants using Pb and Zn treatments had significantly higher chlorophyll content than those with Cd and Cu treatments (Figure 3).

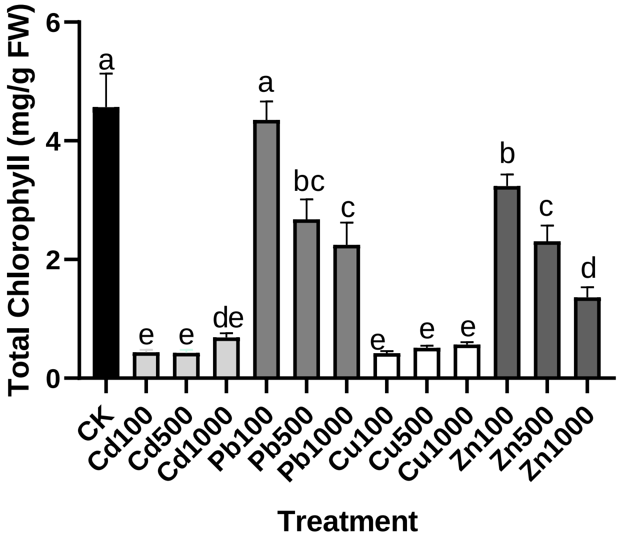

Figure 3. Chlorophyll content in leaves of *M. cordata* under control (CK) and different concentrations of Cd, Pb, Cu, and Zn after 20 days of exposure. Values are means ± SEs (n = 3) of three different experiments. Means denoted by different letters refer to the significant differences ($p < 0.05$, Duncan's test).

3.2. The Accumulation and Translocation of Pb, Zn, Cd, and Cu in M. cordata

After the plants were exposed to a range of concentrations of Pb, Zn, Cd, and Cu treatment for 20 days, the contents of Pb, Zn, Cd, and Cu in the roots, stems, and leaves of *M. cordata* were, respectively, detected (Table 1). With an increase in the treatment concentration of Pb, Zn, Cd, and Cu, the corresponding metal content in the roots, stems, and leaves of *M. cordata* increased. Overall, the metal content in the roots was higher than that in the stems and leaves of *M. cordata*. We found that the Pb content in the roots of *M. cordata* under 500 μmol·L^{-1} Pb was the highest, while the Cu content in the roots under 1000 μmol·L^{-1} Cu was the highest. The Zn content in the leaves with 100 and 500 μmol·L^{-1} Zn was the largest, reaching 698 μg·g^{-1}, while the contents of Pb and Cd in leaves were below 50 μg·g^{-1}. The total content of Zn in the leaves under all Zn concentrations was, respectively, 18.4, 12.6, and 4.8 times higher than that of Pb, Cd, and Cu under corresponding treatments; the Pb content in the stems and leaves was the lowest, and the total Pb content in the stems under all Pb concentrations was, respectively, 4%, 3.8%, and 3.3% that of Zn, Cd, and Cu under corresponding treatments.

By analyzing the accumulation of Pb, Zn, Cd, and Cu in the roots and aboveground parts (the sum of the stems and leaves) of a single plant, we can better understand the transport mechanism of Pb, Zn, Cd, and Cu in plants. We found that the accumulation of Zn in the shoots of *M. cordata* under 100 μmol·L^{-1} Zn was the largest, reaching 1430 μg per plant, while the accumulation of Zn in the roots was 508 μg per plant; therefore, the Zn translocation factor was up to 4.4 (Table 2). The accumulation of Pb in the roots far exceeded that in the shoots, especially under 100 μmol·L^{-1} Pb; the Pb content per plant was the largest, reaching 738 μg, whereas the concentration of Pb in the shoots was 60 μg·plant^{-1} and its TF was the lowest, only 0.08 (Table 2). The accumulation of Cu in the roots and shoots of *M. cordata* was similar to that of Pb (Table 2). Furthermore, the accumulation of Cd in the aboveground of *M. cordata* under the 100 μmol·L^{-1} Cd treatment exceeded that in the roots, and TF was 1.92 (Table 2). The total accumulation of Zn in the leaves of each plant under all Zn treatments was, respectively, 16.1, 3.3, and 8.8 times higher than that of Pb, Cd, and Cu under corresponding treatments.

Table 1. Contents of Pb, Zn, Cd, and Cu in roots, stems, and leaves of *M. cordata* under control (CK) and different concentrations of Cd, Pb, Cu, and Zn after 20 days of exposure.

Treatment	Metal Detected	Content in Roots ($\mu g \cdot g^{-1}$)	Content in Stems ($\mu g \cdot g^{-1}$)	Content in Leaves ($\mu g \cdot g^{-1}$)
CK	Pb	ND [1]	ND	ND
Pb100		4678.7 b ± 211.1	37.0 a ± 3.9	31.2 b ± 4.6
Pb500		5921.3 a ± 260.2	32.3 a ± 2.9	31.0 b ± 3.9
Pb1000		2030.8 c ± 116.4	38.0 a ± 2.1	47.1 a ± 3.5
CK	Zn	885.5 c ± 54.0	82.4 c ± 3.4	82.7 b ± 4.1
Zn100		1801.2 b ± 138.2	708.6 b ± 39.4	698.3 a ± 24.4
Zn500		3023.3 a ± 235.3	906.1 a ± 75.2.0	698.3 a ± 31.9
Zn1000		3322.2 a ± 266.0	1032.0 a ± 54.2	617.2 a ± 72.6
CK	Cd	ND	ND	ND
Cd100		1587.9 c ± 98.7	655.6 b ± 31.4	44.3 b ± 9.0
Cd500		4179.9 b ± 314.7	1014.4 a ± 46.4	43.3 b ± 4.6
Cd1000		6180.5 a ± 264.6	1133.3 a ± 53.7	71.8 a ± 6.4
CK	Cu	57.3 d ± 5.1	4.1 d ± 0.1	10.1 b ± 0.6
Cu100		3668.9 c ± 155.7	155.9 c ± 3.4	16.8 b ± 1.0
Cu500		5164.3 b ± 192.9	531.3 b ± 33.7	26.0 b ± 0.8
Cu1000		6646.8 a ± 254.1	2526.5 a ± 127.8	320.8 a ± 21.0

[1] ND, not detected. Values are means ± SEs ($n = 3$) of three different experiments. Means denoted by different letters refer to the significant differences ($p < 0.05$, Duncan's test).

Table 2. Metal content and translocation factor (TF) per plant of *M. cordata* under control (CK) and different concentrations of Cd, Pb, Cu, and Zn after 20 days of exposure.

Treatment	Metal Detected	Metal Content in Roots ($\mu g \cdot Plant^{-1}$)	Metal Content in Shoots ($\mu g \cdot Plant^{-1}$)	TF
CK	Pb	ND [1]	ND	ND
Pb100		738.4 a ± 33.3	55.9 b ± 2.8	0.08 b
Pb500		585.2 b ± 25.7	62.7 a ± 7.0	0.11 b
Pb1000		205.6 c ± 11.8	64.0 a ± 4.2	0.31 a
CK	Zn	200.4 b ± 12.2	162.6 d ± 4.6	0.82 c
Zn100		508.0 a ± 39.0	1430.2 a ± 39.0	2.84 b
Zn500		223.6 b ± 17.4	920.1 b ± 28.7	4.16 a
Zn1000		139.0 c ± 11.1	607.6 c ± 54.8	4.44 a
CK	Cd	ND	ND	ND
Cd100		123.6 c ± 7.7	235.6 b ± 8.3	1.92 a
Cd500		401.0 b ± 30.2	382.1 a ± 18.7	0.96 b
Cd1000		536.5 a ± 23.0	266.8 b ± 10.3	0.50 b
CK	Cu	13.0 c ± 1.1	16.0 d ± 0.8	1.25 a
Cu100		374.2 b ± 15.9	44.2 c ± 0.6	0.12 c
Cu500		489.4 a ± 18.3	97.7 b ± 5.9	0.20 c
Cu1000		415.4 b ± 15.9	192.7 a ± 14.1	0.46 b

[1] ND, not detected. Values are means ± SEs ($n = 3$) of three different experiments. Means denoted by different letters refer to the significant differences ($p < 0.05$, Duncan's test).

In order to further understand metal accumulation in situ, we stained the roots and leaves of *M. cordata* under 100 $\mu mol \cdot L^{-1}$ Pb, Zn, Cd, and Cu treatment for 20 days with dithizone and determined the contents of Pb, Zn, Cd, and Cu in the roots, stems, and leaves under the same conditions. Dithizone can form colored complexes with Pb, Zn, Cd, and Cu; a darker stain was indicative of the presence of more metals in roots or leaves. We found that the root tips of *M. cordata* exposed to 100 $\mu mol \cdot L^{-1}$ Pb were stained the deepest, considering the colored complexes on the root-tip surfaces (Figure 4a); accordingly, the total metal content in the roots of *M. cordata* under the Pb treatment was the highest (Figure 4b). The root-tip staining degree of Pb treatment was successively followed by that of Cu, Cd, and Zn, which were also consistent with the amount of the four metals in the root surfaces of *M. cordata.* Moreover, we found that the colored complexes from Pb and Cu treatments occurred in the mature areas of the root tips, and the Cu treatment induced lateral root formation in advance (Figure 4a). Overall, the effect of leaf staining was not obvious, except that the leaf veins in the leaves of the *M. cordata* exposed to Cd and Zn were stained deeper than others (Figure 5a), which was consistent with the total metal content in the stems, not in the leaves (Figure 5b,c).

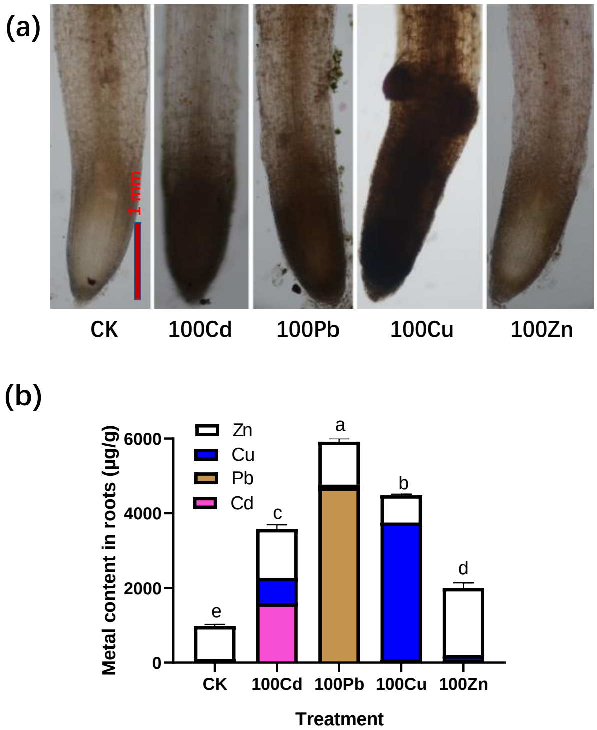

Figure 4. Dithizone staining in root tips (**a**) and metal content in roots (**b**) of *M. cordata* under control (CK) and 100 μmol·L^{-1} Cd, Pb, Cu, and Zn after 20 days of exposure. Staining experiments were repeated at least five times with similar results. Bar, 1 mm. Values are means ± SEs (n = 3) of three different experiments. Means denoted by different letters refer to the significant differences ($p < 0.05$, Duncan's test).

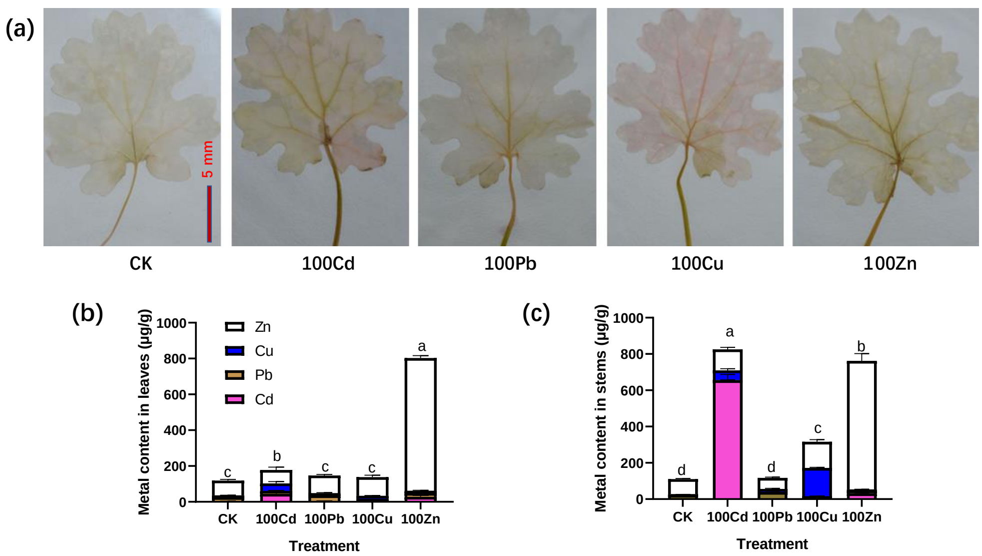

Figure 5. Dithizone staining (**a**) and metal content in leaves (**b**) and stems (**c**) of *M. cordata* under control (CK) and 100 μmol·L^{-1} Cd, Pb, Cu, and Zn after 20 days of exposure. Staining experiments were repeated at least five times with similar results. Bar, 5 mm. Values are means ± SEs (n = 3) of three different experiments. Means denoted by different letters refer to the significant differences ($p < 0.05$, Duncan's test).

3.3. Content Changes in Pb, Zn, Cd, and Cu in M. cordata Exposed to Compound Heavy Metals in the Solution

According to the tolerance indices of *M. cordata* to Pb, Zn, Cd, and Cu, we chose 500 $\mu mol \cdot L^{-1}$ Pb, 500 $\mu mol \cdot L^{-1}$ Zn, 100 $\mu mol \cdot L^{-1}$ Cd, and 100 $\mu mol \cdot L^{-1}$ Cu to perform single-metal treatments or combine them into compound treatments. After 20 days, we analyzed the ratios of the metal contents under compound treatments to those of single-metal treatments. If the ratio was greater than 1, the accumulation increased; otherwise, it decreased. The results showed that the addition of other metals to the metal treatment solution significantly reduced the content of that metal in the plant in most cases (Figure 6). However, there were some exceptions, for example, addition of Zn or Cu to 500 $\mu mol \cdot L^{-1}$ Pb treatment solution resulted in a significant increase in the Pb contents of the stems and leaves (Figure 6b), while the addition of Zn or Cu when using 100 $\mu mol \cdot L^{-1}$ Cd had the opposite effect on the Cd content (Figure 6a). The addition of any metal when using the 500 $\mu mol \cdot L^{-1}$ Zn solution decreased the Zn accumulation in the roots, stems, and leaves of *M. cordata* (Figure 6c). With the addition of Pb or Cd when using 100 $\mu mol \cdot L^{-1}$ Cu, the Cu accumulation in the roots and stems decreased, while the Cu accumulation in the leaves significantly increased (Figure 6d). The compound treatment of these four metals (PZCC) evidently increased the Pb and Cd contents but decreased the Cu and Zn contents in the leaves of *M. cordata,* compared with the single-metal treatments.

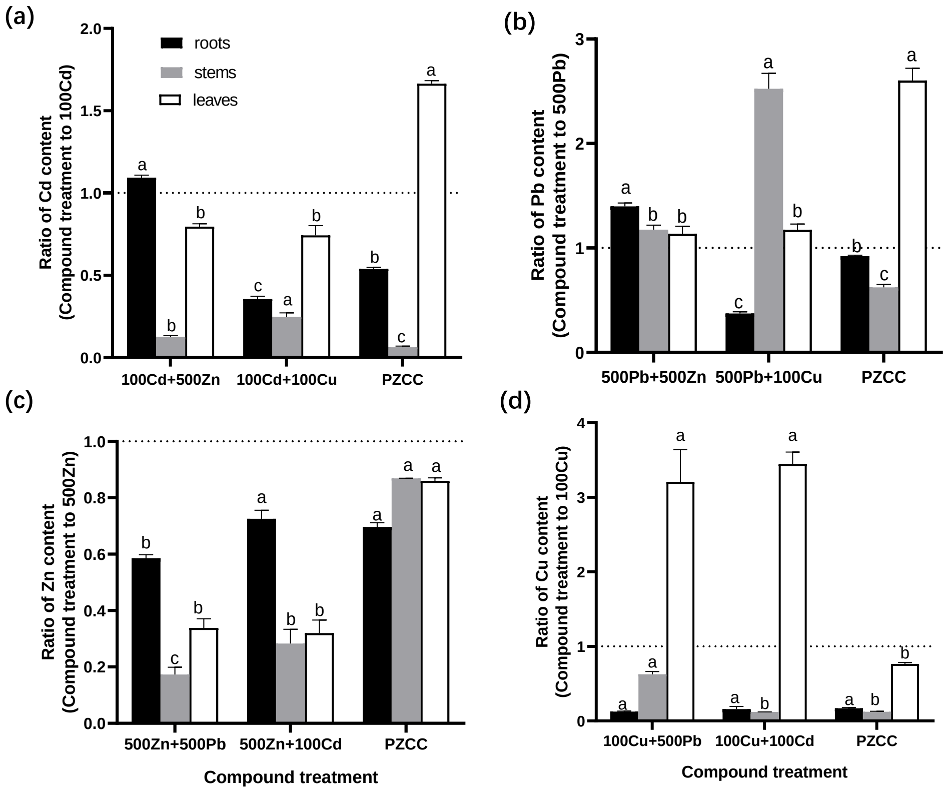

Figure 6. Content ratios of Cd (**a**), Pb (**b**), Zn (**c**), and Cu (**d**) in roots, stems, and leaves of *M. cordata* under compound treatments of 100Cd, 500Pb, 100Cu, and 500Zn. PZCC means the mixed treatment of 500Pb, 500Zn, 100Cd, and 100Cu. Values are means ± SEs (n = 3) of three different experiments. If the mean is greater than 1, it means upregulation by compound treatment; otherwise, it means downregulation. Means denoted by different letters refer to the significant differences (p < 0.05, Duncan's test).

4. Discussion

The tolerance index is determined by the plant growth parameters, including the root length, shoot length, root biomass, and shoot biomass; in particular, root elongation is the most sensitive index of plants to heavy metal stress [28]. In present studies, the

Pb and Zn treatments little inhibited root elongation under 100 and 500 μmol·L^{-1} Pb or 100 μmol·L^{-1} Zn treatment, and when using 100 μmol·L^{-1} Zn, it even increased the root biomass (Figures 1 and 2a). The shoot biomass of *M. cordata* from all the Pb treatments and the 100 μmol·L^{-1} Zn treatment did not decrease when compared with the control (Figure 2b). All the Cd and Cu treatments decreased root elongation, as well as the root and shoot dry weights (Figures 1 and 2), which shows that *M. cordata* has more tolerance to Pb and Zn than to Cd and Cu treatments. Metal-induced changes in chlorophyll contents in the leaves of the plant were similar to the result of the shoot biomass of *M. cordata* (Figure 3). The chlorophyll content can affect the plant's photosynthetic yield and biomass. The greater the biomass of the plant, the greater the capacity of the plant to stabilize and accumulate heavy metals. From this, it can be deduced that *M. cordata* is more suitable as a phytoremediator of the soils contaminated with Pb and Zn.

After being exposed to a range of concentrations of Pb, Zn, Cd, and Cu for 20 days, *M. cordata* accumulated the highest concentrations of Zn and Cd, respectively, in the leaves and stems and accumulated high contents of Pb and Cu in the roots (Table 1). As shown in Table 2,the amount of heavy metal accumulated per plant was in the order of Zn > Pb > Cd > Cu, and per shoot was in the order of Zn > Cd > Cu > Pb. TF was used to evaluate the plants' phytoremediation efficiency; the TF of Zn in *M. cordata* exceeded 4, and the maximum Zn content in the shoots exceeded 1 mg for a single plant in the present study (Table 2). The maximum Cd concentration of the shoots (1133.3 mg·kg^{-1}) in the present study exceeded the criterion necessary for a Cd hyperaccumulator, which was also higher than that reported (91.93 mg·kg^{-1}) in the stems of the *M. cordata* cultivated in the soil by Nie et al. [20]. Even the Cd accumulations in the roots and shoots were more than those observed in the Cd hyperaccumulator *Solanum nigrum* under the same treatment conditions [29]. However, *M. cordata* is still not a hyperaccumulator of Pb, Zn, Cd, and Cu, according to the international standards of hyperaccumulators [2]. The results showed that *M. cordata* is a suitable plant for the phytoremediation of multiple metals, especially in the lead–zinc-contaminated soil. Cai et al. [17] found that the TF of Pb in *M. cordata* treated with the soils from lead–zinc tailing was 0.5, which is higher than that of the present treatment (0.08–0.31), and the TF of Zn in the *M. cordata* treated with lead–zinc tailing was about 1.3, which is lower than that of the present treatment (2.84–4.44). This suggests that the solution culture increased the Zn transport but decreased the Pb transport in *M. cordata* from the roots to the shoots of the plants.

In order to investigate the distribution of metals in the roots and leaves of *M. cordata*, we analyzed the accumulation of metals in situ in the roots and leaves of *M. cordata* under 100 μmol·L^{-1} Pb, Zn, Cd, and Cu treatments with dithizone staining. Dithizone is highly sensitive to Cd and Pb ions, and it is able to form colored complexes with zinc, copper, chromium, iron, nickel, and some other metals [5]. The compounds of Zn, Cu, Cd, and Pb with dithizone have different properties, and their molar extinction coefficients are reported to be in the following order: $Cu^{2+} < Pb^{2+} < Cd^{2+} < Zn^{2+}$ [30]. In the present study, the degree of root-tip staining was consistent with the total metal content in the roots of *M. cordata* (Figure 4). In addition, we found that Pb and Zn induced less damage, whereas Cu induced more damage in the root tips of *M. cordata*, compared with the control, which is associated with root length inhibition. On the other hand, thedegree of leaf staining was inconsistent with metal contents in the leaves, but degree of leaf-vein staining was consistent with theamount of metals in the stems (Figure 5). We also found that Zn was not sensitive enough to dithizone staining in this experiment.

Adamczyk-Szabela et al. [31] found that compound metal treatment can affect the uptake and accumulation of *Melissa ofcinalis* only under medium-stress concentrations of heavy metals. Based on the tolerance indices of *M. cordata* to Pb, Zn, Cd, and Cu, we analyzed the effect of compound treatments on the metal accumulation of *M. cordata* under 500 μmol·L^{-1} Pb, 500 μmol·L^{-1} Zn, 100 μmol·L^{-1} Cd, and 100 μmol·L^{-1} Cu. Our results showed that the addition of other metal ions to a metal treatment solution significantly reduced content of the metal in *M. cordata* in most cases, which was consistent with the accumulation characteristics of *M. ofcinalis* to Pb, Zn, Cd, and Cu in the soil [30]. However, with the addition of Zn or Cu when using 500 μmol·L^{-1} Pb, the Pb content in the stems and leaves significantly increased, while the addition of Zn or Cu when using 100 μmol·L^{-1} Cd had the opposite effect on the Cd content (Figure 6). This suggests that Cd and not Pb ions are transported via the symplastic pathway and compete with Zn or Cu for similar transports. On the other hand, the compound treatment of these four metals (PZCC) increased the Pb and Cd contents but decreased the Cu and Zn contents in the leaves of *M. cordata*; however, the reason for this effect is unclear.

5. Conclusions

In this study, *M. cordata* showed higher tolerance and accumulation ability to Pb and Zn than to Cd and Cu in the solution culture conditions, and most of the Pb content accumulated in the roots, and three-quarters of zinc accumulated in the shoots of *M. cordata*. We first determined the location of metal accumulation in the roots or leaves of *M. cordata* using dithizone staining; the root-tip staining was the deepest color under Pb and Cu treatments, which was consistent with the amounts of the total metal contents in the roots of *M. cordata*. The addition of Zn or Cu when using 500 μmol·L^{-1} Pb increased the Pb content in the leaves of *M. cordata*. We speculate that Pb is transported via a different pathway than Zn or Cu transport pathways.

Author Contributions: All authors contributed to the study's conception and design. Material preparation was performed by H.Z., L.Y. and Y.C. Data collection and analysis were performed by H.Z., W.Z., H.X., Y.C., S.L., D.H., F.W. and X.S. The manuscript was written by H.Z. and L.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Natural Science Foundation of Henan Province (212300410340), the Scientific and Technological Research Project of Henan Province (182102410028), and the Key Project of the Department of Education of Henan Province (16A180004). This work was also supported by the National Key R&D Program of China (2021YFC1809100) and the China Agriculture Research System of MOF and MARA.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data analyzed are included and available in the manuscript.

Conflicts of Interest: I hereby declare that all the authors have participated in this study and the development of the manuscript. All the authors have read the final version and given their consent for the article to be published in *Applied Sciences*.

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