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Manganese uptake and interactions with cadmium in the hyperaccumulator—*Phytolacca Americana L.*

Kejian Peng [a,b], Chunling Luo [c], Wuxin You [a], Chunlan Lian [d], Xiangdong Li [c], Zhenguo Shen [a,*]

[a] *College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, People's Republic of China*
[b] *Hunan Research Academy of Environmental Sciences Changsha 410004, People's Republic of China*
[c] *Department of Civil and Structural Engineering, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong*
[d] *Asian Natural Environmental Science Center, The University of Tokyo, 1-1-8 Midori-cho, Nishitokyo, Tokyo 188-0002, Japan*

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Abstract

In the present study, the accumulation of Mn and other metals by *Phytolacca Americana L.* from contaminated soils in Hunan Province, South China, was investigated. Results showed that the average concentrations of Mn in the leaves and roots reached 2198 and 80.4 mg kg^{-1} (dry weight), respectively, with a maximum 13,400 mg kg^{-1} in the leaves. A significant correlation was found between Mn concentrations in the plant leaves and those in the corresponding soils. Hydroponic experiments were also conducted to study the Cd uptake ability and interactions between Mn and Cd in the plant. It was found that *P. americana* hyperaccumulated not only Mn, but also Cd in the leaves. In the presence of Cd, adding Mn to the solution significantly improved the plant growth and reduced the concentrations of Cd in all organs of the plant.

Keywords: *Phytolacca americana*; Phytoremediation; Manganese; Cadmium; Hyperaccumulation

1. Introduction

Soil and water are contaminated with heavy metals in many parts of the world due to a variety of human activities. One of the major pollution sources is the mining and smelting of metalliferous ores. Manganese is an essential micronutrient for plants. It plays a major role in photosynthesis, respiration and activation of several enzymes, including superoxide dismutase, NADPH-specific decarboxylating malate dehydrogenase and nitrate reductase [1]. However, at a high concentration, this element may be toxic to plants. The incidence of Mn toxicity shows a strong dependence on plant species and soil properties, such as pH [2].

Two strategies have been proposed for plant resistance to heavy metal stresses in the environment [3]. One is to avoid excessive metal uptake, and restrict its transport to the shoots. In some plant species, it has been found that large amounts of Mn are retained in roots with smaller amounts translocated to shoots [4–6]. However, Mn retention in the roots may not be the cause for the Mn tolerance of other species [7–10]. Manganese tends to accumulate in shoots rather than in roots. The second strategy is related to the internal tissue tolerance by which metals are accumulated in plant shoots by either compartmentation in vacuoles or complexation by organic ligands [11–13]. Manganese is transported through transpiration stream in the xylem to shoots where it is mainly accumulated in the first leaves of wheat and lupin, and is not redistributed via the phloem within the root system or within the shoot [14,15]. At a subcellular level, excess Mn might be stored in the vacuoles [16–18], cell walls [19,20], and possibly, chloroplasts [21,22] depending on plant species. Previous study suggested that excessive Mn can induce oxidative stress [22–24] catalyzing the oxidation of the physiologically active Fe^{2+} to the inactive Fe^{3+} form.

Plants growing on polluted soils may accumulate elevated levels of heavy metals in comparison with those on uncontaminated soils. Plants with shoot concentrations of >100 mg kg^{-1} Cd, >1000 mg kg^{-1} Ni, Pb and Cu, or >10,000 mg kg^{-1} Zn and

* Corresponding author. Tel.: +86 25 84396391; fax: +86 25 84396542.
E-mail address: zgshen@njau.edu.cn (Z. Shen).

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Mn (based on dry weight) have been classified as hyperaccumulators [25]. In recent years, there has been increasing interest in the study of hyperaccumulators because the plants might be used to remove toxic metals from contaminated soils by harvesting metal-rich aboveground parts of plants. Plant species differ considerably in their Mn concentration in normal leaf [26]. Fourteen plant species have been found to accumulate above 10,000 mg kg^{-1} Mn in shoot dry matter (DM) [11,27,28]. Recently, Xue et al. [27] found that *Phytolacca acinosa* Roxb. had not only remarkable tolerance to Mn, but also extraordinary uptake and accumulation capacity for this element. The maximum Mn concentration reached 19,300 mg kg^{-1} in the leaves of plants grown on Xiangtan Mn mine tailings. Under hydroponic condition, *P. acinosa* was able to tolerate 5000 μM Mn without showing visible toxic symptoms. In the 12,000 μM Mn treatment, the leaf Mn concentration reached 36,380 mg kg^{-1} DM. Our previous studies showed that *Phytolacca Americana L.*, a closely related species with *P. acinosa*, may accumulate large amounts of heavy metals in its aerial tissues, especially for Cd [28]. It is suggested that *P. americana* may be a potential Cd hyperaccumulator. Recently, Tie et al. [29] reported that *P. americana* L. collected from Mn mine tailings exhibited high Mn concentrations with a range of 5160–8000 mg kg^{-1} in the leaves. It is not clear whether *P. americana* is able to co-accumulate Cd and Mn although the interactions between Cd and Mn in other plants had been reported [19,30,31]. In addition, some metal transporters belonging to the Nramp (natural resistance-associated macrophage protein) and ZIP (zinc-regulated transporter/iron-regulated transporter-related protein) families have been identified, and they are able to transport Fe, Zn, Mn and Cd [32,33].

P. americana is a perennial weed that occurs worldwide. According to our investigation, it is often found in disturbed habitats, particularly in contaminated areas of South China. The first-year plants can reach over 2 m in height. The present work aims to evaluate the Mn accumulation ability of *P. americana* plants growing in a mining contaminated area of South China. In addition, hydroponic experiments were conducted to investigate the effects of excess Mn on plant growth and interactions between Mn and Cd in plants.

2. Materials and methods

2.1. Plant and soil sampling in Xiangxi area

The field investigation was carried out in the Xiangxi Autonomous Prefecture (109°10′00″E–109°22′30″E, 27°44′30″N–29°28′00″N) located in the northwest of Hunan Province, China. Xiangxi has a mean annual temperature of 13.5–16.7 °C with an average annual rainfall of 1419 mm. The region has an average altitude of 800–1200 m with a subtropical mountainous moist climate.

The plant and soil samples were collected from four contaminated and one unpolluted sites: two wastelands around the Xiangxi Zn and Mn smelter in Jishou city (Datianwan I and II); one mine tailing ruin of the Tuanjie Pb–Zn mine in Huayuan county; one wasteland site around the Zhengxin Mn smelter in Huayuan county; and one uncontaminated site in Yongshun county as a baseline site of the Xiangxi region. Plant samples included the leaf, stem and root of *P. americana*. Besides, some seeds of *P. americana* were also collected at the same time for the subsequent hydroponic experiments. At least 10 individual subsamples of leaf, stem and root from each plant were collected at each sampling location, and then they were mixed to give a representative leaf, stem and root sample for a complete plant. The collected plant samples were washed thoroughly with tap water and rinsed with deionized water, then blotted dried with tissue paper, weighed fresh, dried at 80 °C to constant weight, and dry weights were also recorded. The soils in which the plant was growing were also collected for the chemical analysis. The sampling depth for soil was about 0–20 cm. The pH and Mn concentration of the soils are presented in Table 1.

2.2. Hydroponic experiment

2.2.1. Experiment 1

This experiment was conducted to study Mn uptake and accumulation in the population of *P. americana* from Datianwan site II. The seeds of *P. americana* were selected for health and uniformity and germinated in a mixture of perlite and vermiculite in plastic dishes moistened with tap water for 15 days. After germination the seedlings were transplanted to a vessel containing 2.5 L 1/2 Hoagland nutrient solution at pH 5.6. Twelve days later, the seedlings were treated with different Mn concentrations: 9.1 (control), 500, 1000, 2000, 5000 and 10,000 μM. The Mn was supplied as $MnCl_2$ into Hogland solutions. Each treatment was replicated in three different vessels, with each vessel having five plants. The nutrition solutions were renewed every 2 days. After further 15-day growth, the plants were harvested. Shoots and roots were separated and washed thoroughly with tap water and rinsed with deionized water, then blotted dried with tissue paper, weighed fresh, dried at 80 °C for 24 h, and dry weights were also recorded.

Table 1
The ranges and mean concentrations of Mn in the soils collected at different sites in the Xiangxi area

Soil	*n*	pH	Exchangeable Mn (mg kg^{-1}) range (a.m.)	Total Mn (mg kg^{-1}) range (a.m.)
Datianwan I	30	3.75–7.08 (6.45)	5.13–55.6 (22.6)	616–3350 (1500)
Datianwan II	9	6.41–6.97 (6.59)	14.6–52.1 (35.2)	1490–13,200 (4290)
Tuanjie	18	6.52–7.83 (6.94)	4.28–50.2 (33.5)	300–4060 (2110)
Huayuan	9	6.29–6.81 (6.51)	2.96–52.1 (44.9)	5600–13,900 (10600)
Yongshun	3	6.88–7.33 (7.13)	1.48–3.11 (2.21)	253–683 (495)

n: the number of sample in the location; a.m.: arithmetic mean.

Seedlings were grown under controlled-environment conditions: 12 h day length with a light intensity of 350 μmol photons m^{-2} s^{-1} supplied by fluorescent tubes, about 25 °C/20 °C day/night temperature, and 60–70% relative humidity.

2.2.2. Experiment 2

Experiment 2 was conducted to study the interactions between Cd and Mn on the growth and metal uptake of *P. americana*. After germination the seedlings were transplanted to a vessel containing 2.5 L 1/2 Hoagland nutrient solution at pH 5.6 for 12 days. Seedlings were then treated with different concentrations of Cd and Mn. The experiment was arranged in a 3 × 4 factorial design with three Cd concentrations and four Mn levels, giving 12 different treatments. Three Cd treatments were 0, 10 and 50 μM Cd supplied as $CdCl_2$. Four Mn treatments were 9.1, 1000, 2000 and 5000 μM Mn as $MnCl_2$. Each treatment was replicated three times, with each vessel containing five plants. The nutrition solutions were renewed every 2 days. The plants were harvested on day 22 after the treatment.

2.3. Sample preparation and analysis

Dried plant materials were ground using an agate mortar. Plant materials were digested with concentrated HNO_3 and $HClO_4$ (87:13, v/v), and the total concentrations of Cd, Mn and other elements such as Zn, Cu and Fe were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, Perkin-Elmer Optima 3300 DV).

Water-soluble Mn in *P. americana* tissues was extracted with 1.0 mM MES-Tris buffer at pH 6.0 for 5 h [34]. The extraction solution was filtered through a 0.45 μm filter paper (Whatman [Maidstone, UK] 42) and analyzed for Mn concentrations by atomic absorption spectrophotometer model TAS-986 (Purkinje General Co. Ltd., Beijing).

Soil samples collected from the rhizosphere of *P. americana* were air-dried at room temperature and ground using an agate mortar. They were digested with a mixture of concentrated HF: HNO_3: $HClO_4$ (4:1:1, by volume). The total Mn concentration in solutions was determined using ICP-AES. Exchangeable Mn was extracted by 50 mM HCl for 2 h with 1:5 soil to solution ratio, and was determined using the atomic absorption spectrophotometer. Soil pH was measured with deionized water in 1:2.5 (w/v) using a pH meter.

Certified standard reference material of SRM 1573 (apple leaves) of the National Institute of Standards and Technology, U.S.A., was used in the digestion and analysis as part of the QA/QC protocol. Reagent blank and analytical duplicates were also used where appropriate to ensure accuracy and precision in the analysis. The recovery rates were around 92 ± 5% for all the metals in the plant reference material. Statistical analyses of the experimental data in the present study, such as correlation and significant differences, were performed using SPSS® 11.0 statistical software (SPSS Inc., Chicago, U.S.A.).

3. Results and discussion

3.1. Mn accumulation by P. americana at contaminated sites

The concentrations of Mn in normal plant shoot ranged from 20 to 500 mg kg^{-1} [26]. Some plant species with exceeding the criterion of 10,000 mg kg^{-1} Mn have been regarded as Mn hyperaccumulators [26,27]. Table 2 shows the concentrations of Mn in the leaves and roots of *P. americana* grown in contaminated soils. The average Mn concentrations were 2198 and 80.4 mg kg^{-1} in the leaves and roots, respectively. The maximum Mn concentration in the leaf DM was 13,400 mg kg^{-1} at the Datianwan II site, followed by 11,300 mg kg^{-1} from the same sampling location. These values were lower than the maximum Mn concentration in the leaf of *P. acinosa* from Xiangtan Mn tailings reported by Xue et al. [27]. The bioaccumulation factor is defined as a ratio of metal concentration in the dry plant fraction to that in the corresponding soil [3,35]. The high bioaccumulation factor and efficient transfer of metal from roots to above-ground parts were usually typical features for hyperaccumulation plants. In the leaves of *P. americana*, the average bioaccumulation factor was 0.58, with a range from 0.06 to 3.90, and 17% plants with a bioaccumulation factor >1.0. Moreover, the leaves of *P. americana* had greater Mn concentrations than the roots in all plant specimens. These data showed that Mn could be efficiently taken up by *P. americana* roots and transported to leaves.

The relationship between the concentration of Mn in the leaves of *P. americana* and the concentration of total Mn in

Table 2
The concentrations (mg/kg DW) of Mn and bioaccumulation factors in *P. americana* collected from contaminated and uncontaminated sites

No.	Datianwan I			No.	Datianwan II			No.	Tuanjie			No.	Huayuan			No.	Yongshun		
	Leaf	Root	BF		Leaf	Root	BF		Leaf	Root	BF		Leaf	Root	BF		Leaf	Root	BF
DI1	308	29.4	0.19	DII1	3850	61.2	1.53	T1	171	nd	0.31	H1	4900	141	0.40	Y1	149	nd	0.22
DI2	355	30.3	0.37	DII2	8080	684	3.90	T2	384	40.2	1.28	H2	4990	51.5	0.45	Y2	113	nd	0.21
DI3	495	4.63	0.36	DII3	1850	53.5	0.52	T3	161	7.40	0.06	H3	1220	32.8	0.09	Y3	194	nd	0.10
DI4	388	16.3	0.39	DII4	11300	561	0.86	T4	439	32.9	0.16	H4	1090	0.11	0.13	–	–	–	–
DI5	2930	32.2	2.25	DII5	237	0.03	0.06	T5	617	nd	0.32	H5	2550	122	0.31	–	–	–	–
DI6	379	9.24	0.11	DII6	13400	23.2	2.26	T6	376	5.28	0.18	H6	1330	55.7	0.23	–	–	–	–
DI7	97.8	3.74	0.08	DII7	2800	145	1.35	T7	122	nd	0.07	H7	2000	30.8	0.15	–	–	–	–
DI8	329	6.87	0.41	DII8	741	40.3	0.12	T8	238	30.9	0.10	–	–	–	–	–	–	–	–

No.: sampling location code; BF: bioaccumulation factor = metal concentration in leaf/metal concentration in soil; nd: not detectable.

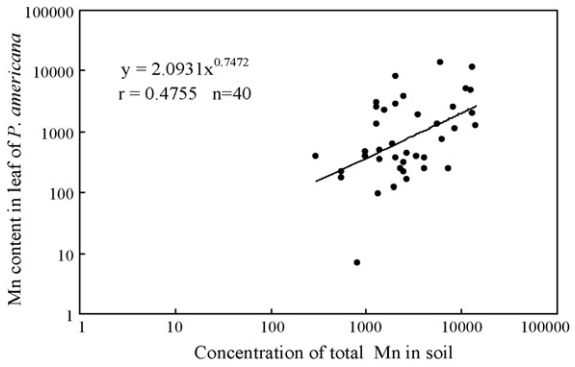

Fig. 1. The relationship between the concentrations (mg kg^{-1}) of Mn in the leaf of *P. americana* and the corresponding soils.

soil is shown in Fig. 1. On a power function, Mn concentration in leaves was significantly related with that in soils. This indicated that soil Mn could influence the metal uptake by plants, although it can also be affected by several other soil factors such as pH, Eh, organic matter and microbial activity [36].

As seen from Table 3, there was no significant correlation between the concentrations of Mn and Fe in the leaves of *P. americana*. In the leaf samples with Mn concentration >500 mg kg^{-1}, the concentrations of Fe were within the normal ranges described by Reeves and Baker [26] (60–600 mg kg^{-1}), and the ratios of Fe/Mn concentrations in leaves were less than 0.8. According to Kabata-Pendias and Pendias [37], the ratio of Fe/Mn in vegetal tissues should be between 1.5 and 2.5. The low Fe/Mn ratio in *P. americana* showed that these plants possess a high capacity for selective Mn uptake from soil. Moreover, the concentrations of Cr were mostly below the detection limit in the samples, and the maximum Cr concentration was 0.76 mg kg^{-1} in the leaf samples (data not shown), which was within the normal values of 0.2–5 mg kg^{-1} reported by Reeves and Baker [26]. No significant correlation was found between Mn and Al, Cu or Zn in plant leaves (Table 3).

Previous studies showed that the average concentration of Cd was 55.2 mg kg^{-1} in these *P. americana* leaf samples [28]. The maximum Cd concentration of 402 mg kg^{-1} was found in *P. americana* collected at the Datianwan I site. These results showed that *P. americana* was able to hyperaccumulate both Mn and Cd in the leaves. However, there was no significant correlation between Mn and Cd concentrations in the leaves (Table 3).

A significantly positive correlation was found between Mn and S or Mg in the leaves of *P. americana* (Table 3). The roles of S compounds, such as glutathione and phytochelatin, in the detoxification of heavy metals were well documented [12,13]. The increased uptake of Mg and S may be one of the tolerance mechanisms to Mn toxicity for *P. americana*. Küpper et al. [38] also found that the concentrations of Mg and S were significantly increased in the shoots of *Arabidopsis halleri*, a Zn hyperaccumulator, due to the phytotoxicity of Cd.

Table 4
The dry weights (mg/plant) of *P. americana* treated with various Mn solutions for 15 days under hydroponic conditions

Mn (μM)	Root	Stem	Leaf
9.1	73.2 ± 19.2	77.8 ± 15.9	226 ± 4.45
500	76.2 ± 14.5	91.4 ± 18.4	256 ± 39.4
1000	77.2 ± 16.7	99.3 ± 15.2	263 ± 52.7
2000	82.5 ± 12.6	99.5 ± 14.0	291 ± 25.8
5000	78.7 ± 8.08	97.2 ± 11.9	295 ± 31.0
10000	67.2 ± 2.86	89.5 ± 9.59	279 ± 9.93
ANOVA *F* ratio Mn	0.45^{NS}	0.99^{NS}	1.70^{NS}
Error d.f.	12	12	12

3.2. Plant growth and Mn uptake under hydroponic culture conditions

Most plant species expressing tolerance to high Mn have the ability to maintain normal growth under a high shoot Mn concentration [13,33,39]. In the present study, increasing Mn supply from 9.1 to 10,000 μM did not produce any significant effect on the DM yields of *P. americana* roots, stems and leaves (Table 4). The concentrations of total and extractable Mn in stem, leaf and root of *P. americana* are shown in Fig. 2. With the increasing Mn supply from 9.1 to 10,000 μM in the nutrient solution, the concentrations of Mn in the roots, stems and leaves of *P. americana* progressively increased. The maximum concentrations of total Mn in the roots, stems and leaves reached 9310, 3550 and 11,600 mg kg^{-1} DW, respectively, when the plants were grown at 10,000 μM exogenous Mn supply. Among different organs,

Table 3
The relationship of major and trace elements in the leaves of *P. americana* from the contaminated soils

	Cd	Mn	Zn	Cu	Ti	Fe	Ca	Mg	Al	S
Mn	−0.040									
Zn	0.733**	0.256								
Cu	0.478**	0.202	0.677**							
Ti	−0.160	−0.330*	0.070	−0.203						
Fe	−0.144	−0.087	0.155	0.088	0.640**					
Ca	0.096	−0.372*	0.210	0.047	0.478**	0.282				
Mg	−0.519**	0.481**	0.148	−0.024	0.040	0.286	−0.313**			
Al	−0.257	−0.157	0.033	0.096	0.870**	0.807**	0.367*	0.204		
S	0.377**	0.454**	0.407**	0.147	0.260	0.290	−0.422**	0.003	−0.391*	
P	−0.387*	−0.143	−0.452**	−0.395*	0.008	0.058	−0.324*	0.091	0.017	0.005

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS: not significant.

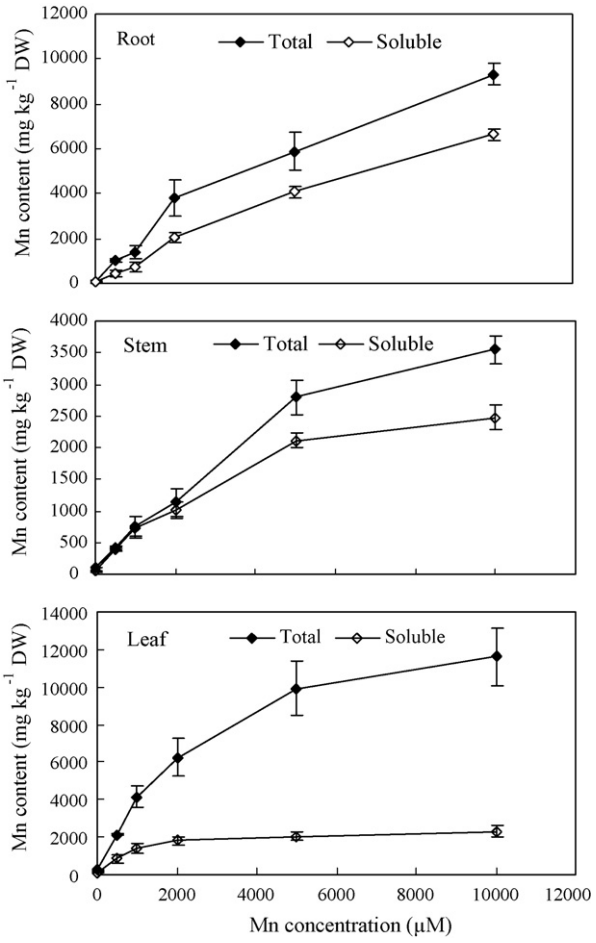

Fig. 2. The total and soluble Mn concentration of *P. americana* treated with manganese for 15 days under hydroponics conditions.

total Mn concentrations were the highest in the leaves and lowest in the stems at all levels of Mn treatments. The results further demonstrated that Mn can be efficiently absorbed by root systems of *P. americana* and transferred from roots to above-ground organs.

It has been suggested that metals are stored in water-soluble forms in the hyperaccumulators, such as *Thlaspi caerulescens*, *Berkheya coddii* and *Arabidopsis halleri* [40–42]. In the present study, the percentage of Mn that was soluble in water (1 mM MES buffer) was considerably higher in the roots than in the shoots (Fig. 2). In the roots, this percentage increased from 43% in the 500 μM Mn treatment to >70% in the 5000 μM Mn treatment (Fig. 2a), whereas in the leaves it decreased as the concentration of Mn in solution increased, in particular from 500 to 10,000 μM (Fig. 2c). In the roots, detoxification of Mn would therefore require the sequestration of soluble Mn species. It has been documented that the vacuole is the major Mn storage compartment in roots of maize [43] and Douglas fir [17]. Baldisserotto et al. [44] found large and dark vacuolar precipitates of Mn and phenolic compounds inside the upper epidermis cells of *Trapa naans*. Another possibility may be the formation of low solubility complexes between Mn and P ions inside the plants [7]. But evidence from SEM/EDAX analyses showed that there was an average vacuolar Mn concentration >500 mM in the upper-layer palisade mesophyll of *Gossia bidwillii*, a Mn hyperaccumulator [18]. In the present study, however, it is not clear which soluble or insoluble compounds are responsible for complexion with Mn in the roots and shoots of *P. americana*. Organic acids, amino acids, phytochelatins and phenolic compounds might be potential ligands for detoxification of Mn, as observed for several metals [12,13,17,44].

3.3. Interactions between Mn and Cd

Root, stem and leaf dry weights of *P. americana* plants in different Cd and Mn treatments are shown in Table 5. In general, the addition of 10 or 50 μM Cd caused leaf chlorosis and significantly decreased the biomass of root, stem and leaf. When there was no Cd supply, the addition of Mn at 1000 and 2000 μM did not have significant effect on the root, stem and leaf dry weights of *P. americana* plants, in comparison with the control group (9.1 μM Mn) (see Table 5). When the Mn concentration increased to 5000 μM, the dry weights of root, stem and leaf were significantly affected. In the presence of 10 and 50 μM Cd, increasing Mn concentration from 9.1 to 5000 μM in the solution significantly increased the root, stem and leaf biomass. No Cd toxicity symptoms were observed. Therefore, increased

Table 5
The dry weights (mg/plant) of *P. americana* treated with Cd and Mn solutions for 22 days under hydroponic conditions

Treatment (μM)	Root			Stem			Leaf		
	0 Cd	10 Cd	50 Cd	0 Cd	10 Cd	50 Cd	0 Cd	10 Cd	50 Cd
9.1 Mn	304	93.5	76.0	84.3	22.4	17.1	524	160	102
1000 Mn	372	96.5	90.2	74.1	24.6	22.2	499	209	135
2000 Mn	328	134	83.7	78.1	39.6	22.9	495	310	166
5000 Mn	210	141	112	62.4	40.7	29.2	411	332	216
ANOVA *F* ratio									
Mn		3.81*			1.72^{NS}			4.93**	
Cd		295***			318***			265***	
Mn × Cd		12.6***			8.42***			9.49***	
Error d.f.		24			24			24	
LSD ($P<0.05$)		39.7			9.1			60.5	

*$P<0.05$; **$P<0.01$; ***$P<0.001$; NS: not significant.

Table 6
The concentration of Mn (mg/kg DW) in *P. americana* treated with Cd and Mn for 22 days under hydroponics conditions

Treatment (μM)	Root			Stem			Leaf		
	0 Cd	10 Cd	50 Cd	0 Cd	10 Cd	50 Cd	0 Cd	10 Cd	50 Cd
9.1 Mn	120	56.7	52.1	38.7	nd	nd	227	154	162
1000 Mn	1630	1270	1020	900	712	874	4160	3760	4550
2000 Mn	2720	2390	2110	1050	1340	1620	7220	6330	7460
5000 Mn	6030	4800	4310	2590	3540	2840	10300	9520	10700
ANOVA *F* ratio									
Mn		408.36***			560.71***			1457.28***	
Cd		17.87***			8.26**			17.40***	
Mn × Cd		3.97**			10.43***			2.22^{NS}	
Error d.f.		24			24			24	
LSD ($P<0.05$)		529.6			268.4			562.5	

**$P<0.01$; ***$P<0.001$; NS: not significant; nd: not detectable (< the detection limit, Mn: 0.1 mg kg^{-1}).

Mn supply could alleviate the inhibiting effect of Cd on the plant growth of *P. americana*, which might be related to Mn and Cd status in the plants.

In the presence of 9.1 or 1000 μM Mn, the addition of Cd decreased Mn concentrations in the roots, stems and leaves of the plant, except a slight increase was observed in the leaves of the treatment with 50 μM Cd and 1000 μM Mn (Table 6). When the concentration of Mn was increased to 2000 and 5000 μM in solution, the addition of Cd significantly decreased Mn concentration in the roots and increased Mn concentration in the stems. For the leaves, the 10 and 50 μM Cd treatments had different effects on the concentration of Mn in plant. The addition of 10 μM Cd significantly decreased the leaf Mn concentration. In contrast, the 50 μM Cd treatment tended to increase the leaf Mn concentration. The fact that the plants supplied with 50 μM Cd produced a higher Mn concentration in the leaves than those plants with no Cd addition might be attributed to the severe biomass reduction due to the Cd toxicity (Table 6). A significant reduction of Mn uptake was also observed in other plants with Cd treatment, such as lettuce and pea [19,30]. But Ramos et al. [31] found that Mn concentration in lettuce shoots increased when the level of Cd became toxic, as a mechanism of defense against this toxicity. In that study, the concentrations of Mn and Cd in the shoots and roots were much lower than the values obtained in the present study.

The concentrations of Cd in different organs of *P. americana* were in the order: root > leaf > stem (Table 7). The concentrations of Cd in the leaves were 242 and 714 mg kg^{-1} DW, respectively, when the plants were grown in 10 and 50 μM exogenous Cd solutions with the supply of 9.1 μM Mn. The Mn and Cd concentrations reached 10,700 and 408 mg kg^{-1} DW, respectively, in the leaves of plants exposed to 5000 μM Mn with 50 μM Cd. The results showed that the maximum Cd concentration in the leaves of *P. americana* was 1150 mg kg^{-1} found in the 200 μM Cd treatment [28]. These results demonstrated that *P. americana* can hyperaccumulate not only Mn, but also Cd in the leaves. Increasing Mn supply from 9.1 to 5000 μM in nutrient solutions resulted in a progressively decreased Cd concentrations in all organs. The result indicated that Mn could inhibit Cd uptake by *P. americana*. Similar results were also observed in soybean and tomato plants [45,46]. These results suggest that the ameliorative effect of Mn on the Cd-treated plants may be due to the decreased Cd uptake by plants.

Several transporter gene families have been implicated in Mn^{2+} transport, including members of the Nramp and ZIP family, which can also transport Cd^{2+} [33]. It would therefore be

Table 7
The Cd concentration (mg/kg DW) of *P. americana* treated with Cd and Mn for 22 days under hydroponic conditions

Treatment (μM)	Root			Stem			Leaf		
	0 Cd	10 Cd	50 Cd	0 Cd	10 Cd	50 Cd	0 Cd	10 Cd	50 Cd
9.1 Mn	nd	710	1310	nd	149	437	nd	242	714
1000 Mn	nd	502	1110	nd	80.8	351	nd	176	666
2000 Mn	nd	415	1020	nd	60.8	283	nd	137	570
5000 Mn	nd	310	646	nd	39.8	172	nd	102	408
ANOVA *F* ratio									
Mn		70.53***			225.04***			114.40***	
Cd		1148.2***			2821.5***			3454.6***	
Mn × Cd		22.67***			89.53***			44.79***	
Error d.f.		24			24			24	
LSD ($P<0.05$)		88.0			17.6			30.2	

***$P<0.001$; nd: not detectable (< the detection limit, Cd: 0.1 mg kg^{-1}).

expected that Mn and Cd ions use the same transport proteins in metal uptake by *P. americana*. Further work is still needed to determine the mechanisms of Mn influx into the cell, and Mn accumulation in the leaves.

4. Conclusion

The plant species *Phytolacca americana* showed a high potential in co-accumulating high concentrations of Mn and Cd in shoots. Considering its fast growth and high biomass, this plant may be used in the remediation of Mn–Cd contaminated soils. Its practical application is worth further exploring on a field scale. The interactions between Mn and Cd in plant uptake indicated that Mn and Cd ions might use the same transport proteins for metal uptake by *P. americana*. Further study at molecular level is needed to clarify the mechanisms involved in Mn and Cd uptake, which would also provide more information to improve the efficiency of metal accumulation by the plant.

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