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Mitigation mechanism of Cd-contaminated soils by different levels of exogenous low-molecular-weight organic acids and *Phytolacca americana*

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Phytolacca americana L. (pokeweed) is a promising plant for phytoremediation of cadmium (Cd)-contaminated soil, with its large biomass and fast growth rate. Pot experiments were conducted to investigate the effects of low-molecular-weight organic acids (LMWOA) at different levels (10, 20, 30, 40 mmol kg^{-1}) on the growth, oxidative stress and antioxidant system of pokeweed. Their role in Cd transportation and accumulation and the ameliorating effects of Cd-induced oxidative stress were also studied. The results showed that the tolerance threshold of Cd stress for pokeweed was 50 mg kg^{-1} dw. Moreover, Cd transfer can be effectively enhanced from root to shoot by lower concentrations (10 and 20 mmol kg^{-1} dw) of organic acids. Cd adsorption of pokeweed was obviously increased (312.5%, 142.9%, 305.9% in leaves and 130.9%, 103.6%, 119.9% in roots, respectively) when 10 mmol kg^{-1} dw citric acid (CA), 20 mmol kg^{-1} dw malic acid (MA) and 10 mmol kg^{-1} dw oxalic acid (OA) were added to the Cd-contaminated soil. The cadmium transfer coefficient of pokeweed was found to be effectively improved by certain concentrations of LMWOA. In conclusion, this study provides an important reference for further application of the pokeweed to soil remediation.

1. Introduction

Soil contamination has been the subject of continuous environmental and human health concern and has been universally recognized in recent decades.[1,2] The decontamination of soils polluted by heavy metals, especially cadmium, is one of the most knotty problems for soil remediation.[3,4] Cadmium, as a nonessential element, is of particular concern due to its high toxicity and accessibility for concentration from soil to plants, which then further harms the food chain.[5] Besides, Cd has been documented as a human carcinogen and has become a big threat to mankind.[6] Recent news showed that the content of cadmium in rice in some areas exceeded the standard limit, which could provoke kidney damage in humans.[7] There is an urgent need to avoid Cd toxicity using effective and economical technological solutions. Phytoremediation, the use of plants to minimize damage, is known as a potential strategy that comes with many advantages such as being economical, highly effective and environmentally sustainable.[8–10] Furthermore, a special merit of phytoremediation is that the soil function can be maintained and reactivated.[11]

Phytoremediation primarily makes use of an extraordinary plant that is capable of assimilating and tolerating a large amount of heavy metals in their shoots, *i.e.*, the ability to hyperaccumulate metals.[12,13] In recent years, more and more hyperaccumulator plants have been found and used for cleaning up toxic metals such as Zn, Ni, Pb, Mn, Cu and As in soil.[14–16] Furthermore, some plants have been found to have excellent ability to accumulate Cd. *Thlaspi caerulescens*, a well-known Zn hyperaccumulator plant, has been reported to accumulate remarkably high concentrations of Cd in their aerial parts, reaching 3000 mg kg^{-1}.[17–19] Another possible Cd hyperaccumulator is *Arabidopsis halleri*, which is able to accumulate up to 6000 mg kg^{-1} in shoots under hydroponic culture conditions and more than 100 mg kg^{-1} under natural conditions.[20,21] *Rorippa globulosa*, a weed, once showed a Cd concentration of 107.0 and 150.1 mg kg^{-1} in stems and leaves, respectively, when cadmium concentrations in the soil was 25.0 mg kg^{-1}.[22,23] However, not all of the hyperaccumulators above were satisfactory for the required threshold value of phytoremediation. There are still some limiting factors of hyperaccumulators for their application in phytoremediation. For example, the low biomass of most hyperaccumulators restricts them from obtaining a relatively high total accumulation of heavy metal. Furthermore, the peculiarity of slow growth of

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these hyperaccumulators further lowers their phytoremediation efficiency.

The success of any phytoremediation technology depends on appropriate plant species that can accumulate high concentrations of heavy metals and produce sufficient biomass. However, some species recorded as Cd hyperaccumulators are not ideal for phytoremediation due to their slow growth or small biomass production. *Phytolacca americana L.* (pokeweed) is a large, semi-succulent herbaceous perennial plant with rapid clonal growth that can grow up to about 3 meters height.[24] It is widely distributed in the wild and in wastelands, with the intrinsic biological advantages of high yield, broad tolerance, and phenotypic plasticity.[24,25] In addition, previous research has shown that *P. americana* is a well-known hyperaccumulator of Mn and Cd. Other studies indicated that *P. americana* had a high potential synergistic interaction of Cd and Mn in shoots.[26] However, research on the long-term soil culture of pokeweed under cadmium stress is relatively rare. Although the pokeweed has proved to have a hyperaccumulation effect on Cd, its ability in the actual application still needs to be strengthened. To achieve this purpose, the usual strategy is to add exogenous substances, *i.e.* chelating agents.

The rational use of chemical-mobilizing agents to enhance phytoextraction of soil metals should be properly designed and ensure eco-environmental safety, because the residual metal–chelate complexes might leach into the groundwater to cause potential risks over extended periods of time.[27] Compared with chemical chelating agents, *e.g.* EDTA, EDDS *etc.*, low molecular weight organic acids (LMWOA) are friendlier to the environment due to their biodegradation without secondary pollution. Previous studies suggested that low molecular weight organic acids, especially citric, oxalic, and malic acids, are able to form soluble complexes and chelates with metal ions.[28,29] In addition, low molecular weight organic acids were confirmed to have a good effect on strengthening phytoextraction in some plants.[30] For further practical applications of pokeweed in soil remediation, the objectives of this study were to investigate the growth response of pokeweed and its Cd uptake, distribution and accumulation at varied LMWOA (citric, oxalic, and malic acids) supply levels under cadmium stress.

2. Materials and methods

2.1. Plant materials and soil preparation

The pokeweed seeds were collected from the Xiangtan Mn mine area of Hunan province, China, and then germinated in a large plastic container containing about 12 kg uncontaminated soil and keeping about 80% of the maximum field water-holding capacity for 8 weeks. The uncontaminated control soil used in this study was collected from the surface layer (0–20 cm) in Yuelu Mountain, which is located in Changsha, China. The soil samples were air-dried at room temperature and passed through a 2 mm soil sieve for analysis. The basic physicochemical properties are as follows: organic C 18.5 g kg^{-1}; total N 0.870 g kg^{-1}; total P 0.254 g kg^{-1}; total K 15.6 g kg^{-1}; CEC 16.7 cmol kg^{-1}; Cd undetected (<0.025 mg kg^{-1}).

2.2. Soil treatments and growth experiment

In the first stage, the soils were artificially contaminated by adding precise amounts of Cd with 0, 10, 30, 50 and 100 mg kg^{-1} dw soil (from solutions of $Cd(NO_3)_2 \cdot 4H_2O$). The mixed soil was allowed to equilibrate for a period of 4 weeks and then placed into plastic pots (2 kg per pot). Three homogeneous pokeweed seedlings were transplanted to each pot and grown in a greenhouse equipped with supplementary lighting in a 14 h photoperiod at 25 °C day/20 °C night and a relative humidity (RH) of 70–75%. The test plants and the corresponding soils were harvested at the 10th week.

After the first stage of the experiment, we found that 50 mg kg^{-1} dw soil is a threshold for pokeweed growth. Then, repeating some of the above steps, cadmium concentration was simulated in soil at 50 mg kg^{-1} dw. After the seedlings were transplanted into the pots for 4 weeks, the soils were treated with 0, 10, 20, 30 and 40 mmol kg^{-1} citric acid, oxalic acid and malic acid (each treatment was independently replicated three times), and the test plants and the corresponding soils were harvested at the 10th week.

2.3. Sample preparation and analysis

Upon harvest, the roots were immersed into 20 mM Na_2-EDTA for 15 minutes to remove metal ions attached to root surfaces. The harvested plants were separated into root, stem and leaf, oven-dried at 70 °C to a constant weight, and ground into powder for metal analysis. The dried plant samples were wet-digested with HNO_3–$HClO_4$ (3 : 1, v/v). After digestion, all concentrations of Cd were determined by atomic absorption spectroscopy (Analyst 700, PerkinElmer, USA).

2.4. Determination of lipid per-oxidation

An improved thiobarbituric acid method was used to determine the malondialdehyde (MDA) content of leaves.[28] Plant tissue (0.2 g) was homogenized with 10 ml 10% (w/v) trichloroacetic acid (TCA) in a mortar. The homogenate was centrifuged at 10 000 g for 10 min. Then, a 2 ml aliquot of the supernatant and 2 ml of 10% TCA containing 0.5% thiobarbituric acid (TBA) was added. The mixture was incubated at 100 °C for 10 min and then cooled quickly in an ice bath. The contents were centrifuged at 10 000 g for 15 min, and the absorbance of the supernatant was measured at 532 nm and corrected for nonspecific absorbance at 600 nm. The concentration of MDA was calculated using 155 mM^{-1} cm^{-1} as extinction coefficient.

2.5. Determination of chlorophyll content

Frozen leaf tissues were homogenized in 80% ice-cold acetone in the dark and then centrifuged at 2000 g for 10 min. Afterwards, chlorophyll content was determined spectrophotometrically on the supernatant at 646 and 663 nm, as described by Lichtenthaler.[31]

2.6. Enzyme and non-enzymatic antioxidant analysis

The activity of antioxidant enzyme peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) were determined with kits purchased from Nanjing Jian Cheng Bioengineering Institute, Nanjing, China.

2.7. Statistical analysis

All results from the experiment are presented as mean values ± S.E. of three replications. Graphical work was carried out by using Origin v.8.0. Statistical significance was conducted by *t*-test at a probability level of $P < 0.05$.

3. Results and discussion

3.1. Cd concentration in plant tissues

The Cd concentrations of various components of pokeweed are shown in Fig. 1. In the blank control group (no Cd added), the pokeweed grew well, and Cd is undetected in plant tissues after the plants were harvested at the 10th week. The average plant height in the blank control group is 15 cm taller than the Cd_{50} experiment group. This means that the metal cations such as Ca, Mg and K in soil showed no significant effect on plant growth. Besides, the background soil also showed no obvious effect on the experiment results. The concentration in leaves and roots in the lowest Cd level (10 mg kg^{-1}) treatment reached 31 mg kg^{-1} and 40.5 mg kg^{-1}, respectively. With the increase of Cd concentration, the accumulations in plant components increased simultaneously. Compared with the lowest Cd level (10 mg kg^{-1}), there was a 2.6-fold and 7.9-fold increase of Cd assimilated by plant leaves and roots, respectively, in the 100 mg kg^{-1} Cd level. These results demonstrated that pokeweed has a strong ability to assimilate and accumulate Cd ion. However, from observations during the whole experiment, toxic symptoms of stress occurred in plants when the concentration of soil cadmium reached 50 mg kg^{-1}, which became more serious with the increase of Cd concentration in soil. This illustrates that a Cd level of 50 mg kg^{-1} in soil could be the threshold for pokeweed.

Fig. 2–4 show three different irrigation solutions of LMWOAs in the soil with 50 mg kg^{-1} Cd. The concentration of Cd in plant tissues varied, correlating to the irrigation solutions in soil. Compared to the control group, the absorption of Cd by the pokeweed shoots increased 590.5%, 171.4% and 419.3%, respectively, in 10 mmol kg^{-1} citric acid, malic acid and oxalic acid. This result showed that the application of chelating agent increased Cd concentrations in some plant tissues to different degrees.

Cd accumulation was vigorously promoted by the low level (10 mmol kg^{-1} CA), with a strongly striking amount (235.25 mg kg^{-1}) in the leaves, which is 3 times compared to the control (77.50 mg kg^{-1}). When 20 mmol kg^{-1} CA was added, the concentration of Cd in plant leaves, stems and roots, respectively, are 204.78 mg kg^{-1}, 210.62 mg kg^{-1} and 280.01 mg kg^{-1}. In the same situation, 91.33 mg kg^{-1}, 154.50 mg kg^{-1}, 257.25 mg kg^{-1} and 58.54 mg kg^{-1}, 94.12 mg kg^{-1}, 198.67 mg kg^{-1} were detected in plant leaves, stems and roots, respectively, when 30 mmol kg^{-1} and 40 mmol kg^{-1} CA were added. After the four increasing citric acid (CA) levels were added, different effects on pokeweed were obvious. Results indicated that the transportation of cadmium was much easier with the presence of low concentrations of citric acid. It can be seen from the chart that CA can increase the absorption of Cd and stimulate its transportation from root to shoot.

The probable reason for the above results was that the lower concentration of citric acid formed organic complexes with Cd and increased metal mobility in the plant. Citric acid has been previously shown to chelate Cd and have an ability to abrogate phytotoxic effects. The research from Peterson and Alloway[32] indicated that organically complexed Cd was more easily translocated than equal amounts of the ionic form.[32] Besides, compared with organically complexed molecules, the free trace metal ions are more toxic.[33] However, with further increase of citric acid concentration in soil, Cd concentrations in plants tissues gradually decreased, and the absorption of cadmium was even inhibited by 40 mmol kg^{-1} CA treatment in some

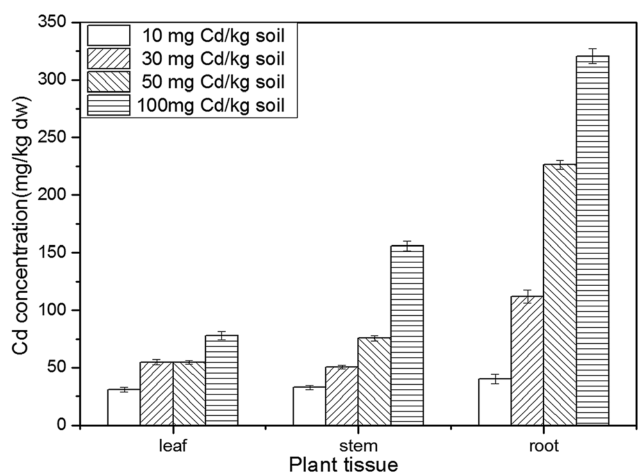

Fig. 1 Cd concentration in plant tissues, as a function of 50 mg kg^{-1} Cd concentration in soil, for plants irrigated with distilled water. Vertical bars refer to the standard error of mean values ($n = 3$).

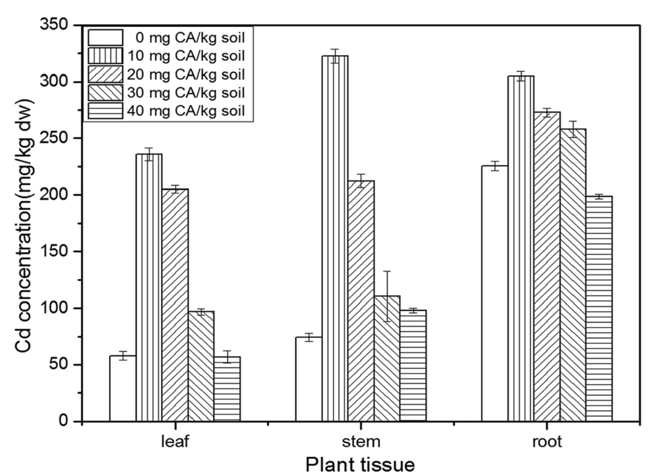

Fig. 2 Cd concentration in plant tissues, as a function of 50 mg kg^{-1} Cd concentration in soil, for plants irrigated with citric acid (CA). Vertical bars refer to the standard error of mean values ($n = 3$).

pokeweed tissues. This might be because the acid–base balance of the soil is broken by the superfluous citric acid. High concentrations of CA can increase phytotoxicity to cultivars.[34] Previous research has also indicated that the effect of citric acid on the phytoremediation of Cd-contaminated soil is mainly due to the increased mobility caused by the pH variability.[35] In the presence of an appropriate concentration of CA, the pH values decrease modestly, which increases the formation of Cd complex. Thus, the absorption of Cd significantly increased in pokeweed. In this study, pH could be lowered excessively by the application of a high concentration of citric acid, which negatively affected the absorption of Cd.

When malic acid was added at the levels of 10, 20, 30 and 40 mmol kg^{-1} dw, the amount of cadmium accumulated in plants leaves was 92.50, 108.56, 68.62 and 53.24 mg kg^{-1} dw. Compared with control group (77.5 mg kg^{-1}), the Cd adsorption increase, respectively, is 60.87%, 88.80% and 19.34% in 10, 20 and 30 mmol kg^{-1} dw malic acid. However, with the increase in concentration of citric acid to 40 mmol kg^{-1} dw, Cd adsorption in plants leaves was lowered by 7.41%. In Fig. 3, we can see that the best concentration of malic acid for enhancing phytoextraction is 20 mmol kg^{-1}. Perhaps, this optimal concentration could be partly explained by the rise of Cd content attributed to Cd absorption, which simultaneously harms the system functions upon ion absorption; thus a balanced result occurs when the supplemented Cd reaches the optimal concentration.

Fig. 4 depicts that when oxalic acid was added at a level of 10, 20, 30 and 40 mmol kg^{-1} dw, there were obvious increases in Cd adsorption in leaves and stems of pokeweed by 392.18%, 289.57%, 152.00%, 125.39% and 282.90%, 242.33%, 234.19%, 115.68%, respectively. This shows that oxalic acid has a potentiation effect on the absorption and translation of cadmium.

With the addition of low-molecular-weight organic acids (LMWOA) in soil, Cd interacts with organic ligands, which leads to the formation of mobile, organically bound Cd (Cd-citric acid, Cd-malic acid and Cd-oxalic acid). From the above results, the low concentration of organic acids can effectively enhance the absorption of cadmium by pokeweed. Besides, appropriate concentration of organic acids can also enhance the transfer of cadmium from the underground part to the aboveground. There was no obvious effect of high concentrations of organic acids on strengthening the absorption of cadmium in pokeweed; in fact, an inhibitory effect appeared when high concentrations of organic acids were added. In comparing the best addition level of the abovementioned organic acids (Fig. 5), citric acid stands out in adsorbing and transferring the most cadmium by pokeweed, followed by oxalic acid, then malic acid. In brief, an appropriate concentration of organic acids is essential in the remediation of Cd-contaminated soils by pokeweed.

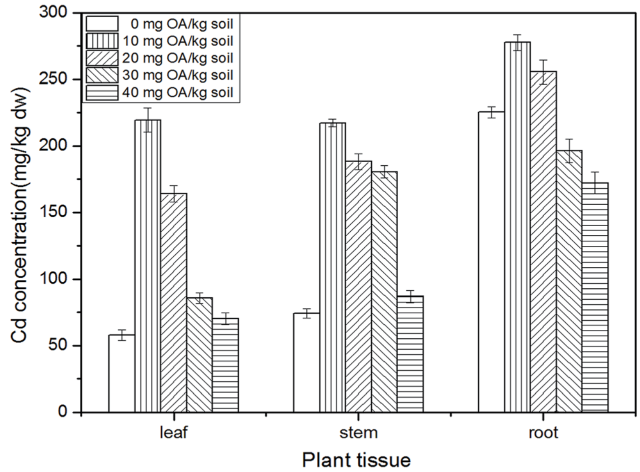

Fig. 4 Cd concentration in plant tissues, as a function of 50 mg kg^{-1} Cd concentration in soil, for plants irrigated with oxalic acid (OA). Vertical bars refer to the standard error of mean values ($n = 3$).

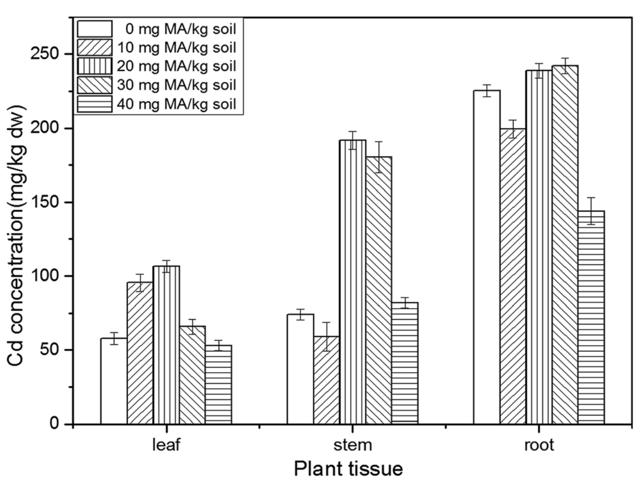

Fig. 3 Cd concentration in plant tissues, as a function of 50 mg kg^{-1} Cd concentration in soil, for plants irrigated with malic acid (MA). Vertical bars refer to the standard error of mean values ($n = 3$).

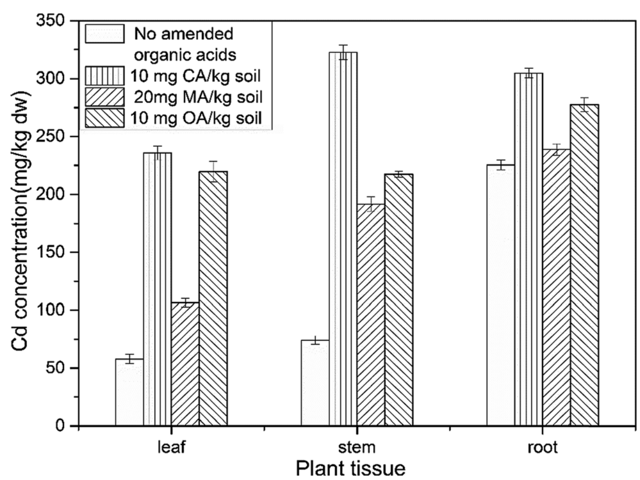

Fig. 5 Cd concentration in plant tissues, as a function of 50 mg kg^{-1} Cd concentration in soil, for plants irrigated with distilled water, 10 mmol kg^{-1} oxalic acid (OA), 20 mmol kg^{-1} malic acid (MA) and 10 mmol kg^{-1} oxalic acid (OA). Vertical bars refer to the standard error of mean values ($n = 3$).

3.2. Plant chlorophyll (Chl)

The chlorophyll (Chl) concentration (mg g^{-1}) on the basis of fresh weight (FW) is regarded as a key indicator of plant growth and tolerance to metal stress in the environment. As shown in Table 1, with the addition of 10 mg kg^{-1} of Cd, the chlorophyll content was increased compared with the blank control group. However, it was decreased strongly when the cadmium concentration was over 30 mg kg^{-1}. The chlorophyll content can be used as an obvious symptom to monitor the Cd-induced damage in pokeweed. After organic acids were added, the Chl a, Chl b, and total chlorophyll concentration of pokeweed were changed by varying degrees when compared with the control group. LMWOA in low concentration (10 and 20 mmol kg^{-1}) can alleviate cadmium stress and increase the chlorophyll content, while higher treatment levels of LMWOA (30 and 40 mmol kg^{-1}) increased stress in plants. Besides, there was less chlorophyll content compared to the control group. In addition, chlorophyll content represents plant photosynthesis. Heavy metal stress resulted in the decrease of chlorophyll, thus limiting plant growth and plant photosynthesis.

3.3. Lipid per-oxidation

The determination of MDA can offer a facile means of assessing lipid per-oxidation in biological materials. Variations in the MDA content are presented in Fig. 6 and 7. The trend of MDA content was found to be consistent with the variation of Cd concentration. High concentrations of Cd treatment contribute to the increase in MDA content tremendously. Such signs could partly illuminate the high concentration of cadmium stress stimulated and the resulting per-oxidative damage of membranes in pokeweed.

Preliminary exogenous treatments attenuated the effect of this stress. Compared with the control group, the MDA content was reduced by 38.2%, 42.9%, 44.5% in 10 mmol kg^{-1} dw CA, 20 mmol kg^{-1} dw MA, and 10 mmol kg^{-1} dw OA group, respectively. This indicated that 10 mmol kg^{-1} dw OA provided a greater ability to alleviate lipid per-oxidation. However, there was no apparent distinction among the MDA contents between high exogenous concentration treatment groups and the Cd control group. These results suggested that an appropriate concentration of low molecular organic acids might mitigate Cd-induced oxidative stress in plant due to the formation of less toxic organic acid–Cd chelates.

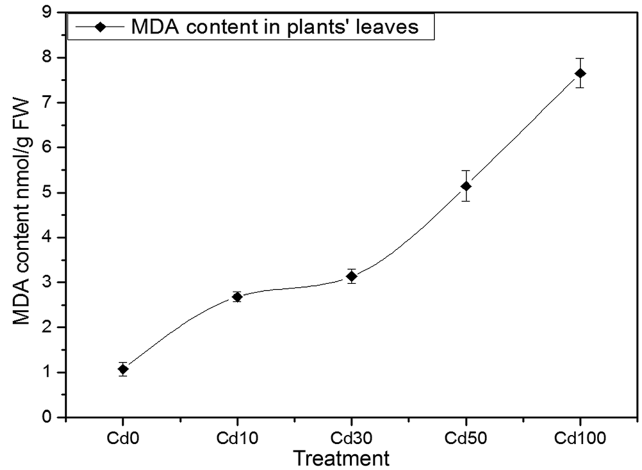

Fig. 6 MDA content in leaves of pokeweed exposed to different levels of Cd. Vertical bars refer to the standard error of mean values ($n = 3$).

Table 1 The chlorophyll content (mg g^{-1} FW) in the leaves of *P. americana* under different treatments

	The chlorophyll (Chl) concentration (mg g^{-1} FW)		
Treatment	Chl a	Chl b	Chl (a + b)
Cd_0	1.37 ± 0.12	0.65 ± 0.05	1.92 ± 0.17
Cd_{10}	1.38 ± 0.11	0.67 ± 0.06	2.06 ± 0.17
Cd_{30}	1.33 ± 0.15	0.64 ± 0.05	1.97 ± 0.20
Cd_{50}	1.26 ± 0.13	0.46 ± 0.04	1.71 ± 0.17
Cd_{100}	0.91 ± 0.09	0.18 ± 0.02	1.08 ± 0.11
$Cd_{50} + CA_{10}$	1.67 ± 0.15	0.75 ± 0.08	2.32 ± 0.23
$Cd_{50} + CA_{20}$	1.53 ± 0.13	0.66 ± 0.05	2.19 ± 0.18
$Cd_{50} + CA_{30}$	1.52 ± 0.12	0.53 ± 0.03	2.08 ± 0.15
$Cd_{50} + CA_{40}$	1.42 ± 0.11	0.25 ± 0.01	1.67 ± 0.12
$Cd_{50} + MA_{10}$	1.21 ± 0.08	0.59 ± 0.05	1.83 ± 0.13
$Cd_{50} + MA_{20}$	1.42 ± 0.14	0.68 ± 0.05	2.1 ± 0.19
$Cd_{50} + MA_{30}$	1.11 ± 0.10	0.57 ± 0.04	1.68 ± 0.14
$Cd_{50} + MA_{40}$	0.95 ± 0.07	0.53 ± 0.06	1.53 ± 0.13
$Cd_{50} + OA_{10}$	1.56 ± 0.16	0.74 ± 0.08	2.31 ± 0.24
$Cd_{50} + OA_{20}$	1.46 ± 0.13	0.72 ± 0.07	2.17 ± 0.20
$Cd_{50} + OA_{30}$	1.40 ± 0.12	0.62 ± 0.05	2.06 ± 0.17
$Cd_{50} + OA_{40}$	1.21 ± 0.11	0.52 ± 0.03	1.73 ± 0.14

3.4. Antioxidant system

A previous study indicated that the toxicity of Cd uptaken by plants may be attributed to oxidative damage caused by reactive oxygen species (ROS).[36] ROS includes the superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2).[37] ROS production induced by Cd is generally deduced from alterations in the antioxidant system. Antioxidant enzymes and certain

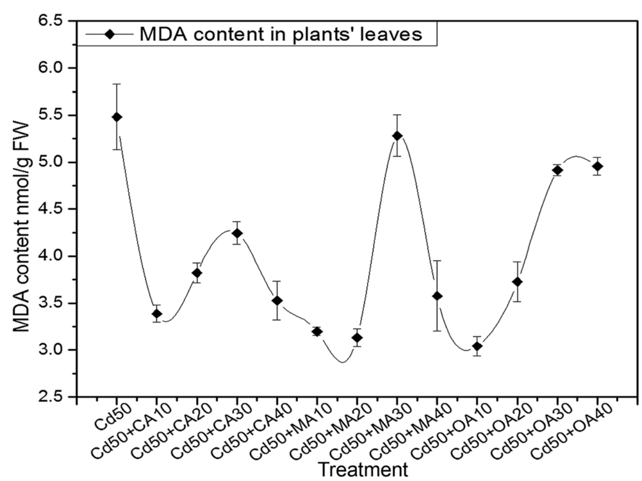

Fig. 7 MDA content in leaves of pokeweed exposed to different levels of citric, malic and oxalic acid application at 50 mg kg^{-1} Cd level. Vertical bars refer to the standard error of mean values ($n = 3$).

metabolites play a significant role in the plant resistance against Cd-induced oxidative stress. To scavenge ROS, plants possess an ordered antioxidative defense system, which contains enzymatic and non-enzymatic antioxidants.

As shown in Fig. 8 and 9, there was an increase of superoxide dismutase (SOD) activity in plants' leaves with increased cadmium concentration until 50 mg kg^{-1}. Within this range, the active oxygen removal system was provoked in plant. But further increased level (100 mg kg^{-1}) of Cd resulted in a slight reduction of SOD. This may come from the excessive accumulation of $O_2^{\bullet -}$, which affected the structure and functions of SOD. A certain degree of cadmium stress in plants has been proven to contribute to the production of superoxide, and it can bring about the activation of existing enzyme pools or increased expression of genes encoding SOD.[38] However, different changes happened when different levels of LMWOA were added. The results showed that SOD activity increased with the decreased concentration of organic acid. This may be related to the fact that the proper concentration of organic acids effectively improved the pokeweed plant's ability to scavenge free radicals. It is probable that the detoxification in the plant is due to SOD shifting the superoxide radical ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2), thus easing per-oxidation of membrane lipids and sustaining the stability of the cell membrane, which serves as the first line for scavenging ROS to evade excess oxidative impairment.[39] Besides, plants are dependent on the enzyme superoxide dismutase (SOD) to detoxify this reactive oxygen species.[40] Therefore, the enhanced SOD activity could provide a more powerful capacity for the plant to scavenge ROS, which then elevates its tolerance against Cd-induced stress.

POD and CAT can be instrumental in decreasing H_2O_2 accumulation and maintaining cell membrane integrity by eliminating MDA. From the general trend in Fig. 10–13, POD and CAT activities showed analogous descending tendency despite some differences between them. There was an increase of peroxidase (SOD) and catalase (CAT) activity in leaves at the Cd treatment level of 30 mg kg^{-1}, but the increase was reversed when Cd concentration reached 50 mg kg^{-1}. This could be attributed to the low concentration of cadmium causing little stress on the growth of plants, while the high levels of cadmium caused more severe stress to the plant so that the enzyme mechanism in the plant was adversely affected, and thus also its stress resistance. In normal conditions, there is dynamic equilibrium between the production and cleaning of reactive oxygen species in plants. Free oxygen radicals will increase and bring about cell membrane per-oxidation when plants are under coercion or aging conditions. When plants suffer from low concentrations of cadmium, the activity of POD and CAT increases, mainly due to induction and speeding up of the physiological characteristics of resistance in the plant. However, with a continued rise in Cd concentration, the activity of CAT and POD was gradually reduced, which may be the result of

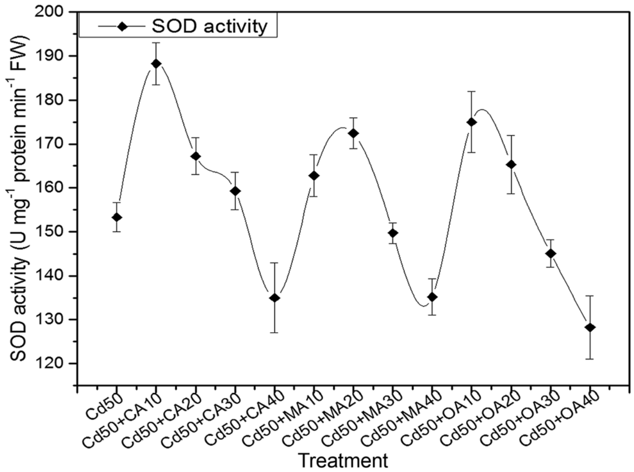

Fig. 9 SOD activities in leaves of pokeweed exposed to different levels of citric, malic and oxalic acid application at 50 mg kg^{-1} Cd level. Vertical bars refer to the standard error of mean values ($n = 3$).

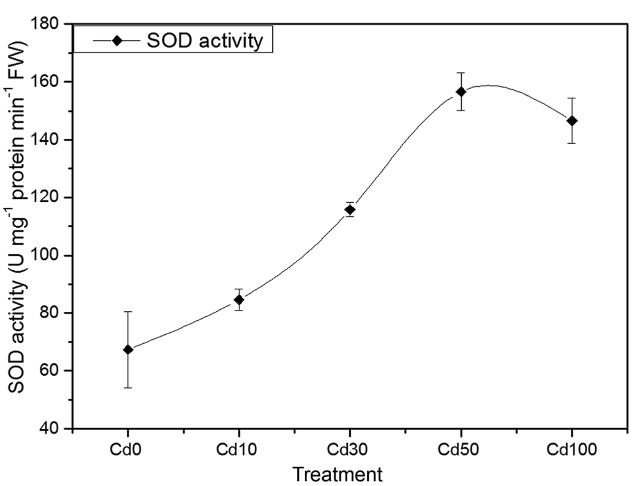

Fig. 8 SOD activities in leaves of pokeweed exposed to different levels of Cd. Vertical bars refer to the standard error of mean values ($n = 3$).

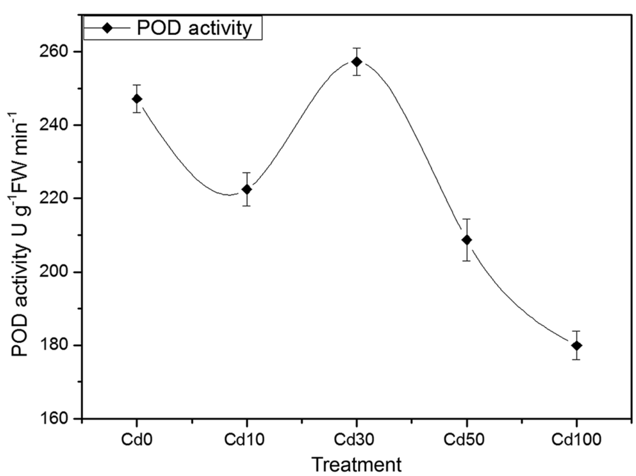

Fig. 10 POD activities in leaves of pokeweed exposed to different levels of Cd. Vertical bars refer to the standard error of mean values ($n = 3$).

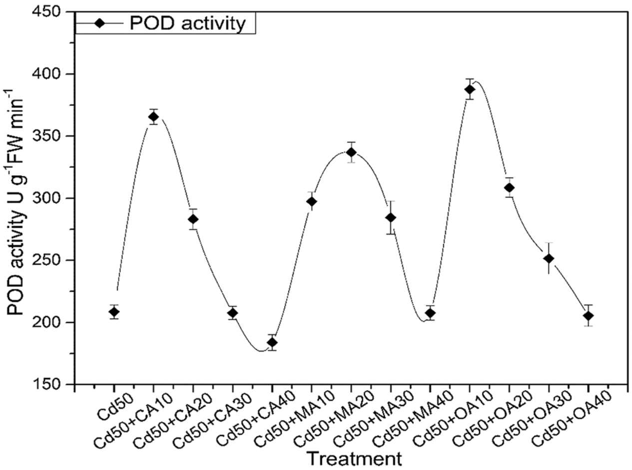

Fig. 11 POD activities in leaves of pokeweed exposed to different levels of citric, malic and oxalic acid application at 50 mg kg^{-1} Cd level. Vertical bars refer to the standard error of mean values ($n = 3$).

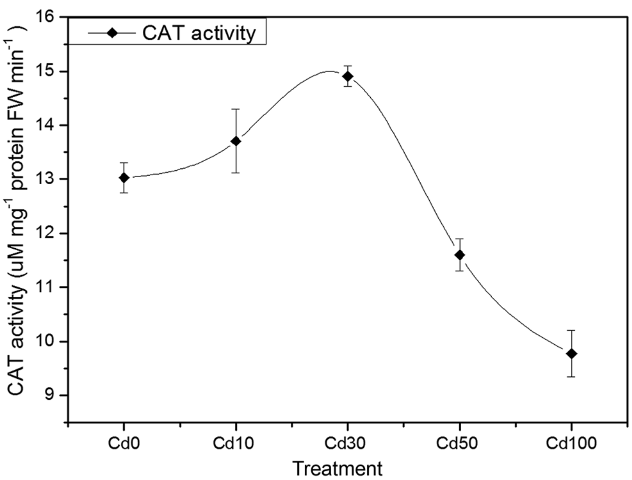

Fig. 12 CAT activities in leaves of pokeweed exposed to different levels of Cd. Vertical bars refer to the standard error of mean values ($n = 3$).

cadmium toxicity, which enhanced the deactivation and degeneration of POD and CAT.

Furthermore, there are various changes in POD and CAT activity when the plant is irrigated with different levels of LMWOA. In general, low levels of organic acid exhibited better alleviation, especially with 10 mmol kg^{-1} dw CA. Compared to the control group, POD and CAT activity were increased by 13.8% and 75.5%, respectively. However, the high concentration of organic acid resulted in a decline of POD and CAT activity in pokeweed when compared to the control group. This might be because exogenous addition of organic acids strengthened the decomposition process of H_2O_2 into water and oxygen, facilitated the efficiency of scavenging ROS, and alleviated oxidative stress from Cd. However, high concentrations of organic acid might have affected the soil pH value, placing the plants under more serious stress and causing the inhibition of

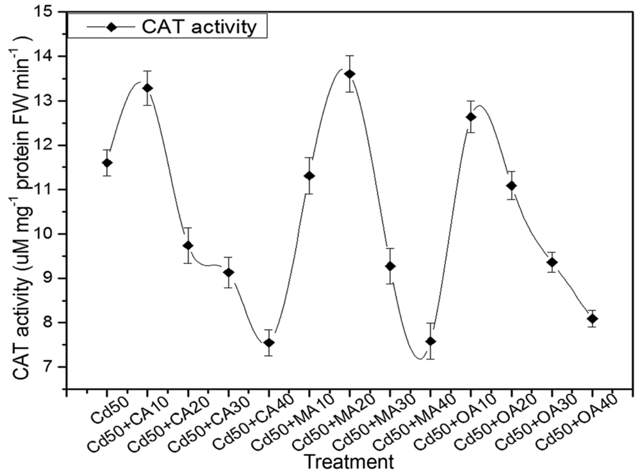

Fig. 13 CAT activities in leaves of pokeweed exposed to different levels of citric, malic and oxalic acid application at 50 mg kg^{-1} Cd level. Vertical bars refer to the standard error of mean values ($n = 3$).

enzyme synthesis or a modification in the assemblage of enzyme subunits.

4. Conclusions

Pokeweed began to show obvious toxic symptoms at 50 mg kg^{-1} when Cd concentration was increased from 10 to 100 mg kg^{-1}. Malondialdehyde (MDA) and superoxide dismutase (SOD) were markedly increased in the plant at the lower level. When it was exposed to high levels of cadmium, pokeweed showed a high accumulation of Cd, which indicated the great potential of this plant in the remediation of Cd-contaminated soils. Optimal growth of pokeweed was observed when the soil was irrigated with 10 mmol kg^{-1} CA. The chart showed that CA enhanced phytoextraction in pokeweed and stimulated transportation of Cd from root to shoot. This study showed that low concentrations of exogenous citric acid (10 mmol kg^{-1}), malic acid (20 mmol kg^{-1}) and oxalic acid (10 mmol kg^{-1}) had a better capability of enhancing Cd uptake and transport, alleviated the physiological toxicity of Cd, and had great effect on the availability of cadmium in the soils. Compared with malic acid and oxalic acid, citric acid proved to be a better choice in alleviating the toxic effect of cadmium and enhancing the adsorption of cadmium by pokeweed. Additionally, the results of this experiment offer a potential illustration for how exogenous organic acid ameliorates Cd-induced *Phytolacca americana L.* (pokeweed) growth inhibition and verifies its role in repairing heavy metal-contaminated areas. However, deeper experiments on the molecular and genetic level are needed in further investigations.

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