RSC Advances

Mitigation Mechanism of Cd Contaminated Soils by Different Levels of Exogenous Low-molecular-weight Organic Acids with *Phytolacca Americana*

Huan Liu[a,b], Yun-guo Liu[a,b,*], Guang-ming Zeng[a,b], Jie-li Xie[a,b], Bo-hong Zheng[c], Xiao-fei Tan[a,b], Da-Fei Wang[a,b], Zhi-chao Sun[a,b], Jian Nie[a,b], Zheng-jiang Jiang[a,b], Chao Gan[a,b], Wei Liu[a,b], Shu-fan Wang[a,b]

[a] College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China

[b] Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, PR China

[c] School of Architecture and Art Central South University, Changsha 410082, PR China

*Corresponding author: Yun-guo Liu; Tel.: + 86 731 88649208; Fax: + 86 731 88822829; E-mail address: Liuyunguohnu@163.com

Abstract

Phytolacca americana L. (pokeweed) is a promising plant for phytoremediation on cadmium (Cd) contaminated soil with 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 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. And the Cd translation can be effectively enhanced from root to shoot by lower concentration (10 and 20 mmol kg^{-1} dw) of organic acids. Cd adsorption of pokeweed were obvious 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 concentration of LMWOA. In conclusion, this study provided an important reference for further application of the pokeweed to the actual soil remediation.

Key words: Phytoremediation; Pokeweed; Cadmium; LMWOA; Soil contamination

1. Introduction

Soil contamination has been the subject of continuous environmental and human health concern which is 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 poisonousness and accessibility to be concentrated from soil to plants, then further does harm to the food chain.[5] Besides, Cd has been documented as a human carcinogen and became 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 to humans.[7] There is an urgent need to avoid Cd toxicity with effective and economical technological solution. Phytoremediation was known as a potential strategy using plants to minimize the damage which comes with many advantages such as economically applicable, high-effective and environmentally sustainable.[8-10] Furthermore, a special merit of phytoremediation is that the soil functioning can be maintained and reactivated.[11]

Phytoremediation is primarily make use of an extraordinary plants which is capable of assimilating and tolerating a large amounts of heavy metals in their shoots, i.e. hyperaccumulation of metals.[12, 13] In recent years, more and more hyperaccumulator plants were found and used for cleaning up toxic metals such as Zn, Ni, Pb, Mn, Cu and As in soil.[14-16] And there are some plants that have been found to have excellent ability of accumulating Cd. *Thlaspi caerulescens* as a proverbial Zn hyperaccumulator plant, have been reported to accumulate remarkable 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* as a weed plant once showed the concentration of Cd with 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 these so-called hyperaccumulators above were satisfied with the required threshold value of phytoremediation. There are still some limiting factors of hyperaccumulators for the application on phytoremediation. For example, the low biomass of most hyperaccumulators restricts them to obtain a relatively high total accumulation of heavy metal. And the peculiarity of slow-growing of hyperaccumulators further lowers the 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, there are some species recorded as Cd hyperaccumulators which 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] Besides, they are widely distributed in wild and wasteland with the intrinsic biological advantages of high yield , broad tolerance, and phenotypic plasticity.[24, 25] In addition, previous research has shown that *P. americana* was a well-known hyperaccumulator to Mn and Cd. Another studies indicated that *P.americana* had a high potential synergistic interaction of Cd and Mn in shoots.[26] However, the long-term soil culture of pokeweed under cadmium stress is relatively rare. Although the pokeweed proved to have super accumulation effect on Cd, but there still needs to find a way to strengthen its ability in the actual application. In order to achieve this purpose, the normal way is to add exogenous substances, i.e. chelating agent.

The rational use of chemical-mobilizing agents to enhance phytoextraction of soil metals should be properly designed and make sure the eco-environmental safety since the residual metal-chelatecomplexes might leach into the groundwater to cause potential risks over extended periods of time.[27] Compared with chemical chelating agent i.e. EDTA, EDDS etc, the low molecular weight organic acids (LMWOA) are more friendly to environment due to their biodegradation and non-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 was confirmed to have a good effect on strengthening phytoextraction by 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 seeds of pokeweed were collected from 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 (0-20cm) in Mountain-Yuelu, which was located in Changsha, China. The soil samples were air-dried at room temperature and passed through a 2mm soil sieve for analysis. The basic physicochemical properties were listed 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 contaminated artificially by adding precisely 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 put to equilibrate for a period of 4 weeks and then put into plastic pots (2 kg per pot). Three homogeneous seedlings of Pokeweed were transplanted to each pot and grown in a greenhouse equipped with supplementary lighting in a 14-h photoperiod at 25 ºC day/20 ºC night and a relative humidity (RH) of 70–75%. The test plants and the corresponding soils were harvested at the10th week.

After the experiment of first stage, we found that 50 mg kg^{-1} dw soil is a threshold for Pokeweed growth. Then repeating some of the above steps, simulation of cadmium concentration in soil was 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 the10th 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 attaching to root surfaces. The harvested plants were separated into root, stem and leaf, oven dried at 70 ºC 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, Perkin Elmer, USA).

2.4. Determination of lipid peroxidation

The improvement of 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 the mortar. The homogenate was centrifuged at 10,000 g for 10 min. Then 2 ml of the aliquot of the supernatant, 2 ml of 10% TCA containing 0.5% thiobarbituric acid (TBA) was added. The mixture was incubated at 100 ºC for 10 min and then cooled quickly in an ice-bath. The contents were centrifuged at 10000 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 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) content were determined with kits purchased from Nanjing Jian Cheng Bioengineering Institute, Nanjing, China.

2.7. Statistical analysis

All results from the experiment were 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 showed in Fig. 1. In the blank control group (no Cd was added), the pokeweed grew well and Cd is undetected in plant tissues after the plant were harvested at the10th week. The average plant height in 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, as background soil, it is also showed no obvious effect on the experiment results. The concentration in leaves and roots in lowest Cd level (10 mg kg^{-1}) treatment reached to 31 mg kg^{-1} and 40.5 mg kg^{-1} respectively. With the increase of Cd concentration, the accumulations in plant components increased simultaneously. It is respectively 2.6-fold and 7.9-fold increase of Cd assimilated by plant leaves and roots in 100 mg kg^{-1} Cd level compared with the lowest Cd level (10 mg kg^{-1}). These results demonstrated that pokeweed is of strong ability to assimilate and accumulate Cd ion. However, from the observation during the whole experiment, toxic symptoms of stress occurred in plants when the concentration of soil cadmium reached to 50 mg kg^{-1}, which became more serious with the increase of Cd concentration of soil. This illustrated that the Cd level at 50 mg kg^{-1} in soil could be the threshold to pokeweed.

Fig. 2 to Fig. 4 showed three different irrigation solutions of LMWOAs in the soil of 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 shoots of pokeweed increased 590.5%, 171.4% and 419.3% respectively in the level of 10 mmol kg^{-1} citric acid, malic acid and oxalic acid. The result showed that the application of chelating agent increased the Cd concentrations in some of plants tissues to different degrees.

The Cd accumulation was vigorously promoted by the low level (10 mmol kg^{-1} CA) with a strongly striking amount (235.25 mg kg^{-1}) by the leaves, which was 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 obviously found. It indicated that the transportation of cadmium was much easier with the existence of low concentration of citric acid. It can be seen from the chart that CA can increased the absorption of Cd and stimulated their transportation from root to shoot.

The probable reason of above results was that the lower concentration of citric acid formed organically complexes with Cd and increased the metal mobility in plant. Citric acid has been studied previously to chelating Cd and once showed its remission ability to phytotoxic effects. The research from Peterson and Alloway [32]indicated that organically complexed Cd was more easily translocated than semblable amounts of the ionic form.[32] Besides, compared with organically complexed molecules, the free trace metal ions are more toxic.[33] However, by further increasing of citric acid concentration in soil, the Cd concentrations in plants tissues gradually decreased. And the absorption of cadmium even inhibited by 40 mmol kg^{-1} CA treatment in some tissues of pokeweed. This might be because the acid-base balance of the soil is broken by the superfluous citric acid. High concentration of CA can increase the phytotoxicity to cultivars.[34] And former research 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 appropriate concentration of CA, the pH values decreases modest that increased the formation of Cd complex. Thus the absorption of Cd significantly increased in pokeweed. In this study, pH could be lowered excessively by application of high concentration of citric acid that negatively affected the absorption of Cd.

When the malic acid was added at the level 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 increased respectively are 60.87%, 88.80% and 19.34% in the level of 10, 20 and 30 mmol kg^{-1} dw malic acid. However, with the increasing concentration of citric acid to 40 mmol kg^{-1} dw, the Cd adsorptions in plants leaves were lowered by 7.41%. In the Fig. 3, we can see that the best concentration of malic acid in enhancing phytoextraction is 20 mmol kg^{-1}. Perhaps, the existence of the optimal concentration could be partly explained that the rise of Cd content contributes to the Cd absorption, and simultaneously harms the system functions in ion absorption, it obtains a balanced result when the Cd supplemented reaches to the optimal concentration.

Fig. 4 depicted that when the oxalic acid was added at a level of 10, 20, 30 and 40 mmol kg^{-1} dw, there are obvious increases of Cd adsorption in leaves and stems of pokeweed by 392.18.6%, 289.57%, 152.00%, 125.39% and 282.90%, 242.33%, 234.19%, 115.68% respectively. This shows that oxalic acid have a potentiation effect of absorption and translation of cadmium.

With the addition of low-molecular-weight organic acids (LMWOA) in soil, there is an interaction of Cd with organic ligands which lead to the formation of mobile organically-bound Cd (Cd-citric acid, Cd-malic acid and Cd- oxalic acid). From the above results, low concentration of organic acids can effectively enhances the absorption of cadmium by pokeweed. Besides, appropriate concentration of organic acids can also enhance the transfer of cadmium from underground part to the ground. While there was no obvious effect of the high concentration of organic acids on strengthening the absorption of cadmium in pokeweed, even more, inhibitory effect appeared when high concentration of organic acids were added. By comparing the best addition level of above several organic acids in Fig. 5, citric acid stood out in adsorbing and transferring of cadmium by pokeweed, oxalic acid followed by, then is malic acid. In brief, appropriate concentration of organic acids is very essential in the remediation of Cd contaminated soils by pokeweed.

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 environments. 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. But it was decreased straightly when the cadmium concentration 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 Chla, Chlb, and total chlorophyll concentration of pokeweed were changed with 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 content of chlorophyll, while higher treatment levels of LMWOA (30 and 40 mmol kg^{-1}) increased stress in plants. Besides, there was less chlorophyll content compared to control group. In addition, chlorophyll content is the symbol of photosynthesis of plant. Heavy metal stress resulted in the decrease of chlorophyll, and then limiting plant growth and plant photosynthesis.

3.3 Lipid peroxidation

The determination of MDA can offer a facile means of assessing lipid peroxidation in biological materials. Variations in the content of MDA are presented in Fig.6 and Fig.7. The trend of MDA content was found consisting with the vibration of Cd concentration. High concentration of Cd treatment contributes to the increase of MDA content tremendously. Such signs could partly illuminate the high concentration of cadmium stimulated stress production and produced peroxidative damage of membranes in pokeweed.

Preliminary supply of exogenous treatment 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. It indicated that 10 mmol kg^{-1} dw OA provided a more doughty ability to alleviate the lipid peroxidation. However, there was no apparent distinction about the MDA contents between high concentration of exogenous treatment group and Cd control group. These results suggested that appropriate concentration of low molecular organic acids might mitigate Cd-induced oxidative stress in plant due to the formation of less toxicity of organic acid-Cd chelates.

3.4. Antioxidant system

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

As shown in Fig. 8 and Fig. 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 came from the excessive accumulation of $O_2\cdot^{-}$, which affected the structure and functions of SOD. A certain degree of cadmium stresses in plant has been proved to contribute to the production of superoxide, and it can bring about the activating of existing enzyme pools or increasing expression of genes encoding SOD.[38] However, different changes happened when different levels of LMWOA were added. The results showed that the SOD activity increased with the decrease in the concentration of organic acid. This may be related to that the proper concentration of organic acids effectively improved the ability of scavenging free radicals in pokeweed. It is proverbial that the detoxification in plant that SOD can shift superoxide radical ($O_2\cdot^{-}$) to hydrogen peroxide (H_2O_2), ease peroxidation of membrane lipids and sustain the stability of cell membrane, which served as the first line of scavenging ROS to evade excess oxidative impairment.[39] Besides, the enzyme superoxide dismutase (SOD) are considered to be the dependent of plants to detoxify this reactive oxygen species.[40] Therefore, the enhanced SOD activity could provide a more powerful capacity of plant to scavenge the ROS, and then elevate the tolerance against Cd-induced stress.

POD and CAT can be instrumental in decreasing H_2O_2 accumulation and maintaining cell membrane integrity through eliminating MDA. From the general trend on the Fig. 10 to Fig. 13, POD and CAT activities showed analogous descending tendency despite there are some differences between them. There was an increase of peroxidase (SOD) and catalase (CAT) activity in leaves at the Cd treatment level at 30 mg kg^{-1}, but the increase was reversed when the Cd concentration reached at 50 mg kg^{-1}. This could be attributed to that the low concentration of cadmium caused little stress on the growth of plants, while high levels of cadmium caused more severe stress to the plant so that the mechanism of enzyme in plant was being adversely affected by resisting the stress. In normal conditions, there is dynamic equilibrium between the production and cleaning of reactive oxygen species in plant. While free oxygen radicals will increase and bring about cell membrane peroxidation when plants under coercion or aging condition. When plants suffer from low concentration of cadmium, the changes in the activity of POD and CAT increased, this mainly due to the physiological characteristics of resistantance in the plant was induced and speeded up by the way of increasing the CAT and POD activity. However, with continue rising of Cd concentration, the activity of CAT and POD reduced gradually, this may be the result of the toxicity of the cadmium which enhanced the deactivation and degeneration of POD and CAT.

Furthermore, there are various changes in POD and CAT activity when plant irrigated with different levels of LMWOA. In general, the low level of organic acid exhibited a better alleviation, especially when 10 mmol kg^{-1} dw CA was exogenously added. Compare to the control group, the POD and CAT activity was increased by 13.8 % and 75.5 % respectively. But 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 properly because that exogenous addition of organic acids strengthened the decomposition process of H_2O_2 into water and oxygen and facilitated the efficiency of scavenging ROS and alleviated oxidative stress from Cd. However, high concentration of organic acid might affect the soil pH value, make the plants under more serious stress and cause the inhibition of 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 exposed from 10 to 100 mg kg^{-1}. Malondialdehyde (MDA) and superoxide dismutase (SOD) was markedly increased in plant at this level. When it was exposed to high levels of cadmium, pokeweed showed high accumulation which indicated a 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 to pokeweed, and stimulated their transportation from root to shoot. It turned out from this study that low concentration 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, and alleviating the physiological toxicity from Cd and showing the effect on the availability of cadmium in the soils. Compared with malic acid and oxalic acid, citric acid is 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 offered potential illustration on how exogenous organic acid ameliorated Cd-induced *Phytolacca americana L.* (pokeweed) growth inhibition and verified its role in repairing heavy metal contaminated areas. However, deeper experiments about the molecular and genetic level are needed in the further investigation.

Acknowledgements

The authors would like to thank financial support from the National Natural Science Foundation of China (Grant No. 41271332 and 51478470), and the Fundamental Research Funds for the Central University, Hunan University.

References

1. I. Raskin, R. D. Smith and D. E. Salt, *Curr. Plant Sci. Biotechnol. Agric.*, 1997, 8, 221-226.
2. G. Saba, A. H. Parizanganeh, A. Zamani and J. Saba, *Int. J. Environ. Res.*, 2015, 9, 309-316.
3. A. Baker, S. McGrath, C. Sidoli and R. Reeves, *Resour. Conserv. Recy.*, 1994, 11, 41-49.
4. Y. Sun, G. Sun, Y. Xu, L. Wang, X. Liang and D. Lin, *Geoderma*, 2013, 193, 149-155.
5. K. Vig, *Adv. Environ. Res.*, 2003, 8, 121-135.
6. S. Satarug, J. R. Baker, P. E. Reilly, M. R. Moore and D. J. Williams, *Arch. Environ. Health,* 2002, 57, 69-77.
7. M. Naseri, A. Vazirzadeh, R. Kazemi and F. Zaheri, *Food Chem.*, 2015, 175, 243-248.
8. N. Witters, R. Mendelsohn, S. Van Passel, S. Van Slycken, N. Weyens, E. Schreurs, E. Meers, F. Tack, B. Vanheusden and J. Vangronsveld, *Biomass Bioenergy*, 2012, 39, 470-477.
9. G. E. Boyajian and L. H. Carreira, *Nat. Biotechnol.*, 1997, 15, 127-128.
10. H. Ali, E. Khan and M. A. Sajad, *Chemosphere*, 2013, 91, 869-881.
11. S. Trapp and U. Karlson, *J. Soil. Sediment*, 2001, 1, 37-43.
12. R. L. Chaney, M. Malik, Y. M. Li, S. L. Brown, E. P. Brewer, J. S. Angle and A. J. Baker, *Curr. Opin. Biotechnol.*, 1997, 8, 279-284.
13. S. Wei, S. Wang, Y. Li and J. Zhu, *J. Soil. Sediment*, 2013, 13, 1069-1074.
14. J. Qu, C. Luo, Q. Cong and X. Yuan, *Environ. Chem. Lett.*, 2012, 10, 153-158.
15. Z. T. Xiong, *Bull. Environ. Contam. Toxicol.*, 1998, 60, 285-291.
16. A. Baker and R. Brooks, *Biorecovery.*, 1989, 1, 81-126.
17. R. R. Brooks, M. F. Chambers, L. J. Nicks and B. H. Robinson, *Trend. Plant Sci.*, 1998, 3, 359-362.
18. J. Escarré, C. Lefèbvre, W. Gruber, M. Leblanc, J. Lepart, Y. Rivière and B. Delay, *New Phytol.*, 2000, 145, 429-437.
19. J. Yanai, F. J. Zhao, S. P. McGrath and T. Kosaki, *Environ. Pollut.*, 2006, 139, 167-175.
20. H. Küpper, E. Lombi, F.-J. Zhao and S. P. McGrath, *Planta*, 2000, 212, 75-84.
21. V. Bert, I. Bonnin, P. Saumitou-Laprade, P. De Laguérie and D. Petit, *New Phytol.*, 2002, 155, 47-57.
22. S. Wei and Q.-X. Zhou, *Environ. Sci. Pollut. R.*, 2006, 13, 151-155.
23. Y. Sun, Q. Zhou, S. Wei and L. Ren, *Front. Env. Sci. Eng.*, 2007, 1, 107-113.
24. Q.-w. Yang, Q. Zeng, F. Xiao, X.-l. Liu, J. Pan, J.-f. He and Z.-y. Li, *Environ. Earth Sci.*, 2012, 68, 1127-1134.

25. C. M. Dou, X. P. Fu, X. C. Chen, J. Y. Shi and Y. X. Chen, *Plant Biology*, 2009, 11, 664-670.
26. K. Peng, C. Luo, W. You, C. Lian, X. Li and Z. Shen, *J. Hazard. Mater.*, 2008, 154, 674-681.
27. B. Nowack, R. Schulin and B. H. Robinson, *Environ. Sci. Technol.*, 2006, 40, 5225-5232.
28. A. Chaoui, S. Mazhoudi, M. H. Ghorbal and E. El Ferjani, *Plant Sci.*, 1997, 127, 139-147.
29. A. Schneider and N. Christophe, J. Environ. Qual., 2011, 40, 1857-1862.
30. C. Chigbo and L. Batty, *Water, Air, Soil Pollut.*, 2015, 226, 1-10.
31. H. K. Lichtenthaler, *Methods enzymol*, 1987, 148, 350-382.
32. P. Peterson and B. Alloway, *Top. Environ. Sci..*, 1979.
33. N. Hue, G. Craddock and F. Adams, *Soil Sci. Soc. Am.J.*, 1986, 50, 28-34.
34. C. Turgut, M. K. Pepe and T. J. Cutright, *Environ. Pollut.*, 2004, 131, 147-154.
35. Y. Chen, Q. Lin, Y. Luo, Y. He, S. Zhen, Y. Yu, G. Tian and M. Wong, *Chemosphere*, 2003, 50, 807-811.
36. C. Radetski, B. Ferrari, S. Cotelle, J.-F. Masfaraud and J. Ferard, *Sci. total environ.*, 2004, 333, 209-216.
37. S. Kanazawa, S. Sano, T. Koshiba and T. Ushimaru, *Physiol. Plant.*, 2000, 109, 211-216.
38. S. Mishra, S. Srivastava, R. Tripathi, R. Govindarajan, S. Kuriakose and M. Prasad, *Plant Physiol. Biochem.*, 2006, 44, 25-37.
39. P. Sharma, A. B. Jha, R. S. Dubey and M. Pessarakli, *J. Bot.*, 2012, 2012.
40. D. J. Kliebenstein, R.-A. Monde and R. L. Last, *Plant Physiol.*, 1998, 118, 637-650.

Figure captions:

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 plant irrigated with citric acid (CA). 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 plant irrigated with malic acid (MA). Vertical bars refer to the standard error of mean values (n=3).

Fig.4. Cd concentration in plant tissues, as a function of 50 mg kg^{-1} Cd concentration in soil, for plant irrigated with oxalic acid (OA). 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 plant 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).

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).

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).

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.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.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).

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).

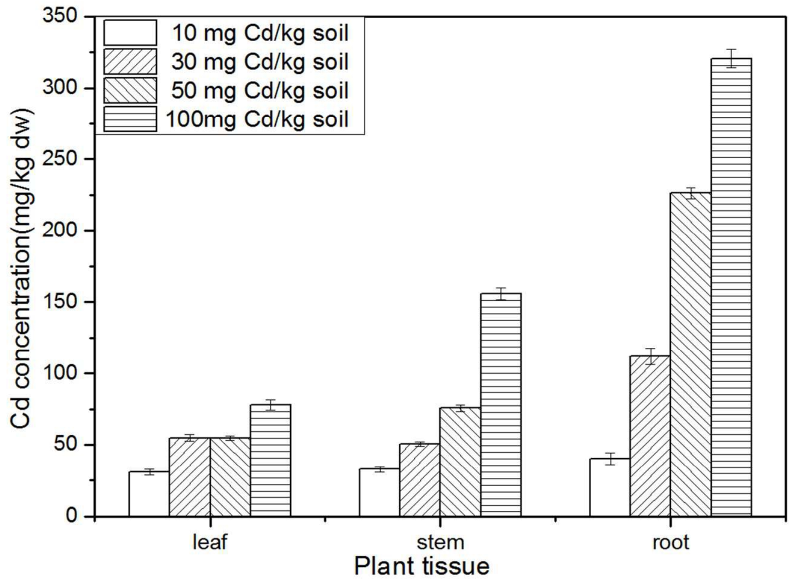

Fig.1. Cd concentration in plant tissues, as a function of 50 mg kg^{-1} Cd concentration in soil, for plants irrigated with distilled water. Vertical bars refer to the standard error of mean values (n=3).

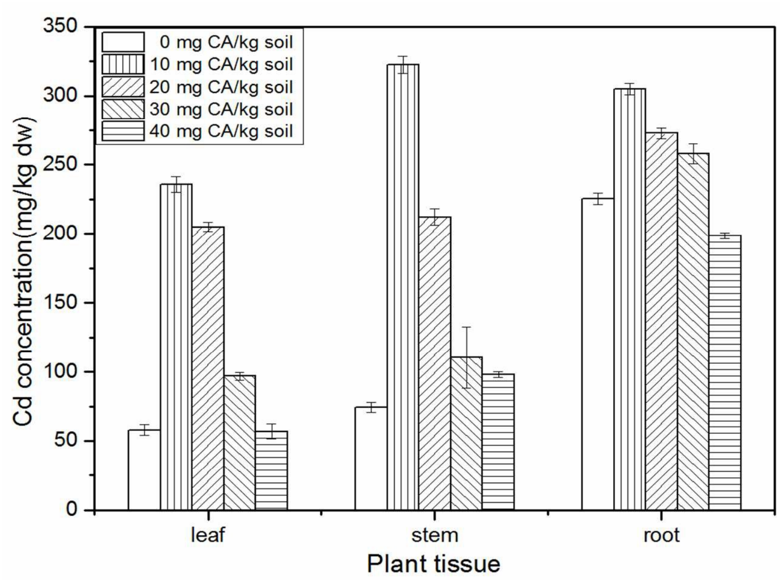

Fig.2. Cd concentration in plant tissues, as a function of 50 mg kg^{-1} Cd concentration in soil, for plant irrigated with citric acid (CA). Vertical bars refer to the standard error of mean values (n=3).

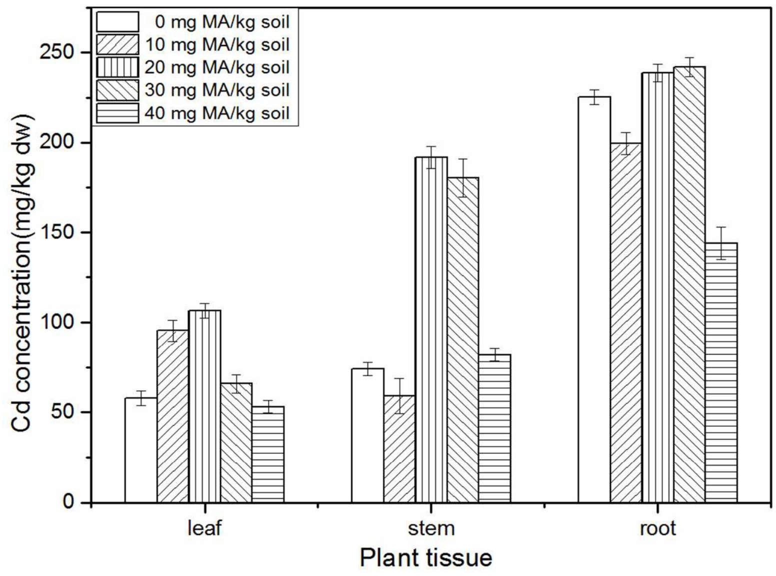

Fig.3. Cd concentration in plant tissues, as a function of 50 mg kg^{-1} Cd concentration in soil, for plant irrigated with malic acid (MA). Vertical bars refer to the standard error of mean values (n=3).

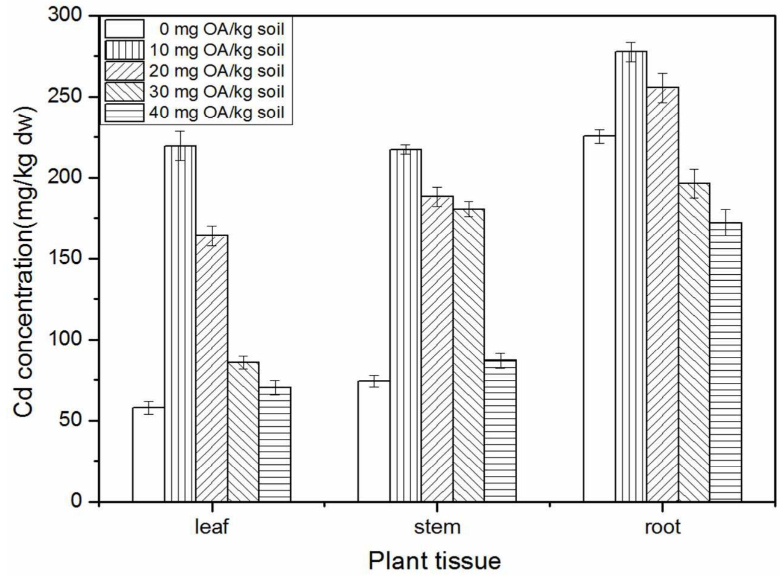

Fig.4. Cd concentration in plant tissues, as a function of 50 mg kg^{-1} Cd concentration in soil, for plant irrigated with oxalic acid (OA). Vertical bars refer to the standard error of mean values (n=3).

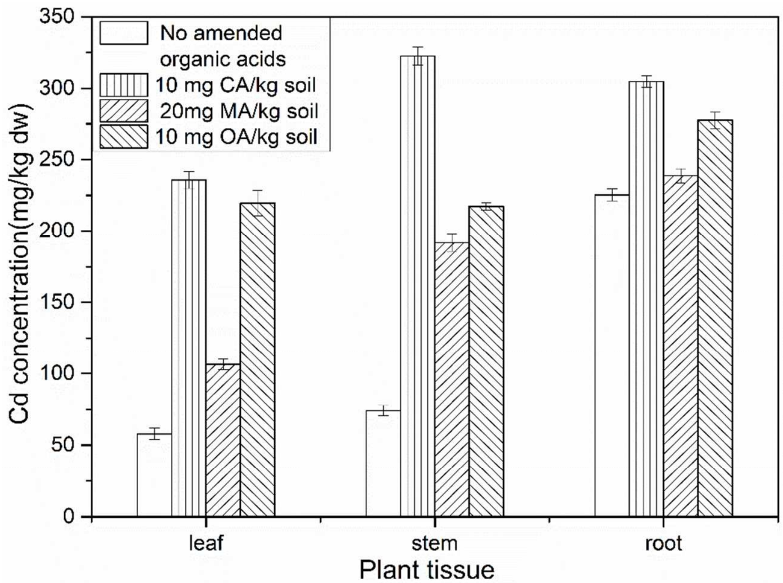

Fig.5. Cd concentration in plant tissues, as a function of 50 mg kg^{-1} Cd concentration in soil, for plant irrigated with distilled water, 10 mmol kg^{-1} oxalic acid (OA), 20 mmol kg^{-1} malic acid (MA) and 10 mmol kg^{-1} oxalic acid (OA). Vertical bars refer to the standard error of mean values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

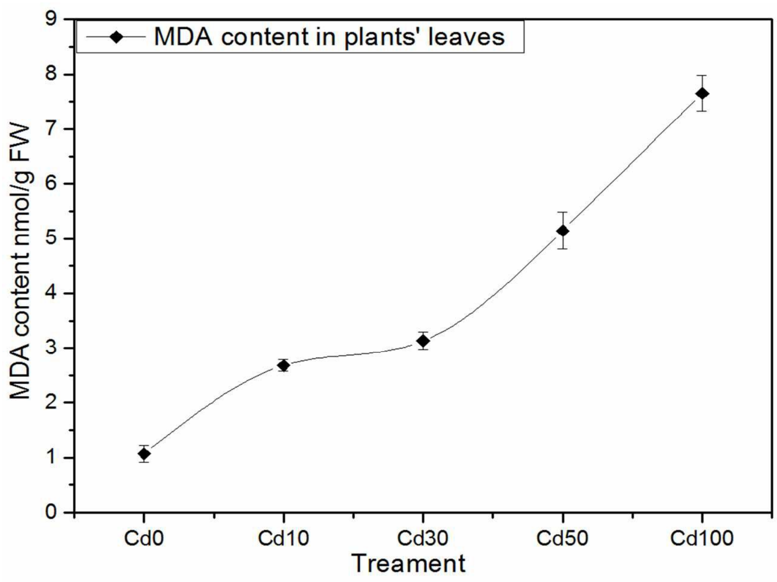

Fig.6. MDA content in leaves of pokeweed exposed to different levels of Cd. Vertical bars refer to the standard error of mean values (n=3).

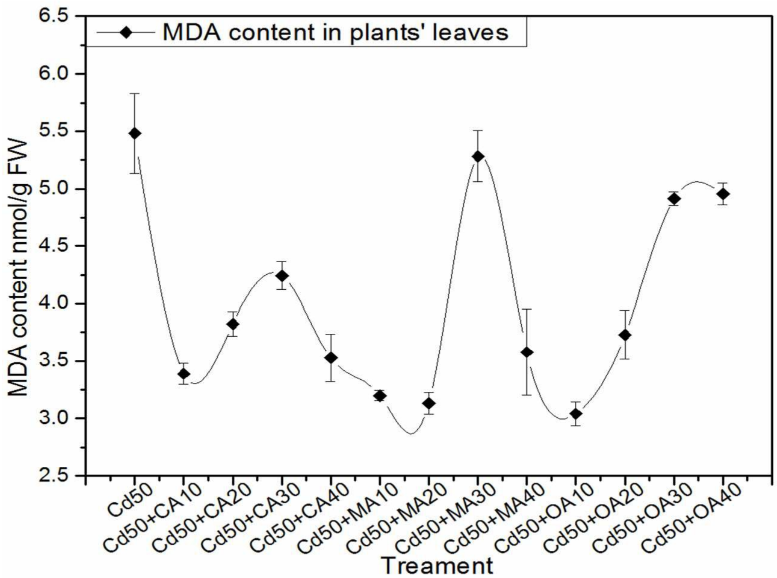

Fig.7. MDA content in leaves of pokeweed exposed to different levels of citric, malic and oxalic acid application at 50 mg kg^{-1} Cd level. Vertical bars refer to the standard error of mean values (n=3).

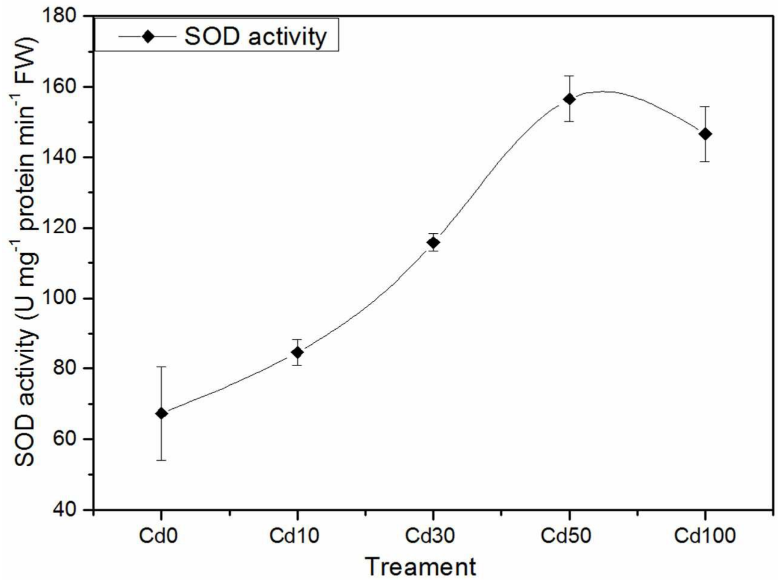

Fig.8. SOD activities in leaves of pokeweed exposed to different levels of Cd. Vertical bars refer to the standard error of mean values (n=3).

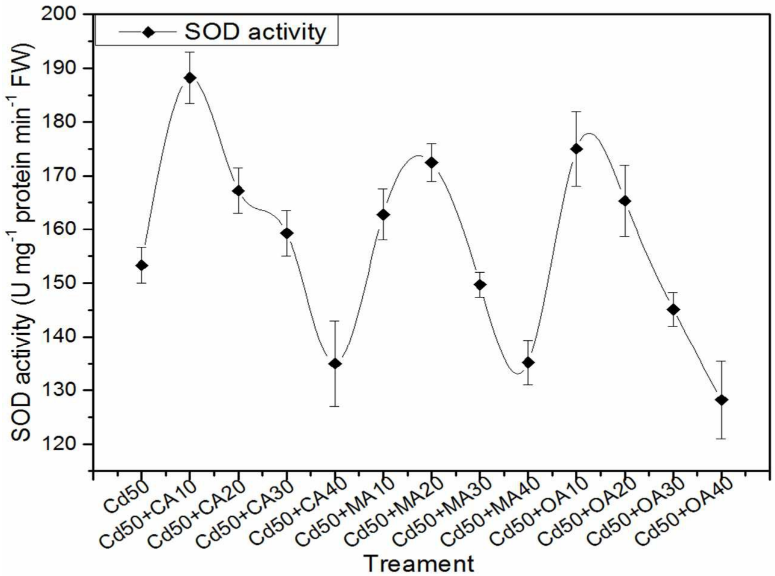

Fig.9. SOD activities in leaves of pokeweed exposed to different levels of citric, malic and oxalic acid application at 50 mg kg^{-1} Cd level. Vertical bars refer to the standard error of mean values (n=3).

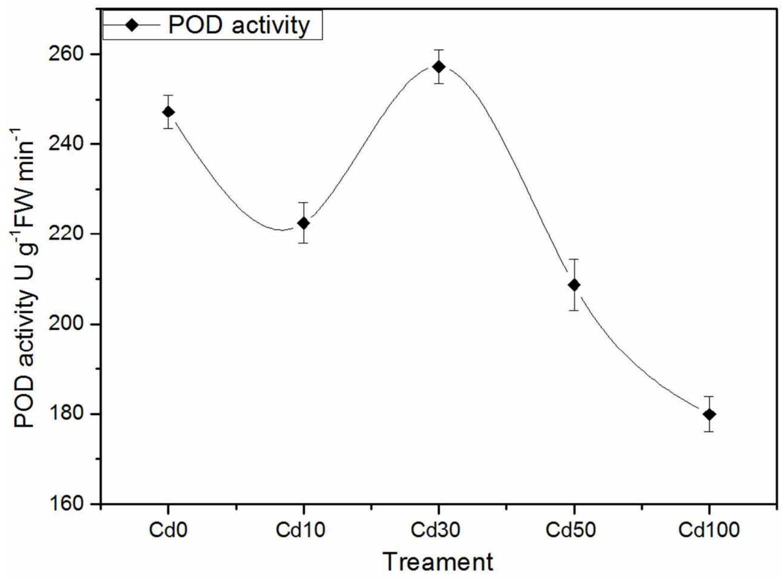

Fig.10. POD activities in leaves of pokeweed exposed to different levels of Cd. Vertical bars refer to the standard error of mean values (n=3).

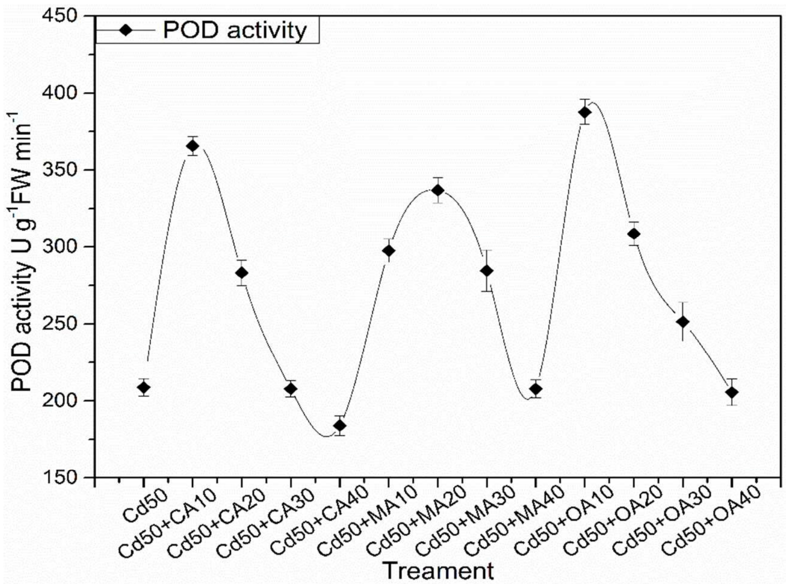

Fig.11. POD activities in leaves of pokeweed exposed to different levels of citric, malic and oxalic acid application at 50 mg kg^{-1} Cd level. Vertical bars refer to the standard error of mean values (n=3).

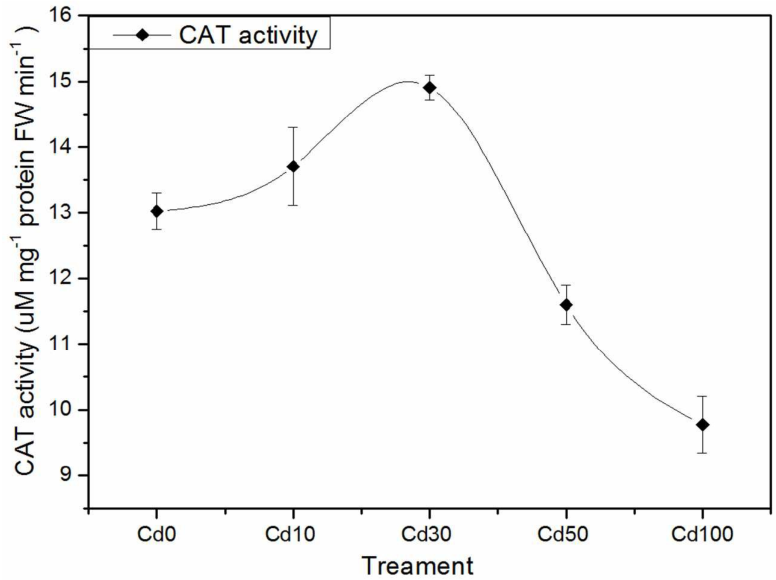

Fig.12. CAT activities in leaves of pokeweed exposed to different levels of Cd. Vertical bars refer to the standard error of mean values (n=3).

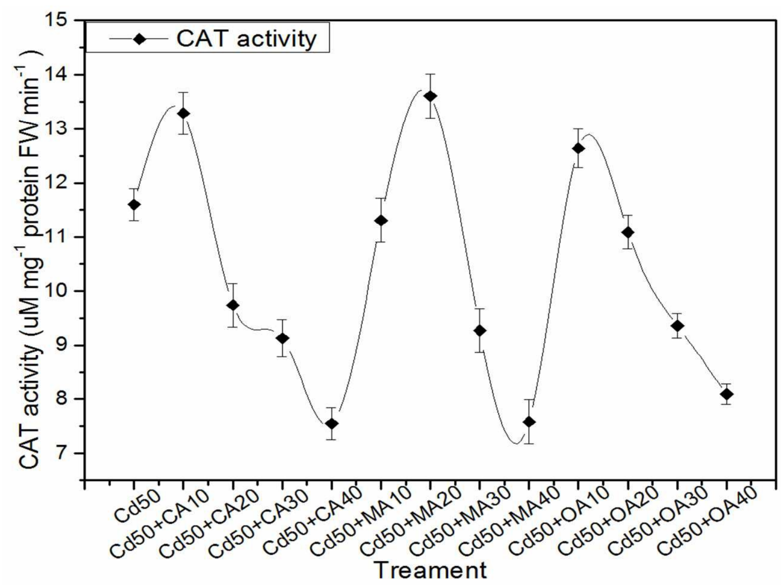

Fig.13. CAT activities in leaves of pokeweed exposed to different levels of citric, malic and oxalic acid application at 50 mg kg^{-1} Cd level. Vertical bars refer to the standard error of mean values (n=3).

Table 1 The chlorophyll content (mg/g FW) in the leaves of *P.americana* under different treatment.

Treatment	The chlorophyll (Chl) concentration (mg g^{-1} FW)		
	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

a Cd_0: without addition of Cd in nutrient solution; Cd_{10}, Cd_{30}, Cd_{50}, Cd_{100}: 10, 30,50, 100 mg kg^{-1} Cd concentration;

Cd_{50}+CA_{10}: 50 mg kg^{-1} Cd concentration+10 mmol kg^{-1} citric acid;

Cd_{50}+MA_{10}: 50 mg kg^{-1} Cd concentration+10 mmol kg^{-1} malic acid;

Cd_{50}+OA_{10}: 50 mg kg^{-1} Cd concentration+10 mmol kg^{-1} oxalic acid;