The Leaching Characteristics of Chelant-Enhanced Phytoextraction of Cd and Pb Contaminated Soil with Simulated Acid Rain

Yayin Lu [1,a],Dinggui Luo[1,2,b*],Guowei Liu[1,c],An Lai[1,d],Jian Gong[1,2,e]

[1]School of Environmental Science and Engineering, Guangzhou University, Guangzhou, Guangdong, China.

[2]Key Laboratory of Water Quality Safety and Protection of the Ministry of Education, Guangzhou University, Guangzhou, Guangdong, China.

[a]mylyy61@163.com, [b]ldggq@163.com, [c]410668170@qq.com, [d]944934795@qq.com, [e]Gongjiangz@163.com

Keywords: Phytoextraction; EDTA-enhanced; Cd- Pb compound contaminated soil; Leakage; *Zea mays* L.

Abstract. A soil column-cultivation leaching experiment was conducted in greenhouse to expose the HMs leaching characteristics in the process of EDTA-enhanced Phytoremediation of Cd- Pb compound contaminated soil by *Zea mays* L.(in seedling stage) under the condition of acid rain. Results showed that: (1) In the chelant-enhanced phytoremediation system , both EDTA and acid rain can accelerate HMs phytoextraction and promote Cd and Pb leakage, but EDTA was more effective than simulated acid rain. (2) In the process of rain leaching, the Cd and Pb concentration in the leachate decreased linearly along with the rainfall increased. The leaching rate of Cd and Pb with EDTA was 5.40- and 87.82-fold of control (without the application of a chelator).In the acid rain- *Zea mays* L.-chelant (EDTA)-enhanced phytoremediation system of Cd-Pb contaminated soil, EDTA and acid rain all have the characteristics of promoting Cd and Pb activation, but the leakage of HMs (especially Pb) depends mainly on the activation rate of EDTA to HMs, and might be closely related to the dosage and usage of chelating agent.

Introduction

Tolerant plants with high biomass could be good candidates for phytoremediation because high biomass can offset the low HMs (heavy metals) uptake rate. On the other hand, chelating agents which can promote the activation of HMs in the soil and strengthen the absorption of tolerant plants has become a research hotspot.

To date, the mostly investigated tolerant plants include: vetiver [1], Chinese cabbage [2], sunflower [3] and *Zea mays* L. [4]. For example, Liu [5] studied the HMs extraction of 29 species of *Zea mays* L. and found that both Zhengdan 958 and waxy *Zea mays* L. not only have high biomass, but also high bioconcentration and translocation factor of Cd and Pb. In addition, the straw of *Zea mays* L. can be used as materials on the production of fuel ethanol [6]. The use of *Zea mays* L. for soil remediation has multiple functions such as production, environmental protection and energy saving.

Most HMs in the soil exist in non-dissolved form, but they can be absorbed and utilized by plants upon dissolution [7]. Chelating agents can be used to promote activation of HMs in the soil [8]. EDTA (belongs to the category of APCAs) has a good activation effect on a variety of metals (e.g. Cd, Cu, Pb and Zn) and can increase the bioavailability by a factor of 203 (*Zea mays* L.), 296

(*Elsholtzia splendens*), 326 (*Zea mays* L.), 15.5 (Crowndaisy) times [9-11].

Chelating agents can be used to promote the release of HMs from soil surface and improve the uptake of HMs by plants. However, they also increase the risk of leaching of HMs, which leads to groundwater contamination [12]. To date, relevant research is limited. Vetiver, *Zea mays* L. cultivation - leaching experiment by Chen et al. [13] showed that EDTA had a greater influence on the downward migration on HMs of the surface soil under a large amount of precipitation. But under the condition of acid rain, HMs leakage with phytoextraction enhanced by chelating agents has rarely been reported.

The southern part of the Yangtze River in China is the main HMs contaminated area, where is also the main distribution of acid rain. Some research showed that the adsorbance of Cd in soil doubled as every 0.5 units increase when pH ranged from 3.8 to 4.9 [14]. Therefore, it is theoretical and practical to explore the characteristic of HMs leakage in chelant-enhanced phytoremediation system under the condition of acid rain.

Based on Cd- Pb compound contaminated soil, *Zea mays* L. (Zhengdan 958) was selected as typical tolerant plant, with seedling stage as the experimental period due to it is one of the main stages of the fast growth and absorption of HMs, EDTA as typical chelating agent, a greenhouse soil column-cultivation leaching test was conducted to expose the HMs leaching characteristics under the condition of acid rain, which will provide a theoretical support to the development and consummation of chelant-enhanced phytoextraction.

Materials and Methods

Experimental Materials

Experimental soil: The experimental soil was collected from the top layer (0-20 cm) of forest land (red loam) located near the Yunfu Iron Pyrite's discharge field in Guangdong Province, China (N 22 ˚59′25.5",E 112 ˚00′40.5"). Physicochemical characterization are measured according to standard methods [15], of which pH is 4.5, contents of organic matter, total N, available N, P and K are 15.4 g/kg, 0.084%, 37.8 mg/kg, 0.90 mg/kg and 12.1 mg/kg, respectively. Concentration of Cd and Pb are detected to be 0.12 and 52.30 mg/kg, respectively.

Plant for experiment: *Zea mays* cv Zhengdan 958 (Qiule Seed Industry Co., Ltd in Gansu Province, China).

Chelating agent: EDTA (Zhiyuan Chemical Reagent Co., Ltd in Tianjin, China).

The other test facilities: plastic flower pots (70-mm diameter by 68-mm height); the atomic absorption spectrophotometer (AAS, iCETM 3500, Fisher SCIENTIFIC, United Kingdom).

Experimental Methods

Experiment preparation

Except for control (CK) and according to the initial Cd and Pb concentration of soil, the studied soil were artificially spiked with solutions of $CdCl_2 \cdot 5/2H_2O$ and $Pb(NO_3)_2$ to bring the final total concentration to 10 mg/kg Cd and 500 mg/kg Pb. These spiked soils were subjected to five cycles of wet (70% of WHC) and dry (air drying) to produce a stabilized and equilibrium condition after HMs were added [16].

Simulated rain was produced according to the studies of Zheng et al. [17], contents of various salts such as NaCl, $(NH_4)_2SO_4$, KNO_3, $MgSO_4 \cdot 7H_2O$ and $CaSO_4 \cdot 2H_2O$ are 1.753, 2.243, 2.141, 5.792 and 24.480 mg/L. respectively. pH of the base solution was adjusted to 4.5 and 6.5 with a mix of HCl、H_2SO_4 and HNO_3.

Pot, Activation and Leaching Experiment

(1) 180g (dry weight) of the polluted soil were filled into the experimental pots, previously which

was fully mixed with a certain amount of chemical fertilizer (N: P_2O_5: K_2O=15:15:15).

(2) Seeds of Zhengdan 958 were germinated for 3 days, 2 seeds were embedded in the experimental pots at a depth of about 2-3cm, and then all pots were placed in the greenhouse at a temperature of 26-37℃, with relative humidity of 60-70% and adding water to keep 70% of the field water-holding capacity by constant weight at 8 am and 5 pm twice a day.

(3) At the 6^{th} -leaf stage of the *Zea mays* L., EDTA (3mmol/kg of soil) was added for a 2 days' activation [18]. Then, a leaching test with acid rain and non-acid rain was conducted. Based on the heavy rain standard, rainfall was set to 50 mm (heavy rain level, 250 mL for this experiment). The concentration of HMs was detected every 50 mL leachate with saturated infiltration.

Experiment Designing

Factors of chelating agent and types of precipitation were considered in this phytoextraction experiment, four experiment groups including: A1 (no chelator)-B1 (Non-Acid Rain); A1 (no chelator)-B2 (Acid Rain); A2 (3 mmol/kg EDTA)-B1 (Non-Acid Rain); A2 (3 mmol/kg EDTA)-B2 (Acid Rain).

Analysis Methods

Analysis of *Zea mays* L.

(1) Physiological characteristics of *Zea mays* L. including height, length of stem, width of stem, length of leaf, width of leaf, length of root and dry weights of roots and shoots are recorded.

(2) The shoots and roots were harvested separately and digested with a mixture of HNO_3 (GR) and H_2O_2 (GR) in order to detect HMs content.

Data Processing and Statistical Methods

(1)The data reported in this paper were the mean values based on the four replicated experiment results. Statistical analysis was performed using the Origin 8.5 software package.

(2) To compare plant accumulations of HMs, bioconcentration factor (BCF) which is the ratio of the HMs concentration in the shoot to that in the soil (BCF_S), and translocation factor (TF) which is defined as the ratio of the metal concentration in the shoot to that in the root, were calculated.

Results and Discussion

Effects of HMs and EDTA on the Growth of *Zea mays* L.

Zhengdan 958 grew well at the 6^{th} -leaf stage (for 15 days) without poisoning symptoms before and after the application of 3 $mmol\cdot kg^{-1}$ of EDTA, which indicated that Zhengdan 958 has the ability to adapt to the concentration of HMs and EDTA. Plant physiological indexes (Table 1) of *Zea mays* L. have only a tiny difference, with all physiological indexes ratio of A2 to A1 varies from 0.98 to 1.10.

Table 1 Physiological Indexes of *Zea mays* L.

No.	Scheme	Height (cm)	Length of Stem (cm)	Width(Diameter) of Stem (cm)	Length of Leaf (cm)	Width of Leaf (cm)	Dry Weight(g/pot)		
							Shoots	Roots	Total
1	A1	37.75	11.25	2.00	47.80	2.30	1.11	0.46	1.57
2	A2	41.60	11.90	2.10	49.40	2.25	1.10	0.49	1.59

BCF and TF of HMs in *Zea mays* L.

In the absence of EDTA (A1), the BCF and TF with acid rain (B2) were all higher than those with non-acid rain (B1), of which the ratios of B2 to B1 were 1.26 and 1.02 for Cd,13.9 and 1.27 for Pb, respectively (Table 2).

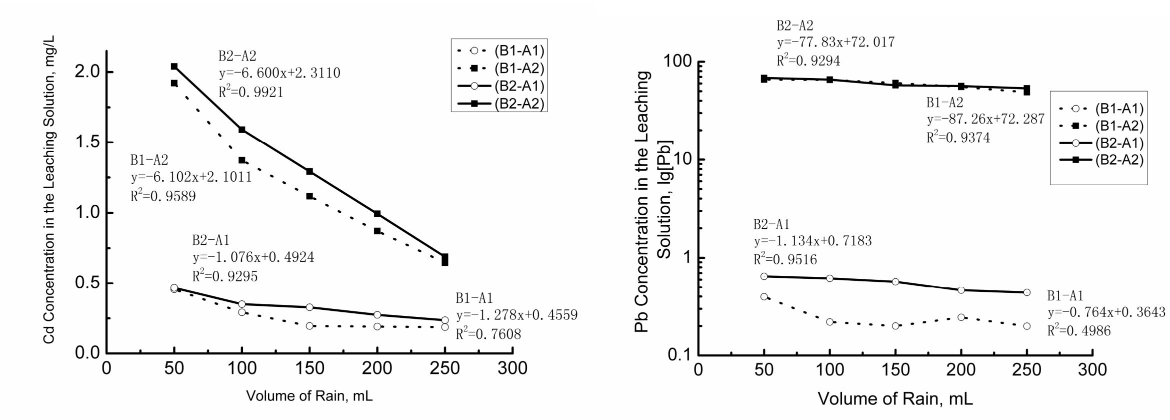
Table 2 Concentration, BCF and TF of Cd and Pb in *Zea mays* L.

No.	Scheme	Cd			Pb		
		C_{Shoots}	BCF_s	TF	C_{Shoots}	BCF_s	TF
1	A1-B1	27.28	2.73	0.43	65.63	0.13	0.26
2	A1-B2	34.50	3.45	0.44	90.79	0.18	0.33
3	A2-B1	51.89	5.19	1.42	478.75	0.96	1.95
4	A2-B2	56.79	5.68	2.00	575.67	1.15	2.65

When EDTA was added to soil (A2), the BCF_s and TF with EDTA were all higher than that of not adding one, of which the ratios of A2 to A1 was 1.76 and 3.93 for Cd, 6.81 and 7.80 for Pb, respectively, which was consistent with Wang et al. [19]. In addition, the BCF_s and TF with acid rain (B2) were all higher than those with non-acid rain (B1), of which the ratios of B2 to B1 were 1.09 and 1.41 for Cd, 1.20 and 1.36 for Pb, respectively, which suggested that acid rain can promote the Cd and Pb activation and accelerate the translocation of HMs from roots to shoots. The main reason can be explained as the acid rain increases the competitive adsorption capacity of H^+ and promotes the desorption of exchangeable HMs in the soil [20], and thus enhances the absorption of HMs by plants.

For further comparison, EDTA was more effective on Pb than Cd, of which the TF was 1.98-fold of Cd.

The Change of the Concentration of HMs in Leachate



(a) (b)

Fig. 1 The change of Cd and Pb concentration in leachate during the process of leaching

Note: Y stands for HMs content (mg/L) in the leachate; X represents the amount of rainfall (L)

Cd and Pb concentration in the leachate decreased gradually along with the simulated rainfall increased (Fig. 1), the changes of which could take advantage of the linear regression equation fitting. Except for the 1^{st} scheme, all r-square of the other schemes were above 0.92 (Fig. 1).

In the absence of a chelating agent (A1), the ranges of HMs concentration in leachate with the application of acid rain was 0.236-0.467 mg/L (0.331 for average) for Cd and 0.440-0.647 mg/L (0.548 for average) for Pb, respectively, which were 1.25- and 2.18-fold that of non-acid rain. When EDTA was added to the soil (A2), the ranges of HMs concentration with acid rain were 0.689-2.040 mg/L (1.321 for average) for Cd and 53.526-68.342 mg/L (60.342 for average) for Pb, respectively, which were 1.114- and 1.02-fold of non-acid rain. These results indicated that acid rain has the characteristic to promote the leaching of Cd and Pb, consistent with the results of Zheng et al. [20].

The average concentration of Cd in leachate with EDTA was 1.253 mg/L which was 4.21-fold of not adding one, which indicated that EDTA can effectively promote the Cd leaching, consistent with the study of Hu et al [21], whose experiments showed that EDTA has the ability to promote the leaching efficiency from 35-53% to 62-80%.

For further comparison, EDTA was more effective on Pb leaching than Cd, which was 35.45-fold of Cd.

Concluding Remarks

In the chelant-enhanced phytoremediation system , both EDTA and acid rain can accelerate HMs phytoextraction and promote Cd and Pb leakage, but EDTA was more effective than simulated acid rain.

In the process of rain leaching, the Cd and Pb concentration in the leachate decreased linearly along with the rainfall increased. The leaching rate of Cd and Pb with EDTA was 5.40- and 87.82-fold of control (without the application of a chelator).

In the acid rain- *Zea mays* L.-chelant (EDTA)-enhanced phytoremediation system of Cd-Pb contaminated soil, EDTA and acid rain all have the characteristics of promoting Cd and Pb activation, but the leakage of HMs (especially Pb) depends mainly on the activation rate of EDTA to HMs, and might be closely related to the dosage and usage of chelating agent.

Acknowledgements

This work was supported by the projects of National Natural Science Foundation of China (41372248), Key Project of of Science and Technology Plan of Guangzhou Colleges (CN) (1201410747), National College Students Innovation Training Project (CN) (201511078009).

References

1. Y.G. Liu, X.C. Song, X. Wang, Z.Y. Min and S.M. Liu. Study of the Cadmium Accumulation and Tolerance of Vetiveria Zizanioides.Journal of Hunan University(Natural Sciences), 37(1):75-79.(2010)

2. H. Grčman, Š. Velikonja-Bolta, D. Vodnik, B. Kos and D. Leštan. EDTA enhanced HMs phytoextraction： metal accumulation, leaching and toxicity. Plant and Soil, 235： 105-114.(2001)

3. M.S. Liphadzi, M.B. Kirkham and K.R. Mankin. EDTA –assisted heavy -metal uptake by poplar and sunflower grown at a long –term sewage-sludge farm. Plant and Soil, 257:171-182.(2003)

4. M. Komárek, P. Tlustos, J. Száková, V. Chrastný and V. Ettler. The use of *Zea mays* L. and poplar in chelant-enhanced phyto-extraction of lead from contaminated agricultural soils. Chemosphere, 67:640-651.(2007)

5. L.W. Liu. Research on the Remediation of Combined Contamination of HMs and Ten Bromine Biphenyl Ether by Different *Zea mays* L. Caltivars. Guangzhou: Jinan University,26-29.(2011)

6. Y.L. Lou, Y.S. Zhang and C.Y. Lin. Effects of forms of nitrogen fertilizer on the bioavailability of HMs in the soils amended with biosolids and their uptake by corn plant. Journal of Zhejiang University (Agric & Life Sci),31:71-77.(2005)

7. K. Tabata, S. Kashiwagi, H. Mori, C. Ueguchi and T. Mizuno. Cloning of a cDNA encoding a putative metal-transporting P-type ATPase from Arabidopsis thaliana. Biochim Biophys Acta, (1326): 1–6.(1997)

8. W.H. Xu, H. Huang, A.H. Wang, Z.T. Xiong and Z.Y. Wang. Advance in studies on activation

of HMs by root exudates and mechanism. Ecology and Environment,15(1):184-189.(2006)
9. R.W. Neugschwandtner, P. Tlustoš, M. Komárek and J. Száková. Phyto-extraction of Pb and Cd from a contaminated agricultural soil using different EDTA application regimes：Laboratory versus field scale measures of efficiency. Geoderma, 144:446-454.(2008)
10. M. Qian, Z.G. Shen and L. Wei. Comparison of EDDS- and EDTA- Assisted Uptake of HMs by Elsholtzia Haichowensis. Journal of Agro-Environment Science,25(1):113-118.(2006)
11. C.L. Luo, Z.G. Shen, Alan J. M. Baker and X.D. Li. A novel strategy using biodegradable EDDS for the chemically enhanced phytoextraction of soils contaminated with HMs. Plant and Soil, (285):67-80.(2006)
12. H. Grčman, Š. Velikonja-Bolta, D. Vodnik, B. Kos, and D. Leštan. EDTA enhanced HMs phyto-extraction: metal accumulation, leaching and toxicity. Plant Soil, (235):105–114.(2001)
13. Y.H. CHEN, L. LIU, G.P. WANG, Q. Guo and D. Zhu. The leaching behavior of HMs in contaminated soils during the process of chelant-enhanced phytoremediation. Journal of Nanjing Agricultural University, 30(4):46-51.(2007)
14. A.E. Boekhold, E.J.M. Temminghoff, S.E.A.T.M. Van Der Zee. Influence of electrolyte composition and pH on cadmium sorp tion by an acid sandy soil. Journal of Soil Science,44 (1)：85-96.(1993)
15. P.K. Lu. Analytical Methods of Soil and Agricultural Chemistry. China Agricultural Science and Technology Press, Beijing.(1999)
16. M.J. Blaylock, D.E. Salt, S. Dushenkov, O. Zakharova, C. Gussman, Y. Kapulnik, B.D. Ensley and I. Raskin. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents.Environ. Sci. Technol,31,860–865.(1997)
17. Y.M. Zheng, K. Zhong, X.Y. Peng, Z.B. Xue, Q.H. Ma and Y. Cai. Chemical Characteristics of Rainwater at Yunfu City in Guangdong Province. Journal of South China Normal University(Natural Science Edition),(1):111-115.(2009)
18. J.M. Zhou, Z. Dang, N.C. Chen, S.G. Xu and Z.Y. Xie. TOC And HMs Dynamic In Contam Inated Soil Solution And Their Correlations With The Addtion Of Chelating Agents. Environmental Chemistry,26(5):602-605.(2007)
19. Z.Q. Wang, C.L. Long, B.L. Fu and J. Zhao. Effects of several chelating agents on corn ' s absorbing HMs. Journal of Guizhou Normal University (Natural Sciences), 27（3）：40-43.(2009)
20. S.A. Zheng, C. Chen, X.Q. Zheng, S.T. Liu, R.G. Shi, X.W. Liu and et al. Effect of simulated rain on leaching of cadmium from 22 typical soils and key factors identification. Environmental Chemistry,32(5):867-873.(2013)
21. P.J. Hu, B.F. Yang, C.X. Dong, L.K. Chen, X.Y. Cao, J. Zhao and et al. Assessment of EDTA heap leaching of an agricultural soil highly contaminated with HMs. Chemosphere,(117):532-537.(2014)