Journal of Hazardous Materials 167 (2009) 38–43

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Growth responses and cadmium accumulation of *Mirabilis jalapa* L. under interaction between cadmium and phosphorus

Zhiguo Yu[a,b], Qixing Zhou[a,c,*]

[a] Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
[b] School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou 510275, China
[c] Key Laboratory of Pollution Processes and Environmental Criteria (Ministry of Education), College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China

ARTICLE INFO

Article history:
Received 16 June 2008
Received in revised form 14 December 2008
Accepted 16 December 2008
Available online 25 December 2008

Keywords:
Phosphorus
Heavy metal
Phytoremediation
Mirabilis jalapa L.
XANES

ABSTRACT

Based on the identification of *Mirabilis jalapa* L. as a new Cd-hyperaccumulating ornamental, the growth response of the plant to interaction between cadmium (Cd) and phosphorus (P), its effect on the Cd accumulation in the species and relevant mechanisms were further investigated by the pot-culture experiment with chemical analyses and the X-ray absorption near edge structure spectra (XANES). It showed that the leaf, shoot and root biomass (as dry matter) increased with an increase in P supplies from 20 to 100 mg kg^{-1} at various tested Cd levels except 10 mg kg^{-1}. The Cd accumulation in the leaves and shoots significantly decreased with increasing P concentrations from 20 to 500 mg kg^{-1} at the Cd concentrations from 10 to 50 mg kg^{-1}, except the Cd level at 100 mg kg^{-1}. It was also found that the translocation factor of Cd in *M. jalapa* L. reached the maximum at different tested Cd levels when the concentration of added P was 100 mg kg^{-1}, but the bioaccumulation factor of Cd decreased with increasing P. This changing law may be responsible for the mechanism of P immobilizing Cd. The investigation using the P–K edge XANES showed the spectra of adsorbed phosphate in the shoots exhibited a stronger white-line peak than that in the leaves and roots, and the oscillation near 2165 eV was more intense. Besides, the P–K edge XANES spectra for *M. jalapa* L. indicated P may exist as Cd-phosphate. Thus, it can be inferred that the addition of P at appropriate contents may be a useful approach to enhance the plant growth and to immobilize Cd in the Cd-contaminated soil. Furthermore, P and Cd may form a deposit in plants to tolerate Cd toxicity for reducing the degree of the structural damage of the plant.

1. Introduction

Pollution with metals and other xenobiotics is a global environmental problem that has resulted from mining, industrial, agricultural practices and military purposes [1]. Agricultural soil contamination is increasingly recognized as a dramatic event in large parts of the developing world, primarily in China [2]. The chemical cleanup of contaminated agricultural soils is costly and sometimes time-consuming. For many pollutants, no feasible low cost technologies are yet available. Plants possess highly efficient systems that acquire and concentrate nutrients as well as numerous toxic metals by metabolic activities, all of which are ultimately powered by photosynthesis. The term phytoremediation has been coined for the concept that plants could be used for low-cost environmental cleanup and this has attracted considerable attention in the past decades [3–5]. As a result, researchers have realized that the development of phytoremediation technologies requires a thorough understanding of the underlying processes at the genetic, molecular, biochemical, physiological and agronomic levels. However, augmentation-assisted phytoextraction is a promising method for the cleanup of contaminated soils by metals [6]. In particular, attention has been paid to the interaction of heavy metals and some nutrient elements in soils or soil–plant systems in order to improve the efficiency of phytoremediation.

As one of the most important nutrient elements, phosphorus (P) can exert its important influences on heavy metal accumulation and its relevant mechanisms in hyperaccumulators based on the interaction between P and heavy metals. It was reported that P could induce zinc requirement in tomatoes under hydroponic environment [7]. Similar results were also reported in *Sedum alfredii* by Sun et al. [8]. By examining relationships of arsenic (As) and phosphate, Singh and Ma [9] found that *Pteris vittata* mobilized As to the aboveground biomass, and maintained greater ratio of P and As in roots. Conversely, Huang et al. [10] showed that a higher concentration of nutrient phosphate suppressed accumulation of As in the fronds of *P. vittata* under the sand culture. However, the interaction

---

[*] Corresponding author at: Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China.
E-mail address: Zhouqx523@yahoo.com (Q. Zhou).

0304-3894/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2008.12.082

of cadmium (Cd) and phosphate has been indicative of differences, such as synergistic, additive or antagonistic effects. According to Karblane [11], and He and Singh [12], it was showed that phosphate inhibited Cd uptake in the pot-culture studies with different corps. On the contrary, phosphate has also been shown to increase plant uptake [13]. Moreover, interaction of Cd and phosphate was investigated under hydroponics. For example, Xiong and Lu [14] showed phosphate enhanced the accumulation of Cd in rice plants, but Yang [15] did not found significant effects on Cd accumulation at all phosphate concentration in maize. A large number of studies have developed for interaction of phosphate and Cd in soils, and nutrient solution. It is, however, important to recognize that, depending on the nature of P compounds and heavy metal species, plant species can cause either increased or decreased uptake of metals. Furthermore, it is essential to investigate Cd accumulation characteristics in new-found hyperaccumulators under phosphate treatments.

Ornamental plants are an important type of higher plants apart from those in the food chain, and are crucial if they have hyperaccumulation properties and can be applied to remediation of contaminated soils. *Mirabilis jalapa* L. (four o'clock) is an annual or perennial ornamental species, widely growing in Asia, Europe, and America. Zhou and Liu (Patent No. CN 200610046244.9) [16] reported the plant species as a new Cd hyperaccumulator using pot-culture and nutrient solution culture experiments. To better understand the mechanisms of Cd and P interaction, *M. jalapa* L. was chosen to investigate the Cd distribution as well as P application. The main objectives of this work were to (1) investigate growth responses of *M. jalapa* L. under different P and Cd treatments; (2) compare Cd distribution in roots, shoots and leaves at all P levels; (3) examine the forms of P in different plant parts by the X-ray absorption near edge structure spectra (XANES). Information obtained from this study can enhance the understanding of the Cd detoxification mechanisms by the new-found Cd-hyperaccumulator *M. jalapa* L.

2. Materials and methods

2.1. Pot-culture experiment

The surface (0–20 cm) soil samples were collected from an agricultural field in the Shenyang Station of Experimental Ecology, Chinese Academy of Sciences (123°41′N and 41°31′E). The tested soil is meadow burozem. It was shown by chemical analyses that organic matter, total N, pH and Cd concentration in this soil were 1.52%, 0.11%, 6.50 and 0.20 mg kg^{-1}, respectively. After the collected soil samples were air-dried and ground to pass through a 4.0 mm mesh, they were used in the pot-culture experiment.

Three Cd levels, namely treatments Cd_1, Cd_2 and Cd_3 (Cd concentration = 10, 50, and 100 mg kg^{-1} DW soil), were jointly applied with four P levels including P_0–P_3 (P concentration = 0, 20, 100, and 500 mg kg^{-1} DW soil). The tested topsoil samples were thoroughly mixed with $CdCl_2 \cdot 2.5H_2O$ and $Ca(H_2PO_4)_2 \cdot H_2O$ at the above-mentioned concentrations, filled into plastic pots, each for three replicates (20 cm in diameter, 15 cm in height, 2.5 kg air-dried soil per pot) and equilibrated for 1 month.

Seeds of *M. jalapa* L. were soaked in water for 12 h. Then the seeds were sowed directly into the prepared soil in May 2007. The treatment pots were placed in a greenhouse with natural light (about 10 h of photoperiod) and normal temperature (16–30 °C). The tested soils were watered to reach 60–80% of the field water-holding capacity. Two weeks after sowing, two seedlings were put into every treatment pot, respectively. At the mature stage (20 weeks after sowing), the plants were sampled from pots for chemical analyses [17].

2.2. Determination of Cd

For Cd analysis, air-dried plant tissues were ground by a mill and then digested with $HNO_3/HClO_4$ (3:1 [v/v]) [18]. The Cd concentration in digested solutions was determined by a flame atomic absorption spectrophotometer (AAS, WFX-120, Beijing Rayleigh Analytical Instrument Corp., China).

2.3. Soil analysis

At the stage of harvesting mature plants, soil samples were also collected for the analysis of pH, water-soluble (solution/soil ratio 2.5:1) and plant-available Cd in soil was extracted by 1.0 M NH_4NO_3 at a soil/solution ratio of 1:8 after 2 h shaking by an end-over-end shaker at room temperature (20 °C). At the end of the shaking period, the supernatant solutions were separated from the soil by centrifugation and filtration for measurement of Cd by AAS [19].

2.4. P speciation analysis

XANES sample preparation and data acquisition was prior to the lyophilization. The samples were first immersed in liquid nitrogen for 45 min until completely frozen, then placed into the Freeze Zone System (ALPHA 1-4/LD-2, CHRIST, made in Germany) and lyophilized at −45 °C and 0.069 mbar pressure. Subsequent to the lyophilization, the samples were ground using a Mixer Mill (MM200, Retsch Corporation, made in Germany) to obtain a homogenous mixture and then packed into sample holders, then placed in a vacuum chamber at 10^{-9} mmHg pressure. The samples were analyzed on beamline 4W7A at the Beijing Synchrotron Radiation Laboratory (BSRL) using a 2-element Si–Li fluorescence detector (Princeton Gamma-Tech) for P–K edge XANES. The beamline setup was described as follows: a Si(1 1 1) double crystal monochromator, a current ranging from 70 to 160 mA, beam energy of 2.5 GeV. The obtained XANES spectra with X-rays was in the energy range between 2100 and 2300 eV with stepwise increased energies in 0.5 eV increments [20].

In addition, dilution of the model, $Ca(H_2PO_4)_2 \cdot H_2O$, white line peak maximum 2150.5 eV, compounds were performed by mixing the model compounds with zinc oxide using a mortar and pestle to give a homogenous mixture with one absorption unit change across the absorption edge.

2.5. Statistical analysis

Values are expressed as means ± standard deviation (S.D.). The data were analyzed for significant differences using one-way ANOVA, taking $p < 0.05$ as significant according to the LSD test. The P–K edge XANES spectra was normalized with ifeffit-ATHENA [21].

3. Results

3.1. Response of plant growth to interaction between Cd and P

During the pot-culture experiment, there were no apparent toxic symptoms occurred in *M. jalapa* L., such as chlorosis from Cd poisoning phenomenon after Cd treatments. However, chlorosis took place in the P_3 level, due to the function of P inhibition on the absorption of nutrient elements, such as iron [22]. On the other hand, compared with the control (P_0), the lateral roots of the plants under P_1–P_3 treatments were promoted, especially under P_2 and P_3 treatments. In addition, the maturity of various plants in this experiment was hastened under all P treatments.

Plant biomass to denote plant growth is an important factor for successful application of phytoextraction since its effectiveness

Table 1
Effects of P and Cd addition on the dry biomass of *Mirabilis jalapa* L. (g pot^{-1} D.W.)[a,b].

Treatment of Cd	Treatment of P	Root	Shoot	Leaf	Aboveground	Total
Cd_1	P_0	8.41 ± 1.42a	7.19 ± 0.88a	6.05 ± 1.30a	14.28 ± 2.58a	22.69 ± 4.00a
	P_1	12.89 ± 0.60a	2.85 ± 0.48b	2.93 ± 0.37b	5.78 ± 0.85b	18.66 ± 1.45a
	P_2	14.04 ± 5.07a	3.30 ± 0.35b	2.37 ± 0.15b	6.01 ± 0.98b	20.05 ± 6.04a
	P_3	8.26 ± 0.99a	4.13 ± 2.44ab	2.81 ± 0.62b	7.22 ± 2.67b	15.48 ± 3.67a
	p	>0.05	>0.05	<0.05	<0.05	>0.05
	F	2.479	4.303	10.207	8.356	1.061
Cd_2	P_0	6.75 ± 3.98b	3.82 ± 1.08a	3.46 ± 0.99a	7.28 ± 2.07a	14.03 ± 6.05b
	P_1	12.55 ± 2.27ab	4.06 ± 2.33a	3.63 ± 1.34a	7.69 ± 3.67a	20.24 ± 1.39ab
	P_2	17.13 ± 0.19a	3.26 ± 0.58a	2.66 ± 0.47a	5.92 ± 1.05a	23.05 ± 0.85a
	P_3	9.62 ± 3.44ab	4.99 ± 1.48a	2.99 ± 1.56a	8.27 ± 2.65a	17.88 ± 0.80ab
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	F	4.782	0.461	0.287	0.309	2.922
Cd_3	P_0	4.03 ± 2.66b	3.57 ± 1.67a	2.99 ± 1.10a	6.56 ± 2.77a	10.59 ± 5.43a
	P_1	10.82 ± 0.84a	3.67 ± 0.13a	2.62 ± 0.54a	6.29 ± 0.67a	17.12 ± 0.17a
	P_2	7.72 ± 0.44ab	3.56 ± 1.83a	2.33 ± 1.29a	5.90 ± 3.13a	13.62 ± 3.56a
	P_3	6.01 ± 0.52b	3.53 ± 0.59a	2.02 ± 0.43a	5.55 ± 1.02a	11.56 ± 0.51a
	p	<0.05	>0.05	>0.05	>0.05	>0.05
	F	8.067	0.004	0.408	0.082	1.568

[a] The values in the same column followed by the same letters are not significantly different, whereas by the different letters are significantly different at $p < 0.05$.
[b] Data are means ± S.D. ($n = 3$).

depends on both plant biomass and elemental concentrations in a plant. The dry biomass of *M. jalapa* at the mature stage are shown in Table 1. Noticeably, the highest dry biomass of roots was at P_2 treatment with various Cd concentrations. Under treatments Cd_1 and Cd_2, the aboveground biomass of the tested plants reduced first and then increased with increasing P contents. There were different biological effects between P and Cd in aboveground parts and roots of *M. jalapa*. Generally speaking, the normal level of P (P_1 and P_2) could promote an increase in the dry biomass of *M. jalapa* under various Cd treatments. However, the high level of P(P_3)could lead to lower biomass of the plants.

3.2. *Cd accumulation affected by interaction between Cd and P*

To evaluate the extent of Cd-induced changes and the associated Cd detoxification systems in the plant, it is important to know the Cd accumulation in the plant species. Effects of P and Cd addition on the accumulation of Cd in different parts (roots, stems and leaves) of *M. jalapa* were shown in Table 2. According to Table 2, the Cd concentration in the aboveground parts was higher than that in the roots under various P treatments. Compared with P_0, the Cd accumulation in different parts was inhibited. In particular, there was the most significant decreased concentration of Cd under Cd_2–P_3 treatment. The Cd accumulation in the roots, shoots and leaves decreased by 69%, 60%, and 74%, respectively. Moreover, there was an inconsistent trend of Cd accumulation in aboveground parts and roots of *M. jalapa* at various P treatments. The Cd accumulation in the roots decreased with an increase in P under various Cd treatments, while the concentration variation of Cd in the leaves and shoots was irregular, but there was a remarkable phenomenon that the content of Cd in plants decreased by at least 50% under different P treatments.

According to Fig. 1, accumulation of Cd in the aboveground parts reduced first, then increased with increasing P contents under treatments Cd_1 and Cd_2. However, the accumulation of Cd was different under treatment Cd_3. Simultaneously, the inhibition of Cd accumulation was not significant. As shown in Fig. 2, the accumulation of Cd in the roots decreased with increasing P in soil under treatments Cd_1 and Cd_2. On the contrary, the Cd accumulation in the roots was enhanced at various P contents under treatment Cd_3.

Table 2
Effects of P and Cd addition on the Cd accumulation in *Mirabilis jalapa* L. (mg kg^{-1})[a,b].

Treatment of Cd	Treatment of P	Root	Shoot	Leaf	Aboveground
Cd_1	P_0	14.65 ± 7.81a	25.70 ± 17.50a	25.22 ± 17.98a	17.74 ± 10.26a
	P_1	5.61 ± 5.65a	11.62 ± 3.35a	8.460.57a	10.07 ± 1.99a
	P_2	2.03 ± 0.68a	12.54 ± 3.85a	9.33 ± 3.88a	9.23 ± 1.45a
	P_3	3.68 ± 2.23a	8.67 ± 6.98a	17.11 ± 4.42a	11.4 ± 3.39a
	p	>0.05	>0.05	>0.05	>0.05
	F	2.579	1.20	1.367	0.969
Cd_2	P_0	40.72 ± 6.53a	82.63 ± 20.04a	104.80 ± 45.13a	93.64 ± 32.40a
	P_1	11.56 ± 1.13b	37.31 ± 14.11b	39.15 ± 3.65b	38.31 ± 8.91b
	P_2	11.64 ± 0.07b	37.35 ± 7.85b	30.80 ± 0.34b	34.01 ± 3.65b
	P_3	12.8 ± 1.18b	32.68 ± 2.66b	26.84 ± 2.69b	26.99 ± 1.32b
	p	<0.05	<0.05	>0.05	<0.05
	F	36.466	6.615	5.218	6.555
Cd_3	P_0	67.93 ± 17.67a	146.48 ± 65.35a	135.74 ± 46.03a	141.14 ± 55.79a
	P_1	38.10 ± 7.93b	150.05 ± 1.39a	104.16 ± 0.74a	127.22 ± 0.90a
	P_2	51.80 ± 4.67ab	167.98 ± 18.46a	124.68 ± 21.18a	146.82 ± 0.67a
	P_3	60.21 ± 2.01ab	155.48 ± 8.12a	121.82 ± 3.83a	138.47 ± 5.57a
	p	>0.05	>0.05	>0.05	>0.05
	F	3.254	0.152	0.53	0.173

[a] The values in the same column followed by the same letters are not significantly different, whereas by the different letters are significantly different at $p < 0.05$.
[b] Data are means ± S.D. ($n = 3$).

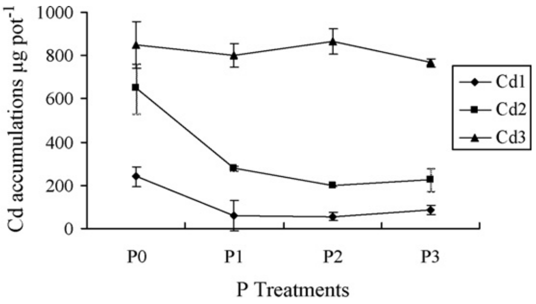

Fig. 1. Accumulation of Cd in leaves and shoots of *Mirabilis jalapa* L. under P and Cd interaction.

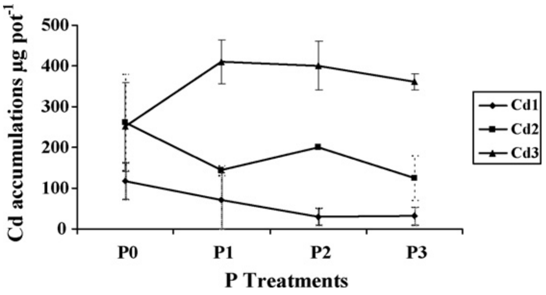

Fig. 2. Accumulation of Cd in the roots of *Mirabilis jalapa* L. under P and Cd interaction.

3.3. *Cd distribution affected by interaction between Cd and P*

The translocation factor (TF), defined as the ratio of the metal concentration in the shoots to that in the roots [23,24], is used to evaluate the effectiveness of a plant in translocating Cd from roots to shoots. To illustrate the metal accumulation efficiency in the plants, the bioaccumulation factor (BF), which is defined as the ratio of the metal concentration in a plant to the metal concentration in the soil, was also calculated [25]. TF and BF were expanded to aboveground/root and aboveground/soil to better understand Cd distribution in the plants.

As observed in Table 3, TF values of Cd in *M. jalapa* increased with increasing P concentrations under various Cd treatments, except treatment P_3. The TF values of Cd in *M. jalapa* were greater than 2.8 under treatments P_1 and P_2, whereas those under treatment P_0 were less than 2.3 in all Cd treatments. However, BF of Cd in *M. jalapa* reduced with increasing P concentrations. The BF values of Cd were greater than 1.0 under treatments Cd_1 and Cd_3, but less than 1.0 under treatment Cd_2.

It indicated that the transferring efficiency of Cd from roots to aboveground parts of *M. jalapa* was enhanced under normal P treatments. In other words, the results suggested that P probably played an important role in the transport of Cd from roots to aboveground parts of *M. jalapa*. High P levels in soil could reduce Cd accumulation from roots to aboveground parts of the plant. On the one hand, it can be interpreted that the Cd accumulation from roots to aboveground parts reduced due to inhibition of nutrient elements (greensickness discussed in plant growth) [24], thus may indirectly affect on photosynthesis and respiration, On the other hand, Cd can be adsorbed by soil particles or form a precipitation with P [26].

Table 3
Bioaccumulation and translocation factors of Cd in *Mirabilis jalapa* L. under Cd–P interaction.

	P_0	P_1	P_2	P_3
Translocation factor				
Cd_1	1.19	3.27	4.68	3.46
Cd_2	2.27	3.37	2.92	2.12
Cd_3	2.04	3.41	2.85	2.30
Bioaccumulation factor				
Cd_1	1.77	1.01	0.92	1.14
Cd_2	1.87	0.77	0.68	0.54
Cd_3	1.41	1.27	1.47	1.38

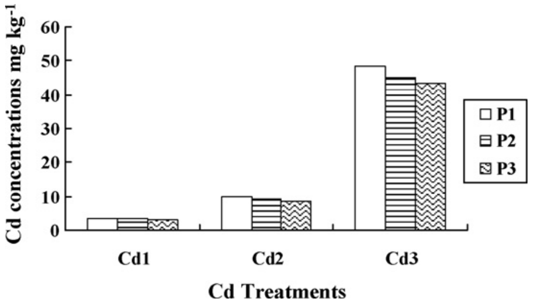

Fig. 3. Plant-available Cd in the tested soil under Cd–P interaction.

3.4. *Plant-available Cd affected by interaction between Cd and P*

It was expected that the addition of P to metal contaminated soil would provide four potential benefits for *M. jalapa*, including reducing the bioavailability of Cd, Pb and Zn, enhancing the bioavailability of As, providing a source of Ca and P, and increasing soil pH. In order to further prove the reliability of the interaction between Cd and P in soil, plant-available Cd in soil was determined. As shown in Fig. 3, plant-available Cd decreased with increasing P concentrations, in particular, when Cd in soil became higher. Concretely speaking, plant-available Cd under treatments Cd_1–Cd_3 decreased from 10, 50 and 100 mg kg^{-1} at the initial stage of the experiment to 3, 10 and 50 mg kg^{-1} at the mature stage of the plants, respectively. Meantime, soil pH increased from 6.82 at the initial stage of planting the ornamental to 7.21–7.33 after 10 weeks of plant growth [27].

3.5. *P speciation in M. jalapa affected by interaction between Cd and P*

XAFS has been newly used in biological and environmental science, it has been considered as a powerful and unique technique to explore the physiological actions of elements in living plants [28,29]. In order to further prove the reliability of interaction between Cd and P in plants, it is very imminent in application of P–K edge XANES to investigate various speciation of P in different parts of *M. jalapa*.

The P standard for XANES analysis was $Ca(H_2PO_4)_2 \cdot H_2O$. The P–K edge XANES spectra either for plants or for model exhibited no characteristic pre-edge feature (Fig. 4). However, the P–K edge XANES spectra for model exhibited a unique post-edge shoulder between 2152 and 2156 eV (Fig. 4). The structure of this shoulder was different from aboveground parts and roots in *M. jalapa*, and it was more well-defined for hydroxyapatite and octacalcium phosphate by Hesterberg et al. [30] as a Ca-phosphate feature peak. Without the post-edge feature in plants, it indicated that Ca-phosphate was not present in *M. jalapa*. On the other hand, there was another weak post-edge feature between 2158 and 2162 eV in leaves and shoots, which was different from that in roots. Noted that distinguishing of P peaks in different parts of *M. jalapa*, it can be interpreted as a mechanism of physiology for P in various parts under Cd treatments. Moreover, the spectrum for shoots exhibits a stronger white-line peak than those of the roots and leaves, and the oscillation near 2165 eV was more intense.

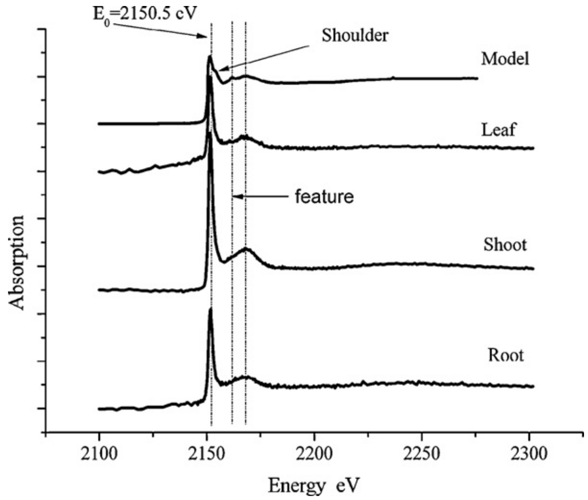

Fig. 4. The difference of the P–K edge XANES spectra between *Mirabilis jalapa* L. and the model under 100 mg kg^{-1} Cd.

4. Discussion

The present study shows that the concentration and amount of Cd accumulated in *M. jalapa* treated with Cd and P were inversely correlated with the concentration of phosphate as a nutrient substance, although there were some differences at the high P level. Moreover, the normal level of P (P_2 and P_3) could promote an increase in the dry biomass of *M. jalapa* under various Cd treatments. However, the concentration and amount of Cd accumulated in *M. jalapa* were both decreased with the increasing dry biomass of *M. jalapa*. This further validated the hypothesis proposed in the previous study that P inhibited the absorption of Cd. In other words, interaction between P and Cd both in soils and plants may account for decreased accumulation of Cd.

As shown in Fig. 3, the concentration of plant-available Cd in soil was reduced with the increasing P concentrations under various Cd and P treatments. This phenomenon may be responsible for the interaction between P and Cd in soil. It can be account for, on the one hand, several mechanisms that Cd adsorption could be induced by $H_2PO_4^-$ in soils [31–33], including (1) an increase in negative charges; (2) co-sorption of $H_2PO_4^-$ and Cd as an ion pair; and (3) surface complex formation of Cd on the P compound. On the other hand, some results showed that addition of P to Cd contaminated soil would stimulatively form $Cd_3(PO_4)_2$ as a deposit. Thus, $Cd_3(PO_4)_2$ can control the solubility of Cd in P-sufficient soils [34,35]. In this work, the results also indicated that increasing the amount of P could decrease the content of plant-available Cd, but in fact the translocation of Cd from the roots to the shoots increased, as showed in Table 3. It meant that most of added Cd could be fixed in soil, which played an important role in decreasing accumulation of Cd, but the interaction between P and Cd could not inhibit the absorption of Cd in *M. jalapa*.

In order to evaluate the interaction between Cd and P in plants, it is important to know the mechanism whether addition of P can promote the translocation of Cd from the roots to the aboveground parts of a plant. In this work, addition of external P could observationally mitigate the structural damage of the ornamental by Cd, and at the same time some black deposits accumulated on the chloroplast [36]. Because of the effect of PO_4^{3-} or HPO_4^{2-}, the phosphate deposits with Cd may form. Thus, it decreased the action of Cd restraining protochlorophyll-isocyanate-reductase and aminohexang-valerianic acid, and both enzymes can become active in the biosynthesis of chlorophyll [37]. A XANES analysis using the P–K edge XANES spectra which is sensitive to P valence in *M. jalapa* showed that the spectrum of the shoots of the plant species exhibited a stronger white-line peak than that of the roots and leaves. It further indicated that P in the shoots was related with some response of physiology under Cd treatments, such as forming a deposit with P. Meantime, the P spectra in different parts of *M. jalapa* was similar with the spectra in the model. It indicated that P may exist in plants as inorganic phosphate. Thus, this may be an important evidence for *M. jalapa* to tolerate Cd due to the deposit formation of P and Cd in plants.

To sum up, the addition of extra P to Cd contaminated soil could bring some significant functions. First of all, the immobility of Cd in soil by interaction between Cd and P decreased the content of plant-available Cd. Secondly, P promoted the translocation of Cd from the roots to the aboveground parts of the species and the formation of some deposits in aboveground parts, which may be the important mechanisms to reduce the degree of the structural damage by Cd. These gave indirect proof for the interaction between Cd and P not only in soil but also in plants, in particular, these may be an aspect of *M. jalapa* to accumulate and tolerate Cd. Thus, more mechanisms should be further investigated.

5. Conclusions

The interaction between P and Cd was investigated either in soil or in *M. jalapa* The investigation indicated that the normal level of P (20–100 mg kg^{-1}) could promote the dry biomass of *M. jalapa* under various Cd treatments, especially when the content of added P was 100 mg kg^{-1}. Due to the interaction between Cd and P in soil, the content of plant-available Cd reduced, and the accumulation of Cd in the roots and aboveground parts of the species decreased with increasing P in soil under treatments Cd_1 and Cd_2. The method using XANES further proved the reliability of the interaction between Cd and P in plants, which indicated that the co-existence of Cd-phosphate may be a way of *M. jalapa* tolerating Cd toxicity. On the other hand, the spectrum for the shoots of *M. jalapa* exhibited a stronger white-line peak than that of the roots and leaves, and the oscillation near 2165 eV was more intense. The principle was illustrated in the K-XANES spectrum for a plant sample, which indicated the difference between roots, shoots and leaves. Further studies are required to investigate relationships between Cd and P in the Cd-hyperaccumulator.

Acknowledgements

The research was financially supported by the Ministry of Science and Technology, People's Republic of China as an 863 project (No. 2006AA06Z386). The authors would also like to thank the Ministry of Education, People's Republic of China for financial support as a grand fostering project (No. 707011). In addition, the Sino-Russion Joint Research Center on Natural Resources and Eco-Environmental Sciences, Chinese Academy of Sciences, and the Key Laboratory of Environmental Remediation and Ecological Health (Zhejiang University), the Ministry of Education, People's Republic of China (No. EREH0710) gave partial financial support for this work. Portions of this research were carried out on beamline 4W7A at the Beijing Synchrotron Radiation Laboratory (BSRL). The authors acknowledge Mr. Lin Jun and Xu Wei for their assistance.

References

[1] R.A. Leigh, A.E. Johnson, Nitrogen concentration in field grown spring barley: an experiment of the usefulness of expecting concentration on the basis of tissue water, J. Agric. Sci. Camb. 105 (1985) 397–406.
[2] J.O. Nriagu, J.M. Pacyna, Quantitative assessment of worldwide contamination of air water and soils by trace metals, Nature 333 (1988) 134–139.
[3] S. Cheng, Heavy metal pollution in China: origin, pattern and control, Environ. Sci. Pollut. Res. Int. 10 (2003) 192–198.

[4] S.P. McGrath, F.J. Zhao, Phytoextraction of metals and metalloids from contaminated soils, Curr. Opin. Biotechnol. 14 (2003) 277–282.
[5] D.E. Salt, R.D. Smith, I. Raskin, Phytoremediation, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49 (1998) 643–668.
[6] T. Lebeau, et al., Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review, Environ. Pollut. (2007) doi:10.1016/j.envpol.2007.09.015.
[7] D.R. Paker, J.J. Aguilera, D.N. Thomson, Zinc–phosphorus interactions in two cultivars of tomato (*Lycopersicon sculontum* L.) grown in chelator-buffered nutrient solutions, Plant Soil 143 (1992) 163–177.
[8] Q. Sun, Z.W. Ni, X.E. Yang, et al., Effects of phosphorus on the growth, zinc absorption and accumulation in hyperaccumulator-*Sedum alfredii* Hance, Acta Sci. Circumstantiae 23 (2003) 818–824 (in Chinese).
[9] N. Singh, L.Q. Ma, Arsenic speciation and arsenic and phosphate distribution in arsenic hyperaccumulator *Pteris vittata* L. and non-hyperaccumulator *Pteris ensiformis* L., Environ. Pollut. 141 (2006) 238–246.
[10] Z.C. Huang, Z.Z. An, T.B. Chen, et al., Arsenic uptake and transport of *Pteris vittata* L. as influenced by phosphate and inorganic arsenic species under sand culture, J. Environ. Sci. 19 (2007) 714–718.
[11] H. Karblane, The effect of organic, lime, and phosphorus fertilizer on Pb, Cs, and Hg concent in plants, in: Proceedings of the Estonian Academy of Sciences Ecology, vol. 6, 1996, pp. 552–566.
[12] Q.S. He, B.R. Singh, Crop uptake of cadmium from phosphorus, Water Air Soil Pollut. 74 (1994) 251–256.
[13] J.P. Singh, J.W.P. Stewart, Phosphorus induced zinc deficiency in wheat on residual phosphorus plots, Agron. J. 78 (1999) 668–675.
[14] L.M. Xiong, R.K. Lu, Distribution of cadmium in rice its influencing factors, Soils 24 (1992) 138–145.
[15] Z.M. Yang, Advances on the study of interactions of phosphorus with zinc and cadmium in plants, Plant Nutr. Fertil. Sci. 21 (1998) 54–58.
[16] Q.X. Zhou, J.N. Liu, A new method to remediate Cd-contamination soil by *Mirabilis jalapa* L., China Patent No. 200610046244.9, 2006.
[17] S.H. Wei, Q.X. Zhou, P.V. Koval, Flowering stage characteristics of cadmium hyperaccumulator *Solanum nigrum* L. and their significance to phytoremediation, Sci. Total Environ. 369 (2006) 441–446.
[18] C. Chaffei, H. Gouia, M.H. Ghorbel, Nitrogen metabolism in tomato plants under cadmium stress, J. Plant Nutr. 26 (2003) 1617–1634.
[19] G.S.R. Krishnamurti, L.H. Smith, R. Naidu, Method for assessing plant-available cadmium in soils, Aust. J. Soil Res. 38 (2000) 823–836.
[20] H. Castillo-Michel, J.G. Parsons, J.R. Peralta-Videa, Use of X-ray absorption spectroscopy and biochemical techniques to characterize arsenic uptake and reduction in pea (*Pisum sativum*) plants, Plant Physiol. Biochem. 45 (2007) 457–463.
[21] B. Ravel, M. Newville, *Athena*, *Artemis*, *Hephaestus*: data analysis for X-ray absorption spectroscopy using *IFEFFIT*, J. Synchrotron Radiat. 12 (2005) 537–541.
[22] A.O. Fayiga, L.Q. Ma, X.D. Cao, Effects of heavy metals on growth and arsenic accumulation in the arsenic hyperaccumulator *Pteris vittata* L., Environ. Pollut. 132 (2004) 289–296.
[23] Q.X. Zhou, Y.F. Song, Principles and Methods of Contaminated Soil Remediation, Science Press, Beijing, 2004 (in Chinese, pp. 215–219).
[24] S. Tu, L.Q. Ma, Comparison of arsenic and phosphate uptake and distribution in arsenic hyperaccumulating and non-hyperaccumulating fern, J. Plant Nutr. 27 (2004) 1227–1242.
[25] C.K. Cohen, T.C. Garvin, D.F. FOX, The role of iron-deficiency stress responses in stimulating heavy-metal transport in plants, Plant Physiol. 116 (1998) 1063–1072.
[26] S.B. Nanthi, C.A. Domy, N. Ravi, Role of phosphorus in (Im)mobilization and bioavailability of heavy metals in the soil–plant system, Rev. Environ. Contam. Toxicol. 177 (2003) 1–44.
[27] A.O. Fayiga, L.Q. Ma, Using phosphate rock to immobilize metals in soil and increase arsenic uptake by hyperaccumulator *Pteris vittata*, Sci. Total Environ. 359 (2006) 17–25.
[28] J.G. Parsons, M.V. Aldrich, J.L. Gardea-Torresdey, Environmental and biological application of extended X-ray absorption fine structure (EXAFS) and X-ray absorption near edge structure (XANE) spectra scopes, Appl. Spectrosc. Rev. 37 (2002) 187–222.
[29] I.J. Pickering, C.P. Roger, J.G. Martin, D.S. Robert, N.G. Graham, E.S. David, Reduction and coordination of arsenic in Indian mustard, Plant Physiol. 122 (2000) 171–178.
[30] D. Hesterberg, W.Q. Zhou, K.J. Hutchison, XAFS study of adsorbed and mineral forms of phosphate, J. Synchrotron Radiat. 6 (1999) 636–638.
[31] R. Naidu, N.S. Bolan, R.S. Kookana, K.G. Tiller, Ionic-strength and pH effects on the adsorption of cadmium and the surface charge of soils, Eur. J. Soil Sci. 45 (1994) 419–429.
[32] N.S. Bolan, R. Naidu, J.K. Syers, R.W. Tillman, Surface charge and solute interactions in soils, Adv. Agron. 67 (1999) 88–141.
[33] N.S. Bolan, R. Naidu, J.K. Syers, R.W. Tillman, Effect of anion sorption on cadmium sorption by soils, Aust. J. Soil Res. 37 (1999) 445–460.
[34] J. Santillan-Medrano, J.J. Jurinak, The chemistry of lead and cadmium in soil: solid phase formation, Soil Sci. Soc. Am. Proc. 39 (1975) 851–856.
[35] J.J. Street, B.R. Sabey, W.L. Lindsay, Influence of pH, phosphorus, cadmium, sewage sludge, and incubation time on the solubility and plant uptake of cadmium, J. Environ. Qual. 7 (1978) 286–290.
[36] H.M. Jiang, J.C. Yang, J.F. Zhang, Effects of external phosphorus on the cell ultrastructure and the chlorophyll content of maize under cadmium and zinc stress, Environ. Pollut. 147 (2007) 750–756.
[37] A.K. Stobart, W.T. Griffiths, The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley, Physiol. Plant 63 (1985) 293–298.