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ORIGINAL PAPER

Phytoremediation of cadmium using plant species of *Athyrium wardii* (Hook.)

S. J. Zhang · T. X. Li · H. G. Huang · X. Z. Zhang · H. Y. Yu · Z. C. Zheng · Y. D. Wang · T. J. Zou · X. Q. Hao · Y. Pu

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Abstract *Athyrium wardii* (Hook.) is a promising herbaceous plant species for phytostabilization of cadmium (Cd)-contaminated sites with large biomass and fast growth rate. However, little information is available on its tolerance mechanisms toward Cd. To further understand the mechanisms involved in Cd migration, accumulation and detoxification, the present study investigated subcellular distribution and chemical forms of Cd in the mining ecotypes and corresponding non-mining ecotypes of *A. wardii* via greenhouse pot experiment. Subcellular fractionation of Cd-containing tissues demonstrated that the majority of the element was mainly located in soluble fraction in cell walls. This indicated that both the vacuoles and cell walls might be evolved the Cd tolerance mechanisms to protect metabolically active cellular compartments from toxic Cd concentrations. Meanwhile, Cd taken up by the plant existed in different chemical forms. Results showed that the majority of Cd in plant was in undissolved Cd–phosphate complexes (extracted by 2 % CH_3COOH), followed by water-soluble Cd–organic acid complexes, $Cd(H_2PO_4)_2$, pectates and protein form (extracted by deionized water and 1 M NaCl), whereas only small amount of Cd in roots was in inorganic form (extracted by 80 % ethanol), which suggests low capacity to be transported to aboveground tissues. It could be suggested that Cd integrated with undissolved Cd–phosphate complexes in cell wall or compartmentalization in vacuole might be responsible for the adaptation of the mining ecotypes of *A. wardii* to Cd stress.

Keywords Subcellular distribution · Chemical forms · Heavy metals · Phytostabilization

S. J. Zhang · T. X. Li (✉) · H. G. Huang · X. Z. Zhang · H. Y. Yu · Z. C. Zheng · Y. D. Wang · X. Q. Hao · Y. Pu
College of Resources and Environmental Science, Sichuan Agricultural University, Huimin Road 211#, Chengdu 611130, Sichuan, People's Republic of China
e-mail: litinx@263.net

S. J. Zhang
Agricultural Quality Inspection and Testing Center, Meishan Agricultural Bureau, Wending Street 169#, Meishan 620010, Sichuan, People's Republic of China

H. G. Huang
Tobacco Production Technology Center, Bijie Tobacco Company of Guizhou Province, Bijie 551700, Guizhou, People's Republic of China

T. J. Zou
Environmental Monitoring Station, Zigong Environmental Protection Bureau, Tanmulin Street 75#, Zigong 643000, Sichuan, People's Republic of China

Introduction

The accumulation of heavy metals in the soil, food chain and drinking water is becoming a major environmental problem, mainly due to human activities, such as application of heavy metal-containing pesticides and fertilizers, irrigation with sewage sludge, application of municipal wastes, mining, refining and through natural rock mineralization processes (He et al. 2008; Mahvi et al. 2008; Ling et al. 2011; Wu et al. 2011; Dede et al. 2012). Cadmium (Cd) is a non-essential heavy metal that is highly toxic to plants, animals and humans even at very low concentrations and is released to the environment as a consequence of industrial and agricultural activities (Domínguez et al. 2009; Feng et al. 2009; Sanità di Toppi and Gabbrielli 1999). An important cause of Cd toxicity is its chemical

similarity with essential elements, in particular Zn, but also Ca and Fe, deregulating the homeostasis of the latter elements or causing their displacement from proteins (Verbruggen et al. 2009). Moreover, excess Cd can profoundly interfere with a series of physiological processes in plants, such as enzyme activity, respiration, photosynthesis and nutrient assimilation (Ashraf et al. 2011; Harada et al. 2002; Vig et al. 2003; Zhang et al. 2010). To avoid Cd toxicity in Cd-contaminated areas, plants have evolved intra- and extracellular mechanisms for metal detoxification including binding and precipitation in the cell wall and/or compartmentalization in vacuoles via metal–organic acid complex (Krämer et al. 2000; Clemens 2001; Hall 2002). Although numerous studies on the interaction between Cd and plants have been conducted, many mechanisms of Cd toxicity in plants remain unclear (He et al. 2008).

There is some evidence that determination of Cd distribution and chemical speciation in plants is essential for understanding the mechanisms involved in Cd migration, accumulation and detoxification (He et al. 2008; Wang et al. 2008). However, the ability to take up, transport and accumulate Cd differs greatly among plant species and even among genotypes (He et al. 2008; Krämer et al. 2000). Wang et al. (2008) observed that in case of *Bechmeria nivea* (L.) Gaud. (ramie), a promising species for Cd phytoextraction with large biomass and fast growth rate, most of Cd was present in the cell wall and soluble fraction, and similar subcellular distribution pattern has been reported in *Phytolacca americana* L. (pokeweed) (Fu et al. 2011) and Cd-resistant *Hordeum vulgare* L. (barely) (Wu et al. 2005). Additionally, chemical speciation of Cd is closely related to its biological function, and different chemical forms of Cd extracted by different extracting agents have distinct toxicity degree and migration of Cd (Fu et al. 2011). However, previous studies have not yet provided consistent results. For instance, Zhou et al. (2008) found that the greatest amount of Cd in *Sedum jinianum* was in the form of pectate- and protein-integrated Cd as well as water-soluble Cd–organic acid complexes and $Cd(H_2PO_4)_2$.

Athyrium wardii (Hook.) is a common perennial plant; specifically, a type of fern that grows in fascicles. It was found to have a high potential in accumulating high concentrations of lead (Pb) and Cd in roots at the contaminated sites in China, which may be useful for the phytostabilization of soils contaminated by these two metals (Zou et al. 2011; Zhang et al. 2012). However, there is little information available regarding the Cd uptake and distribution patterns in response to Cd stress in this plant species. Therefore, the objectives of the present study were to investigate the characteristics of Cd subcellular distribution and chemical forms in the mining ecotypes and corresponding non-mining ecotypes of *A. wardii* and their implication in Cd tolerance. The experiment was carried out in the climatic conditions of Ya'an, PR China from March 15, 2010, to October 10, 2010.

Materials and methods

Plant materials and Cd treatments

Seeds for the mining ecotype (ME) were obtained from the Sanhe Pb/Zn mine tailings, Yingjing County, Ya'an, Sichuan Province, China (102°31′E, 29°47′N) at an elevation of 1,358–1,445 m, in March 2010. The climate is generally subtropical moist monsoon with an average temperature of 15.3 °C and an annual rainfall of more than 1,500 mm. Corresponding non-mining ecotypes (NME) seeds were collected from the Yucheng District, Ya'an (102°51′–103°12′ E, 29°40′–30°14′ N), Sichuan Province, which has climatic and topographic conditions similar to those of the mining area. The ferns were separated into similar size plant segments composed of 5–6 fronds. Healthy plants of similar size were selected and cultured for 2 weeks in quartz sand with 1/10 Hoagland's solution, which was watered every 3 days (Zou et al. 2011).

The pot experiment of *A. wardii* was conducted in plastic boxes (400 × 300 × 140 mm) filled with 5 kg of sterilized soil (sterilized by 0.5 % potassium permanganate solution for 1 h) (Guo et al. 2011). A soil sample of Ochric Aquic Cambosol (1–10 cm) was collected in Daxing, Ya'an, and then, approximately 150 kg soils sieved through a sieve with pore size of 2 mm, which was air-dried until using for the pot experiment. The physico-chemical properties of the soil include 57.8 % sand, 16.0 % clay, 26.2 % silt, 2.14 % organic matter, 1.18 g kg^{-1} total N, 90.9 mg kg^{-1} available N, 44.3 mg kg^{-1} Olsen P, 67.6 mg kg^{-1} available K, a pH of 6.53, and a Cd concentration of 0.35 mg kg^{-1}. The Cd treatments consisted of the addition of 25 and 50 mg Cd kg^{-1} soil and supplied as $CdCl_2{\cdot}2.5H_2O$. The control consisted of the treatment without any Cd. There were 4 replicates (pots) in each treatment and 24 pots in total, which were arranged in a randomized block design and interchanged the positions of the pots every 3 days so as to get uniform environment (light, air, temperature and humidity) for all plants. To simulate the Cd-impacted soils, pot soils were homogenized for 4 weeks with the added Cd prior to the transplantation of seedlings. Five seedlings were grown in each pot. The pot experiment was conducted in a greenhouse with natural light, air, humidity and temperature. The water-holding capacity of filled soil in plastic boxes was maintained at 60–65 % by applying deionized water every 3 days before and during the plant growth. At

the end of the experiment (after 4-week exposure to Cd), the plants were harvested and separated into roots, stems (petioles) and leaves (pinnates), and immediately frozen in liquid N_2 and kept frozen until use.

Tissue fractionation

Frozen materials were homogenized thoroughly and then put into pre-cold extraction buffer containing 50 mM Tris–HCl (pH 7.5), 250 mM sucrose and 1.0 mM $C_4H_{10}O_2S_2$, respectively. Cells were separated into three fractions: cell wall, soluble fraction and organelle-containing fraction using differential centrifugation technique as suggested with some modifications by Weigel and Jäger (1980). The homogenate was centrifuged at 4,000 r min^{-1} for 15 min (model ALLEGRA-64R, Beckman Coulter, Inc., USA) and repeated twice, and the precipitation was designated as 'cell wall fraction' mainly consisting of cell walls and cell wall debris. The resulting supernatant solution was further centrifuged at 16,000 r min^{-1} for 30 min. The resultant deposition and supernatant solution were referred to as 'organelle-containing fraction' and 'soluble fraction,' respectively. All steps were performed at 4 °C. Cell wall and organelle-containing fractions were digested with concentrated HNO_3:$HClO_4$ (5:1, v/v) until the liquid was clear approximately 0.5 h. The digested material was subsequently washed into a 10-ml flask and made up to volume using deionized water. Cd concentrations in different fractions were then determined by atomic absorption spectrometry (AA6300, Shimadzu, Japan). The instrument working conditions were wavelength 228.8 nm, slit 0.7 nm, atomization 1,550 °C, read time 3 s and sample volume 10 μL (Szkoda and Żmudzki 2005).

Extraction of chemical forms

Cd associated with different chemical forms was successively extracted by designated solutions in the following order (Yang et al. 1995):

F1: 80 % ethanol, extraction of aminophenol-bound Cd and inorganic-bound Cd such as nitrate/nitrite, chloride.
F2: Deionized water, extraction of water-soluble Cd–organic acid complexes and $Cd(H_2PO_4)_2$.
F3: 1 M NaCl (sodium chloride), extraction of pectate- and protein-integrated Cd.
F4: 2 % CH_3COOH (acetic acid, HAC), extraction of undissolved cadmium phosphate including $CdHPO_4$, $Cd_3(PO_4)_2$ and other Cd–phosphate complexes.
F5: 0.6 M HCl (hydrochloric acid), extraction of Cd oxalate.
F6: Cd in residual form (no extraction solution).

Frozen tissues were homogenized in extraction solution with a mortar and a pestle, diluted at the ratio of 1:100 (w/v) and shaken for 22 h at 25 °C. After that, the homogenate was centrifuged at 4,000 r min^{-1} for 10 min, and first supernatant solution was obtained in a conical flask. The sedimentation was re-suspended twice in extraction solution and shaken for 2 h at 25 °C, and centrifuged at 4,000 r min^{-1} for 10 min. Then, the supernatants of three suspension and centrifuge steps were pooled for each of the five extraction solutions. Each of the pooled supernatant solutions was then evaporated to constant weight (no liquid) and digested with HNO_3:$HClO_4$ (5:1, v/v) until the liquid was clear approximately 0.5 h. For determination of Cd in residual form, the plant material was digested as described above using HNO_3:$HClO_4$ (5:1, v/v) in the last step of the sequential extraction. Cd concentrations associated with different chemical forms were determined as described previously by atomic absorption spectrometry (AA6300, Shimadzu, Japan).

Statistical analysis

Data were expressed as the means and standard deviations (SDs). The data were statistically analyzed by one-way analysis of variance using SPSS (version 13.0) for Windows. The least significant difference (LSD) was used to test Cd concentration in different fractions and ecotypes. A $p < 0.05$ was considered to indicate statistical significance (Xiao et al. 2009).

Results and discussion

Subcellular distribution of cadmium

Subcellular distribution of Cd in the roots, stems and leaves of the two ecotypes of *A. wardii* without Cd supply in the medium (control) was investigated (Table 1). The results showed that only cell wall fraction in the roots of ME *A. wardii* was detectable, which was 0.11 mg kg^{-1}. Therefore, the proportion in the two ecotypes of *A. wardii* was not calculated. Additionally, subcellular distribution of Cd and its proportions in *A. wardii* under Cd stress are investigated, as shown in Figs. 1 and 2, respectively. Generally, increase in Cd supply from 25 to 50 mg Cd kg^{-1} soil markedly increased Cd concentrations in all subcellular fractions in both the ME and NME of *A. wardii*, whereas, with increase in Cd supply, the Cd concentration remained constant in organelle fraction in the roots and leaves of ME and in cell wall fraction in the roots of NME (Fig. 1). Meanwhile, the majority of Cd was associated with cell wall and soluble fraction, and a minor part of this element was present in organelle fraction. Moreover, with

Table 1 Subcellular distribution of Cd in the roots, stems and leaves of the two ecotypes of *A. wardii* without Cd supply in the medium (control)

Parts	Ecotypes	Subcellular distribution of Cd (mg kg^{-1}, fresh weight)		
		Cell wall	Organelle	Soluble fraction
Roots	ME[a]	0.11 ± 0.01	ND[b]	ND
	NME	ND	ND	ND
Stems	ME	ND	ND	ND
	NME	ND	ND	ND
Leaves	ME	ND	ND	ND
	NME	ND	ND	ND

[a] *ME* mining ecotype, *NME* non-mining ecotype

[b] *ND* not detectable. Lower detection limit for Cd is 0.009 mg L^{-1}. Data are mean ± SD of three individual replicates

increase in Cd supply in the medium, relative accumulation of Cd in soluble fractions was generally increased, whereas decreased in cell wall fractions. However, the proportion of Cd in soluble fraction in root of ME decreased (11.05 %) and relative accumulation of Cd in cell wall increased (23.13 %) with increasing Cd supply in the medium (Fig. 2).

Based on the results, Cd concentrations in tissues of the two ecotypes decreased following the order of roots > stems > leaves due to its long-distance translocation from roots to stems and leaves. Similar trend has also been observed in other plants (Gussarsson 1994; Wang et al. 2008). The previous studies have proved that metal-tolerant plants always accumulate higher concentration of toxic metal in roots and lower in shoots as compared with the non-tolerant ones (Baker 1984). Therefore, higher Cd concentrations in roots than those in other tissues could be considered as an important tolerance mechanism of *A. wardii*. Similar results have been reported in Cd-treated *Lactuca* sp. (lettuces) (Ramos et al. 2002).

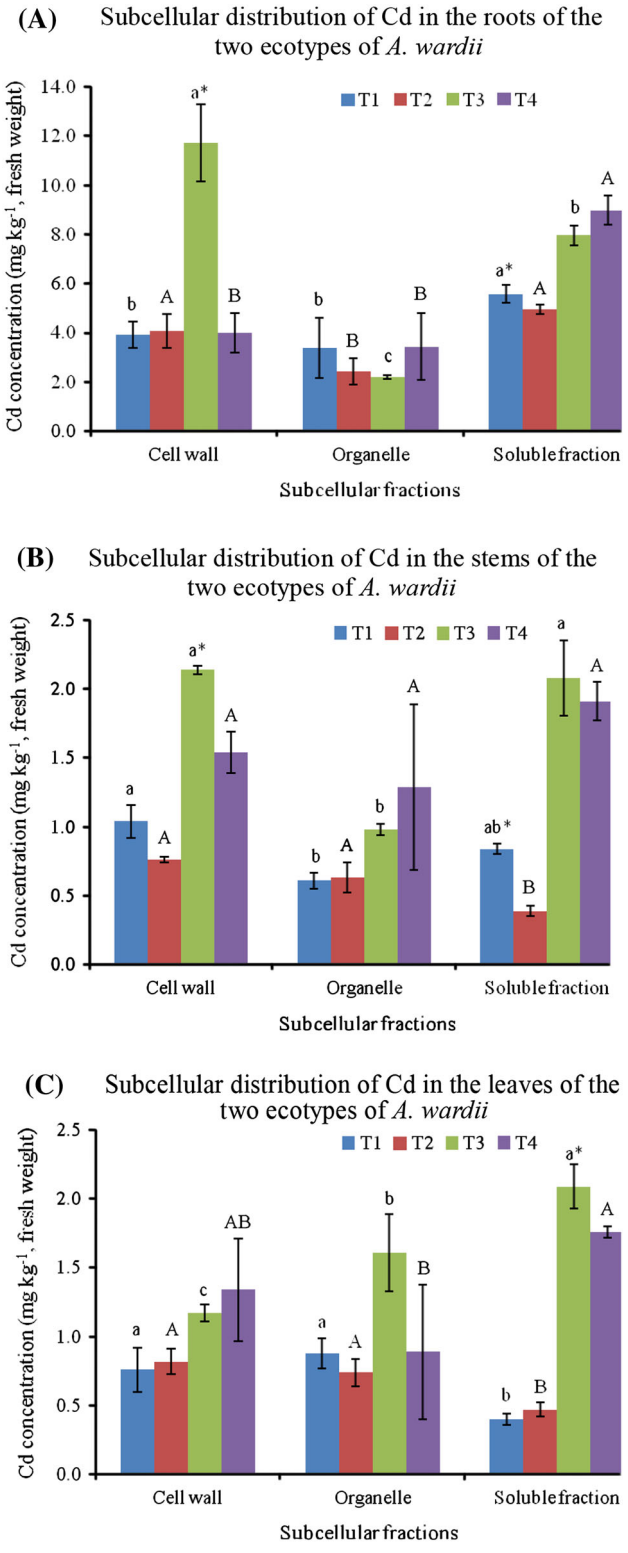
Cd analysis at the subcellular level of tissue in plants demonstrated that large proportion of Cd (24.02–53.54 %) was bound to the cell wall fraction, which seems to function as the first barrier protecting the protoplast from Cd toxicity. Similarly, Wang and co-workers in 2008 revealed that 48.2–61.9 % of the total Cd was bound to the cell walls in ramie. Plant cell walls are principally composed of polyose (including cellulose, hemicellulose and pectin) and protein, providing carboxyl, hydroxyl, amino and aldehyde groups. Therefore, they can bind Cd ions and restrict their transportation across cytomembrane (Fu et al. 2011). Besides, 19.61–43.29 % of the total Cd was stored in the soluble fraction, which consisted mostly of vacuoles and acted as the subdominant site of preferential sink for Cd in

Fig. 1 Subcellular distribution of Cd in the roots (**a**), stems (**b**) and leaves (**c**) of the two ecotypes of *A. wardii*. T1, T2: Mining ecotype and non-mining ecotype of *A. wardii* in 25 mg Cd kg^{-1} soil, respectively; T3, T4: Mining ecotype and non-mining ecotype of *A. wardii* in 50 mg Cd kg^{-1} soil, respectively. Data are mean ± SD of three individual replicates. Mean values labeled with different letters (lower case for ME and upper case for NME) for same ecotype, and Cd level indicates a significant difference ($p < 0.05$). * Significantly different ($p < 0.05$) at different ecotypes for same Cd supply in the medium

all test tissues. Moreover, this strategy could further decrease the amount of Cd interfering with organelles and therefore believe to play an important role in metal tolerance. Sulfur-rich peptides and organic acids are essential for the transportation and storage of Cd in the vacuoles, which can decrease free Cd ions activity and thus reduce toxicity via complexation of Cd with organo-ligands within the storage sites (Bhatia et al. 2005; Sanità di Toppi and Gabbrielli 1999). This result was in line with the result of Wang et al. (2008), who found about 30.2–38.1 % Cd being stored in soluble fraction in ramie.

In addition, it is noted that the percentage of Cd associated with organelles was higher in leaves than in roots and stems for all treatments (Fig. 2), which could be considered as the referential accumulation of Cd in chloroplasts (Ramos et al. 2002). These results are to some extent in line with those found by Wang et al. (2008) in ramie. Moreover, the percentage of Cd in organelles of the ME leaves was higher than NME, indicating the chloroplasts of ME of *A. wardii* might play an important role in Cd accumulation and tolerance.

Meanwhile, the results showed that in the roots of ME of *A. wardii*, following the increase in Cd concentration, the proportion of Cd in the cell wall fraction increased, whereas in NME, the cell fraction decreased with increasing Cd stress, indicating that limitation of root cell walls of ME to subsequently translocate Cd to shoots. These results were in line with those presented for Cd-treated pokeweed (Fu et al. 2011) and soybean plants (Cataldo et al. 1981). However, Cd subcellular distribution pattern presented here is not entirely consistent with other internal detoxification mechanisms exhibited by some plants. As reported by He et al. (2008), Cd was mainly bound to the cell wall of the roots, leaf sheaths and leaves. Ramos et al. (2002) also found that Cd was mainly associated with cell wall fractions in lettuce leaves, which was fairly independent of Cd level in hydroponic solution. However, according to Vázquez et al. (1992), most of Cd was associated with vacuoles of bean roots and no Cd was detected in cell walls. These conflicting results may be attributed to the variable levels of Cd tolerance of the different plants or genotypes studied, which display specific characteristic in metal uptake, translocation and compartmentalization.



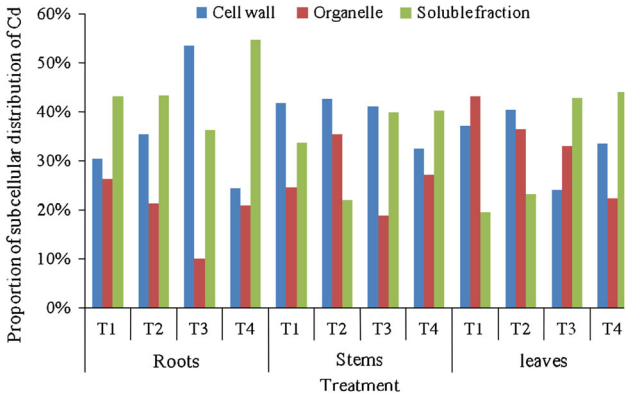
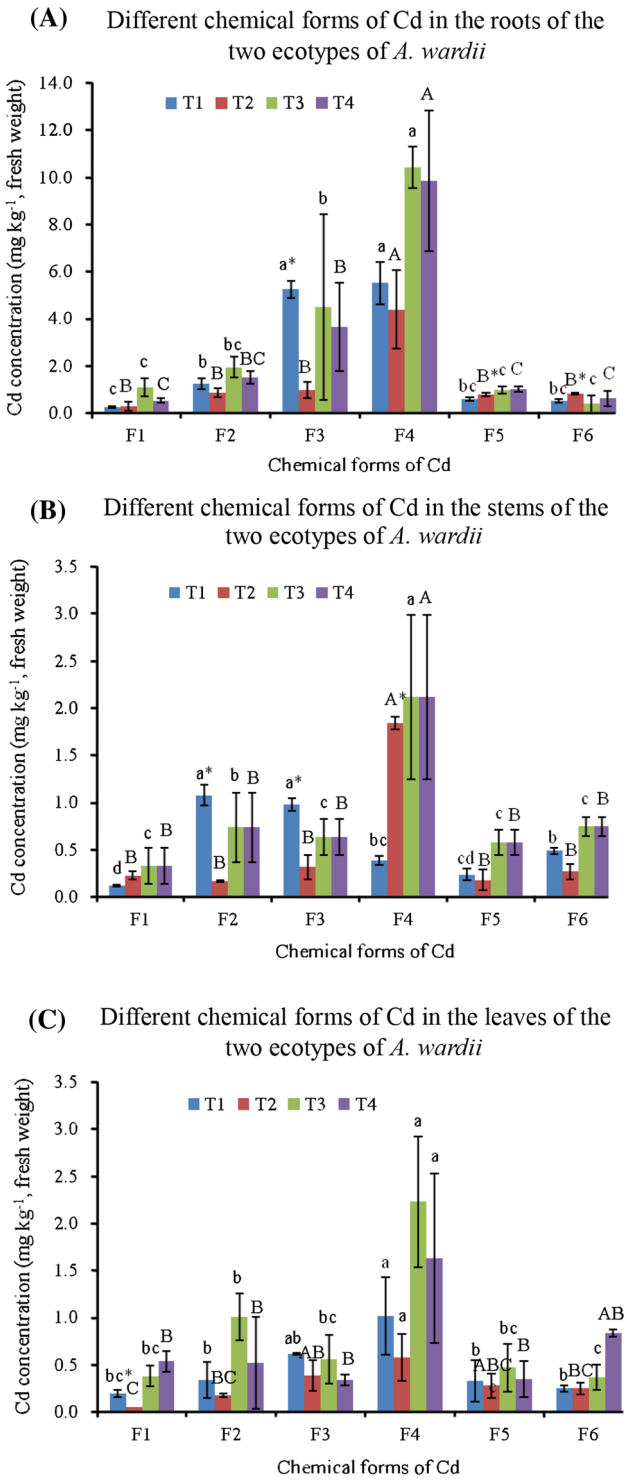


Fig. 2 Proportion of subcellular distribution of Cd in the two ecotypes of *A. wardii*. T1, T2: Mining ecotype and non-mining ecotype of *A. wardii* in 25 mg Cd kg^{-1} soil, respectively; T3, T4: Mining ecotype and non-mining ecotype of *A. wardii* in 50 mg Cd kg^{-1} soil, respectively

Chemical forms of cadmium

Different chemical forms of Cd in tissues can be characterized using different extracting agents. Chemical forms of Cd in the roots, stems and leaves of the two ecotypes of *A. wardii* without Cd supply in the medium (control) were investigated (Table 2). The results showed that only acetic acid (HAC)-extractable Cd in the roots of ME *A. wardii* was detectable, which was 0.09 mg kg^{-1}. Hence, the proportion in the two ecotypes of *A. wardii* was not calculated. In addition, the Cd concentrations bound to different chemical forms in the two ecotypes of *A. wardii* increased in a concentration-dependent manner following Cd exposure (Figs. 3, 4). Generally, for both ME and NME of *A. wardii*, the amount of Cd forms extracted by 2 % HAC was predominant for all treatment levels, whereas extraction of ethanol had the lowest Cd. For sequential extraction of Cd in case of roots, the order of Cd extraction is F4 > F3 > F2 > F5 > F6 > F1, whereas in stems it is F4 > F2 > F3 > F6 > F5 > F1 and in leaves it is F4 > F2 = F3 > F6 > F5 > F1, where F1 = ethanol-extractable Cd, F2 = deionized water-extractable Cd, F3 = sodium chloride (NaCl)-extractable Cd, F4 = acetic acid (HAC)-extractable Cd, F5 = hydrochloric acid (HCl)-extractable Cd, F6 = residual Cd.

As the chemical speciation of heavy metals is closely related to their biological function, the toxicity degree and migration of Cd are dependent on its chemical forms extracted by different designated extraction solutions (Yang et al. 1995; Xu et al. 2012). For instance, inorganic and organic water-soluble Cd (extracted by 80 % ethanol and deionized water, respectively), with higher capacity to migrate, is more deleterious to plant cells than the undissolved cadmium phosphate (extracted by 2 % HAC) and cadmium oxalate (extracted by 0.6 M HCl). Meanwhile, the plants that contained high concentration of Cd and yet showed little or no toxicity, Cd should be in a chemical form(s) that causes low or no phytotoxicity (Fu et al. 2011). In this study, Cd existed in different chemical forms among different plant tissues and genotypes. The majority of Cd



◀ **Fig. 3** Different chemical forms of Cd in the roots (**a**), stems (**b**) and leaves (**c**) of the two ecotypes of *A. wardii*. T1, T2: Mining ecotype and non-mining ecotype of *A. wardii* in 25 mg Cd kg^{-1} soil, respectively; T3, T4: Mining ecotype and non-mining ecotype of *A. wardii* in 50 mg Cd kg^{-1} soil, respectively. F1: ethanol-extractable Cd, F2: deionized water-extractable Cd, F3: sodium chloride (NaCl)-extractable Cd, F4: acetic acid (HAC)-extractable Cd, F5: hydrochloric acid (HCl)-extractable Cd, F6: residual Cd. Data are mean ± SD of three individual replicates. Mean values labeled with different letters (lower case for ME and upper case for NME) for same ecotype, and Cd level indicates a significant difference ($p < 0.05$). * Significantly different ($p < 0.05$) at different ecotypes for same Cd supply in the medium

was integrated with Cd–phosphate complexes (extracted by 2 % HAC). Therefore, it may be assumed that larger percentages of 2 % HAC-extractable Cd is responsible for the adaptation of *A. wardii* to Cd stress. Moreover, water-soluble Cd–organic acid complexes and $Cd(H_2PO_4)_2$ (extracted by deionized water) and pectates and protein form (extracted by 1 M NaCl) were generally higher than the other chemical forms except HAC-extractable Cd for both ME and NME *A. wardii*. Hence, these chemical forms in vacuoles might be another responsible for the adaptation of the *A. wardii* to Cd stress.

However, only 1.87–5.69 % of total Cd in the roots of *A. wardii* was in inorganic form (extracted by 80 % ethanol), suggesting low capacity to be transported to aboveground tissues. Therefore, Cd was hypothesized to be chelated by some specific polar material, such as hydroxyl or carboxyl, to form a non-toxic complex. It may also be assumed that lower percentages of 80 % ethanol-extractable Cd in roots and larger percentages of 2 % HAC-extractable Cd in tissues are responsible for the adaptation of *A. wardii* to high Cd accumulation and stress, which stands for the view point that compartmentalization in vacuoles and sequestration of Cd in cell wall may be crucial for the detoxification of Cd and thus tolerance to metal stress.

Furthermore, genotypic difference may exist in heavy metal tolerance and detoxification. Previous studies found that the ME of *A. wardii* has higher Pb concentration and more bioactivities of antioxidant enzymes as compared with NME (Zou et al. 2011); especially, a Cd-sensitive barley genotype was found to have higher Cd concentration in inorganic and water-soluble forms, while lower in pectate-/protein-integrated Cd in comparison with the other three Cd-resistant genotypes (Wu et al. 2005). Conversely, Cd accumulation seems to be a constitutive trait of pokeweed (Fu et al. 2011). In this study, the proportion of Cd in the cell wall fraction in roots of ME was higher than that of NME in high Cd concentration (Fig. 1), whereas with increasing Cd supply in the medium, undissolved cadmium phosphate (extracted by 2 % HAC) increased significantly in roots of ME of *A. wardii* tissues (Fig. 3). These results indicated that ME may have evolved some mechanisms to accumulate and tolerate Cd in long-term evolution, which enables the ME of *A. wardii* as a promising plant species for phytostabilization of Cd-contaminated soils. However, more detailed experiments are needed to well document the mechanisms of Cd tolerance and detoxification.

Table 2 Chemical forms of Cd in the roots, stems and leaves of the two ecotypes of *A. wardii* without Cd supply in the medium (control)

Parts	Ecotypes	Chemical forms of Cd (mg kg^{-1}, fresh weight)					
		F1[a]	F2	F3	F4	F5	F6
Roots	ME[b]	ND[c]	ND	ND	0.09 ± 0.02	ND	ND
	NME	ND	ND	ND	ND	ND	ND
Stems	ME	ND	ND	ND	ND	ND	ND
	NME	ND	ND	ND	ND	ND	ND
Leaves	ME	ND	ND	ND	ND	ND	ND
	NME	ND	ND	ND	ND	ND	ND

[a] F1: ethanol-extractable Cd, F2: deionized water-extractable Cd, F3: sodium chloride (NaCl)-extractable Cd, F4: acetic acid (HAC)-extractable Cd, F5: hydrochloric acid (HCl)-extractable Cd, F6: residual Cd. Data are mean ± SD of three individual replicates

[b] *ME* mining ecotype, *NME* non-mining ecotype

[c] *ND* not detectable. Lower detection limit for Cd is 0.009 mg L^{-1}. Data are mean ± SD of three individual replicates

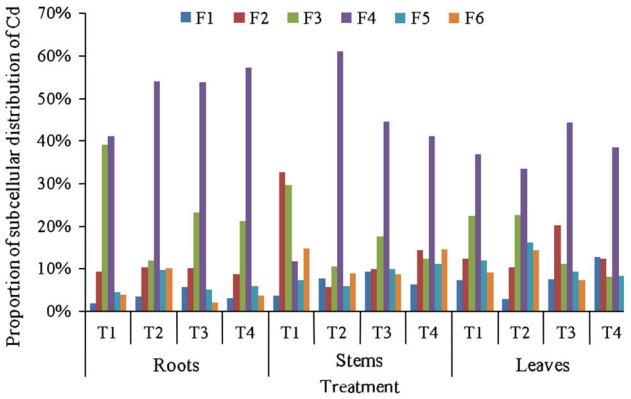


Fig. 4 Proportion of Cd in different fractions of the two ecotypes of *A. wardii*. T1, T2: Mining ecotype and non-mining ecotype in 25 mg Cd kg^{-1} soil, respectively; T3, T4: Mining ecotype and non-mining ecotype in 50 mg Cd kg^{-1} soil, respectively. F1: ethanol-extractable Cd, F2: deionized water-extractable Cd, F3: sodium chloride (NaCl)-extractable Cd, F4: acetic acid (HAC)-extractable Cd, F5: hydrochloric acid (HCl)-extractable Cd, F6: residual Cd

Conclusion

This study showed that large proportion of Cd was bound to the cell wall fraction or stored in the soluble fraction at the subcellular level of *A. wardii* tissues. Besides, the majority of Cd in the plant was undissolved Cd–phosphate complexes, followed by water-soluble Cd–organic acid complexes, $Cd(H_2PO_4)_2$, pectates and protein form, whereas only minority of total Cd in the roots was in inorganic form, which suggests low capacity to be transported to aboveground tissues. From this study, both the vacuoles and the cell walls might be involved in the Cd tolerance mechanisms to protect metabolically active cellular compartments from toxic Cd concentrations. Moreover, the mining ecotypes of *A. wardii* may have evolved some mechanisms to accumulate and tolerate Cd in the roots of this plant, which enables it as a promising plant for phytostabilization of Cd-contaminated soils.

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